Analyse zur globalen Genexpression in Geweben des frühen *Arabidopsis thaliana* Embryos

Dissertation
der Mathematisch-Naturwissenschaftlichen Fakultät
der Eberhard Karls Universität Tübingen
zur Erlangung des Grades eines
Doktors der Naturwissenschaften
(Dr. rer. nat.)

vorgelegt von
Daniel Slane
aus Ichenhausen

Tübingen
2014

Tag der mündlichen Prüfung:	16.12.2014
Dekan:	Prof. Dr. Wolfgang Rosenstiel
1. Berichterstatter:	Prof. Dr. Gerd Jürgens
2. Berichterstatter:	Prof. Dr. Klaus Harter

Danksagung

Aller Anfang ist schwer – und das vor allem in der Doktorarbeit. Und damit meine ich nicht die Doktorarbeit an und für sich, sondern eben das Verfassen der Danksagung. Denn es ist schwer an alle Menschen zu denken, die mich auf diesem Weg begleitet haben, ich werde mich aber bemühen, möglichst allen zu danken.

Vielen Dank dir, Gerd, für die Möglichkeit in der Pflanzenforschung arbeiten zu können und für die wissenschaftliche Freiheit. Ich habe in den Jahren durch die wissenschaftlichen Gespräche sehr viel gelernt und mein Interesse an der Forschung ist weiter gewachsen. Danke auch für die Auswahl der Kollegen, durch die ein äusserst angenehmes Arbeitsklima herrschte.

Prof. Dr. Klaus Harter danke ich für die gelungene Kollaboration und für seine bereitwillige Begutachtung dieser Arbeit.

Exceptionally I thank Jixiang for the great teamwork, the exciting scientific and non-scientific discussions about almost anything. You have always been a good colleague and friend! Stay just the way you are and see you in China!

Kenneth und Joachim, ich danke euch für die gelungene Zusammenarbeit und die Geduld, die ihr Jixiang und mir entgegengebracht habt.

Martin, ich bin sehr froh, dass du in „unser“ Labor gekommen bist. Es ist wichtig einen so guten Wissenschaftler in seinen Reihen zu haben, der immer Zeit für Fragen oder Sorgen hat. Vielen Dank für die gute Zusammenarbeit nicht nur gegen Ende, sondern auch die Jahre zuvor und vor allem in Zukunft.

Vielen Dank, Steffen, für dein schier unerschöpfliches Wissen, deine stetige Bereitschaft ein offenes Ohr zu haben und deine Unterstützung.

Einen grossen Dank an euch tollen Hiwis, vor allem an Phillip und Arvid. Nicht nur wart ihr äussert zuverlässig und fleissig, ich habe auch so manchen Spass mit euch erlebt.

Meine lieben Kollegen und Freunde, seid mir nicht böse wenn ich euch nicht alle namentlich erwähne, aber ihr müsst wissen ich mag euch alle. Martina, dir danke ich für sehr schöne Zeiten vor allem ausserhalb des Labors und ich hoffe dass wir noch

eine Weile erfolgreich zusammenarbeiten werden. Babu, you are simply the best. You are a big-hearted and beloved person and I hope that Ashwini and you will live happily ever after! Allen anderen danke ich für schöne Zeiten, die vielen gemeinsamen Essen, die Tanzabende, das Fussball spielen, das Trinken, das Herumalbern und die unglaublich tolle Laboratmosphäre! Danke für Alles!

Ole und Martin danke ich besonders für die Durchsicht der Doktorarbeit.

Ein ganz grosses Dankeschön gilt dir, lieber Reini. Du bist ein treuer Freund und hast mir die letzten Jahre viel Kraft und Geduld entgegengebracht, nicht nur für die Doktorarbeit.

Meinen Eltern danke ich für die unerschütterliche Unterstützung und Liebe. Danke, dass ihr immer an mich geglaubt habt auch wenn ich meinen ganz eigenen Kopf habe und meine Entscheidungen nicht immer die sinnvollsten waren.

Inhaltsverzeichnis

1. Zusammenfassung

Die Entstehung vielzelliger Eukaryonten bestehend aus unterschiedlichsten Zell- sowie Gewebetypen beruht auf zellulären Differenzierungsprozessen von pluripotenten und nicht endgültig differenzierten Zellen. Damit diese Stammzellen in Zellen mit spezifischen Aufgaben differenziert werden können, ist eine Veränderung der zellulären Genexpression grundlegend. Daher ist das Wissen über unterschiedliche Genexpressionsmuster sowie deren Zustandekommen unerlässlich für ein tieferes Verständnis von entwicklungsbiologischen Aspekten. Bei den Bedecktsamern (Angiospermen) wie der Ackerschmalwand (*Arabidopsis thaliana*) wird der Grundbauplan während der frühen Embryogenese ausgebildet. Dabei sind bereits im sogenannten embryonalen Herzstadium alle drei Achsen (apikal-basale, radiale, bilaterale), das Spross- sowie das Wurzelmeristems, die beiden Keimblätter (Kotyledonen) und das Hypokotyl festgelegt. Zu Beginn der Embryogenese markiert in *Arabidopsis* bereits die erste Zellteilung der Zygote die apikal-basale Symmetrieebene, wobei die beiden daraus resultierenden Tochterzellen in der Folge grundlegend verschiedene Entwicklungsrichtungen einschlagen. Während die Nachkommen der apikale Zelle durch Teilungen in verschiedenen Ebenen einen sphärischen Zellverband, den sogenannten Proembryo ausbilden, teilen sich die Nachkommen der basalen Zelle ausschliesslich horizontal und es entsteht dadurch eine einzige Zellreihe, der sogenannte Suspensor. Aufgrund seiner geringen Größe und der Tatsache, dass der Embryo bei Blütenpflanzen meist sehr tief im maternalen Gewebe eingebettet ist, waren Untersuchungen auf Transkriptomebene in der Vergangenheit kaum möglich.

In dieser Arbeit wurde exemplarisch am frühen *Arabidopsis* Embryo eine Methode entwickelt, mit deren Hilfe Genexpressionsprofile von Zellkernen des Proembryos und des Suspensors sowie auch des gesamten Embryos erstellt wurden. Dafür wurden gewebespezifische Markerlinien in Pflanzen etabliert, deren extrahierte Kerne mittels fluoreszenzbasierter Durchflusszytometrie, sogenanntem fluorescence-activated nuclear sorting (FANS) aufgereinigt wurden. Schliesslich wurde die Boten-RNA (mRNA) der Zellkerne über DNA-Microarrays analysiert. Durch Vergleich mit dem Genexpressionsprofil aus Zellen ganzer, intakter Embryonen vergleichbaren, embryonalen Stadiums konnte die Ähnlichkeit von Kernen und Zellen auf der Ebene der Genexpression gezeigt werden. Die statistisch signifikanten

Unterschiede im Genexpressionsmuster zwischen apikalem und basalem Embryonalgewebe wurden *in vivo* mittels Promoter-Reporter Fusionskonstrukten sowie RNA *in situ* Hybridisierung verifiziert. Überdies konnte gezeigt werden, dass die hier vorgestellte Methode Vorteile gegenüber der vormals angewendeten Laser-Mikrodissektion bietet. Mit dieser Methode sollte es möglich sein, aus schwerzugänglichen Geweben mit geringer Zellzahl auch in anderen Organismen verlässliche Genexpressionsprofile herzustellen. Die Ergebnisse dieser Arbeit liefern ausserdem eine nützliche Datenbank auf transkriptioneller Ebene für zukünftige Studien in der frühen Embryogenese von *Arabidopsis thaliana*.

2. Summary

Formation of multicellular eukaryotes consisting of various cell and tissue types depends on differentiation processes of pluripotent and undifferentiated cells. A prerequisite for these stem cells to be reprogrammed into cells with specific functions are changes in cellular gene expression. Therefore, knowledge about the different expression profiles and their origin is essential for a deeper understanding of development. In flowering plants (angiosperms) like the thale cress (*Arabidopsis thaliana*), the basic body plan is already being shaped during early embryogenesis. In the so called heart stage the embryo already comprises all three body axes (apical-basal, radial, and bilateral), the shoot as well as root meristem, the two cotyledons and the hypocotyl. At the beginning of embryogenesis the first division of the zygote already designates the apical-basal body axis and subsequently the two resulting daughter cells pursue entirely different developmental paths. While descendants of the apical cell form a spherical structure called proembryo through shifts of cell division planes, the basal part is only shaped by horizontal divisions leading to a single cell file called suspensor. Due to its small size and the fact that the embryo in flowering plants is often deeply embedded in the maternal tissue, transcriptomic approaches have been virtually impractical in the past.

Exemplary for the *Arabidopsis* early embryo, this work describes the establishment of a method by means of which nuclear gene expression profiles were generated for the proembryo and the suspensor as well as the whole embryo. For this purpose tissue-specific marker lines were established and the extracted nuclei were purified via so called fluorescence-activated nuclear sorting (FANS). Finally, the nuclear messenger RNA (mRNA) was analyzed with DNA-microarrays. Comparison of the nuclear transcripts with those from cells of entire, intact embryos of similar embryonic stages showed the overall comparability between nuclear and cellular transcriptomes. The statistically significant differences in gene expression patterns between proembryo and suspensor were verified *in vivo* using promoter-reporter fusion constructs and RNA *in situ* hybridization. Moreover, it could be shown that the presented method has advantages compared to previously used laser capture microdissection (LCM). With this method it should be possible to generate reliable gene expression profiles of inaccessible tissues with a limited number of cells also in

other organisms. The results from this work also provide a useful transcriptomic resource for future research on early embryogenesis in *Arabidopsis thaliana*.

3. Einleitung

3.1. Lebenszyklus bei Angiospermen am Beispiel von *Arabidopsis thaliana*

Blütenpflanzen durchleben einen haplodiplontischen Lebenszyklus mit dominierender diploider Generation (Sporophyt) und stark reduzierter haploider Generation (Gametophyt). Im Falle des Kreuzblütlers *Arabidopsis* (*Brassicaceae*) bestehen der weibliche Gametophyt, der sogenannte Embryosack, aus sieben und der männliche, auch als Pollen bezeichnet, aus drei Zellen (Berger and Twell, 2011). Während der reife Pollen eine freie Fortpflanzungseinheit darstellt, ist der Embryosack samt Eizelle tief im maternalen Gewebe eingebettet. In der adulten Pflanze (Abb. 1) kommt es bei *Arabidopsis* während der reproduktiven Phase in den voll ausgebildeten und reifen Blüten zur Selbstbestäubung, Transport der Spermien über den Pollenschlauch und nach ca. 12 Stunden zur sogenannten doppelten Befruchtung. Dabei entsteht im Embryosack durch Verschmelzung eines Spermiums mit der Eizelle die Zygote, das andere Spermium fusioniert mit der diploiden Zentralzelle zum Nährgewebe, dem sogenannten Endosperm (Hamamura et al., 2012). Aus der Zygote entwickelt sich der Embryo im Schutze der Samenanlage, welche wiederum zusammen mit mehreren anderen in den Früchten, den sogenannten Schoten heranreift (Abb. 1). Nach Beendigung der Embryogenese und einer Phase der Samenruhe (Dormanz), kommt es zur Keimung der jungen Pflanze bestehend aus zwei Keimblättern, dem Sprossmeristen, Hypokotyl und einer Wurzel mit Wurzelmeristem (Abb. 1). In den nächsten Wochen der vegetativen Phase werden verschiedene Entwicklungstufen durchlaufen bis hin zur Ausbildung von Blüten und dem Beginn einer neuen Generation (Boyes et al., 2001).

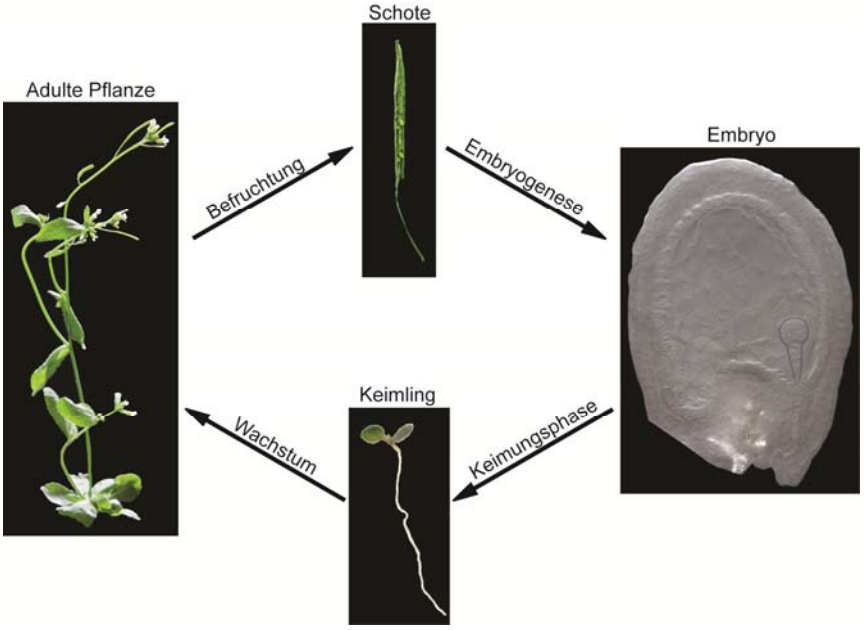

Abbildung 1: Lebenszyklus von *Arabidopsis thaliana*. In den Blüten der adulten Pflanze findet die Selbstbestäubung und kurz darauf die Befruchtung von Eizelle durch Spermium statt. In der Frucht oder auch Schote entwickeln sich mehrere Samenanlagen in deren Inneren auch der Embryo entsteht. Nach Beendigung der Embryogenese und einer Phase der Samenruhe, kommt es zur Keimung und in den Wochen darauf zur Bildung von Blättern (Rosette), einem Spross sowie Blüten.

3.2. Apikal-Basale Musterbildung im *Arabidopsis* Embryo

Beim Grossteil der näher untersuchten Blütenpflanzen teilt sich das erste Sprophytenstadium, die Zygote, horizontal in eine apikale und eine basale embryonale Zelle. Und auch bei den meisten Embryonen der Angiospermen hat dies eine kleinere apikale sowie eine größere basale Zelle zur Folge (Sivaramakrishna, 1978; Johri et al., 1992). In *Arabidopsis* spielen noch vor der asymmetrischen Teilung zwei Prozesse eine wichtige Rolle für die korrekte Musterbildung des frühen Embryos, nämlich sowohl die Polarisierung als auch die Streckung der Zygote. Die Polarisierung ist unter anderen bei *Arabidopsis* sichtbar durch die Lage der Vakuolen im Bereich der Pollenschlaucheintrittsstelle (Mikropyle) und die des Zytoplasmas samt Zellkern darüber in Richtung der sogenannten Chalaza (Mansfield and Briarty, 1991). Auf molekularbiologischer Ebene spielt bei der Polarisierung der Zygote der Transkriptionsfaktor WRKY DNA-BINDING PROTEIN 2 (WRKY2) eine wichtige Rolle, indem er zumindest ein weiteres Gen, nämlich den Transkriptionsfaktor *WUSCHEL RELATED HOMEOBOX 8* (*WOX8*) aktiviert. In *wrky2* Mutanten verliert

die Zygote ihre Polarität und teilt sich daraufhin symmetrisch und in der Folge führt womöglich eine starke Misexpression von *WOX2* im Suspensor zu einer gestörten Embryonalentwicklung (Ueda et al., 2011).

Nach der Polarisierung streckt sich die Zygote ca. um das Dreifache. Für diese Streckung sind mehrere Faktoren verantwortlich, nämlich GNOM (GN), YODA (YDA), SHORT SUSPENSOR (SSP), MAP KINASE 3 und 6 sowie ein weiterer Transkriptionsfaktor GROUNDED/RWP-RK DOMAIN 4 (GRD/RKD4) (Abb. 2). Bis auf GN fungieren diese Gene womöglich in einer frühembryonalen YDA Signalkaskade und Funktionsverlust-Mutationen in all diesen Genen führen zu geringerer Zygotenstreckung und/oder Fehlern bei der asymmetrischen Teilung der

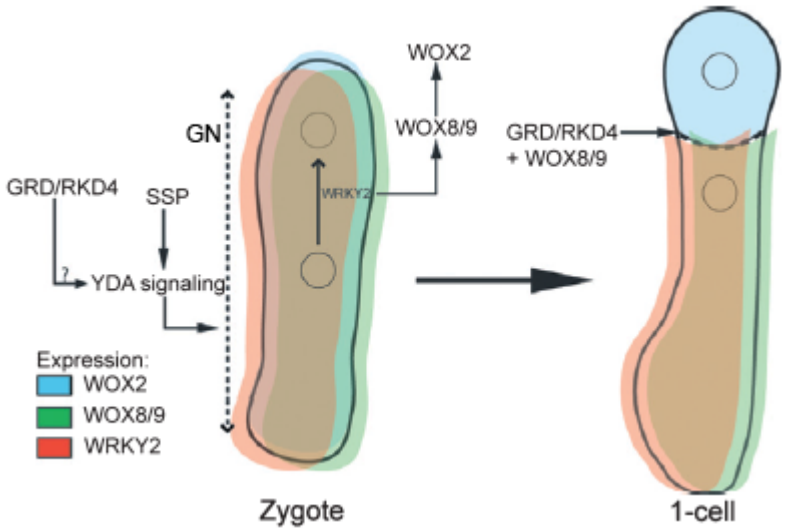

Abbildung 2: Wichtige Faktoren für Zygotenstreckung und asymmetrische Teilung. (modifiziert nach Wendrich and Weijers, 2013).

Zygote (Mayer et al., 1993; Lukowitz et al., 2004; Wang et al., 2007; Bayer et al., 2009; Jeong et al., 2011a; Mao et al., 2011). In *Arabidopsis* leitet die asymmetrische Teilung der Zygote die eigentliche apikal-basale Musterbildung des frühen Embryos ein. Während sich die größere basale Zelle ausschliesslich horizontal teilt und der sich daraus ergebende Zellfaden (Suspensor) bis auf die oberste Zelle (Hypophyse) nicht zum späteren Keimlingsgewebe beiträgt, entsteht aus der kleineren apikalen Zelle fast der gesamte Embryo (Jürgens, 2001). Die erste Teilung der apikalen Zelle ist durch Drehung der Zellteilungsebene im Gegensatz zur basalen vertikal (Webb and Gunning, 1991). Dies wird durch das für die Pflanzenentwicklung wichtige

Phytohormon Auxin vermittelt (vornehmlich Indol-3-Essigsäure). Dabei scheint Auxin in der apikalen Zelle mit Hilfe des Auxin-Efflux-Carriers PIN-FORMED7 (PIN7) durch Transport über die basale Zelle akkumuliert zu werden (Friml et al., 2004), was indirekt durch den gebräuchlichen Reporter DR5::GFP angezeigt wird (Abb. 3). Ausserdem wird der Transkriptionsfaktor *DORNROESCHEN* (*DRN*), der auch ein direktes Zielgen des Auxin-abhängigen ARF Transkriptionsfaktor MONOPTEROS (MP) ist, schon ab dem Ein-Zell-Stadium nur apikal exprimiert (Cole et al., 2009). Interessanterweise verursacht das Fehlen sowohl von funktionalem PIN7 Protein als auch das Fehlen von MP bzw. Proteinstabilisierung des ARF-inhibitorischen AUX-IAA Proteins BODENLOS (BDL) eine fehlerhafte, d.h. horizontale Teilung der apikalen Zelle (Hamann et al., 1999; Friml et al., 2003). Interessanterweise scheint dies auch in *wox2* mutanten Embryonen vorzukommen (Haecker et al., 2004). Da dieser Phänotyp allerdings nicht vollständig penetrant ist, ist die Auxin vermittelte Spezifizierung der apikalen Zelle nicht der einzig entscheidende Faktor. Kürzlich wurde überdies gefunden, dass eine ARF vermittelte Auxinantwort auch im Suspensor wichtig ist. Wird diese Antwort durch Expression eines stabilisiertem AUX-IAA Proteins im Suspensor unterbunden, führt dies zu einer Umkehr der Zellspezifität von Suspensor zu Proembryo und je nach Stärke der Expression sogar zu Zwillingsembryonen (Rademacher et al., 2012). Zwei weitere, für die spätere Embryonalentwicklung wichtige Faktoren sind der Auxin-Efflux-Carrier *PIN1* sowie der GATA Transkriptionsfaktor *MONOPOLE*/*HANABA TARANU* (*MNP*/*HAN*), welche ab dem Zwei-Zell- bzw. ab dem Ein-Zell-Stadium apikal exprimiert sind, deren hauptsächliche Funktionen jedoch erst in den späteren Stadien wichtig sind (Friml et al., 2003; Nawy et al., 2010) (Abb. 3). *HAN* ist für die Aufrechterhaltung der Grenze zwischen Proembryo und Suspensor verantwortlich. In Funktionsverlustmutanten verschwimmt diese Grenze und Expressionsmuster anderer frühembryonaler Faktoren weiter nach oben verlagert werden (Nawy et al., 2010).

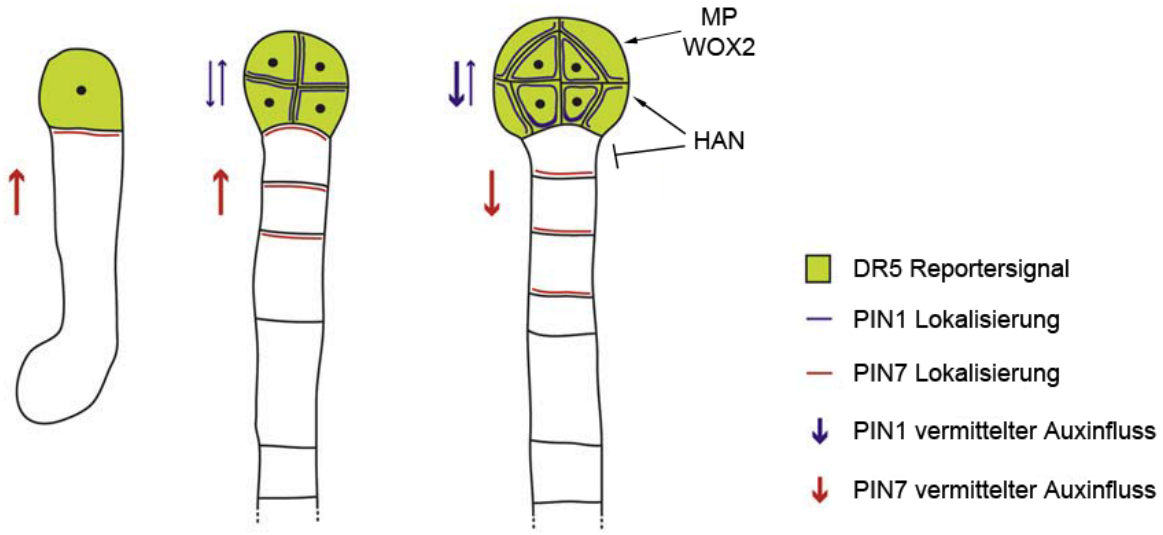

Abbildung 3: Auxin vermittelte Signalantworten in der frühen Embryogenese. Die Richtung des Auxinflusses verläuft je nach Entwicklungsstadium gemäss den Pfeilrichtungen (modifiziert nach Lau et al., 2010).

Neben morphologischen Unterschieden zwischen den beiden Tochterzellen der Zygote – apikale zytoplasmatisch, basale vakuolär – sind in der frühesten apikal-basalen Spezifizierung auch die bereits weiter oben erwähnten WOX Transkriptionsfaktoren von Bedeutung. *WOX2* und *WOX8* sind beide schon im Zygotenstadium exprimiert, im Ein-Zell-Stadium jedoch ist *WOX2* eher apikal und *WOX8* eher basal aktiv (Haecker et al., 2004; Breuninger et al., 2008; Lie et al., 2012) (Abb. 4). Obwohl die genaue Expression von *WOX9* nicht eindeutig ist, führen Funktionsverluste in *WOX9* jedoch zu Fehlern bei der embryonalen Zellteilung und je nach Dosis zum frühen Abbruch der Embryonalentwicklung (Haecker et al., 2004; Wu et al., 2007; Breuninger et al., 2008). In *wox8 wox9* doppelmutanten Embryonen kann überdies wie in *pin7*, *mp* oder *bdl* Mutanten eine horizontale Teilung der apikalen Zelle beobachtet werden (Breuninger et al., 2008). Da *WRKY2* direkt auf *WOX8* und vielleicht auch auf *WOX9* wirkt und zusätzlich *WOX2* in seiner Expression von *WOX8/9* abhängig zu sein scheint, deuten diese Erkenntnise trotz unterschiedlicher Spezifizierung der apikalen und basalen Zellen auf ein eng gekoppeltes Signalsystem hin (Breuninger et al., 2008).

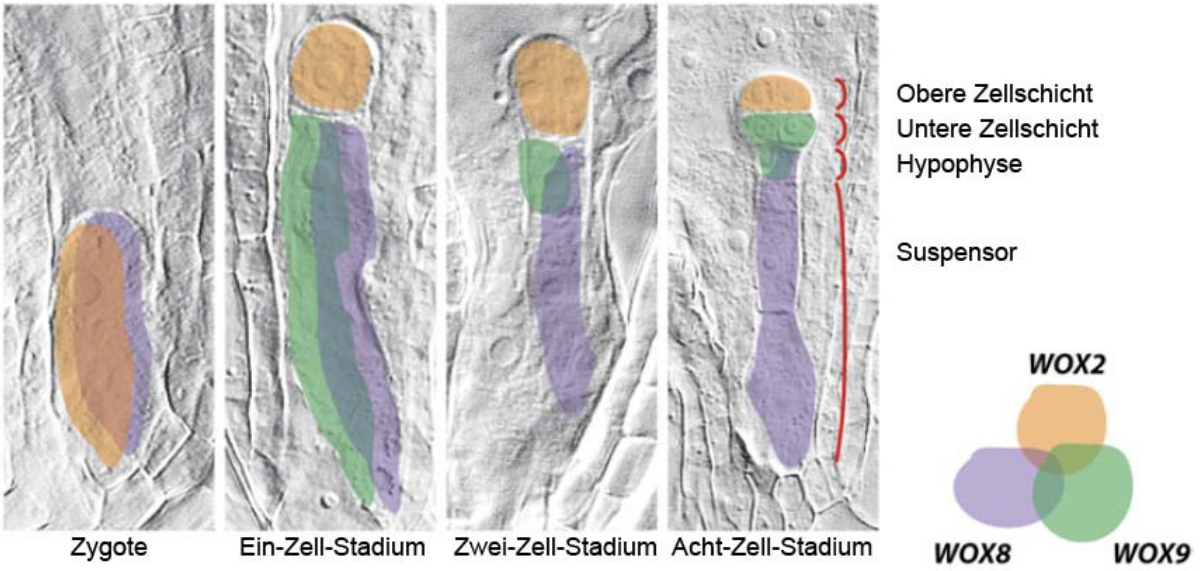

Abbildung 4: Markierung der apikal-basalen Embryonalachse durch WOX-Genexpression. (modifiziert nach Jeong et al., 2011b).

Nach drei weiteren Teilungsrunden haben sich im Acht-Zell-Stadium im Proembryo durch transversale Teilungen eine obere und eine untere Zellschicht gebildet, wodurch sich zusammen mit dem Suspensor drei Ebenen entlang der apikal-basalen Achse ergeben. Aus der obersten entstehen das Sprossmeristem sowie ein Hauptteil der Kotyledonen, aus der mittleren Teile der Kotyledonen, das Hypokotyl, die Wurzel sowie Teile des Wurzelmeristems. Aus der obersten Zelle des Suspensors, der Hypophyse, entwickelt sich der zentrale Bereich des Wurzelmeristems, das sogenannte ruhende Zentrum, und die Wurzelhaube (Jeong et al., 2011b). Dabei nehmen wiederum WOX Gene durch ihr vorwiegendes Expressionsmuster eine markierende Funktion ein: *WOX2* im oberen Bereich, *WOX9* im mittleren Bereich, *WOX8*/*9* in der Hypophyse und *WOX8* in den restlichen Suspensorzellen (Abb. 4).

3.3. Radiale und bilaterale Musterbildung

Beim Übergang vom Acht- zum Sechzehn-Zell-Stadium teilen sich die Zellen des Proembryos ausschliesslich tangential. Dadurch entsteht ein innerer und ein äusserer Zellverband und letzterer wird als Protoderm bezeichnet, welches die Vorläuferzellen des Abschlussgewebes bei Pflanzen darstellt, der sogenannten Epidermis (De Smet et al., 2010). Nach dieser Teilung trennen sich auch die

Expressionsmuster einiger Gene auf, die vorher in allen Zellen des Acht-Zell-Stadiums vorhanden waren. So sind *ARABIDOPSIS MERISTEM LAYER 1* (*ATML1*) und *PROTODERMAL FACTOR 2* (*PDF2*) nur noch auf das Protoderm beschränkt, während *PINHEAD*/*ZWILLE*/*ARGONAUTE* 10 (*PNH*/*ZLL*/*AGO10*) nur in den inneren Zellen exprimiert wird (Lu et al., 1996; Lynn et al., 1999). In *atml1 pdf2* mutanten Pflanzen fehlt die aus dem Protoderm hervorgehende Epidermis (Abe et al., 2003). *ATML1* und *PDF2* codieren für Transkriptionsfaktoren, deren Zielgene nicht bekannt sind, die aber womöglich von WOX2 über eine *WUSCHEL* (*WUS*) DNA-Bindestelle reguliert werden könnten (Lohmann et al., 2001; Abe et al., 2003; Takada and Jürgens, 2007). Interessanterweise weisen *wox2* Mutanten epidermale Zellteilungsdefekte auf, was auch in mp Mutanten der Fall ist und diese Effekte können durch *WOX* Mehrfachmutanten und in Kombinationen mit *mp* verstärkt werden (Haecker et al., 2004; Breuninger et al., 2008). Zusätzlich könnten *ATML1* und *PDF2* in einer positiven Rückkoppelungsschleife interagieren, die auch die Expression anderer protodermal aktiver Gene ermöglichen würde (Abe et al., 2001; Abe et al., 2003). Ebenso treten in doppelmutanten Embryonen von *ale1* und *ale2* (*abnormal leaf shape*) Defekte im Protoderm auf und es kommt zur Misexpression von molekularen Markergenen wie *ATML1*, was auch in den Doppelmutanten *ale2 acr4* (*arabidopsis crinkly 4*) oder *rpk1 toad2* (*receptor-like protein kinase 1* und *toadstool 2*) der Fall ist (Nodine et al., 2007; Tanaka et al., 2007). Genauso wie die Differenzierung der inneren Zellen in den folgenden Embryonalstadien bleibt die Teilung der Zellen im Acht-Zell-Embryo trotz der erwähnten Erkenntnisse bis heute weitesgehend unverstanden.

Die bilaterale Symmetrie entsteht in der frühen Embryogenese durch die Ansätze der beiden Kotyledonen, welche durch das Sprossmeristem voneinander getrennt sind. Die ersten Anzeichen für die Keimblattbildung sind dabei sogenannte DR5-Reporter Maxima in den Vorläuferzellen der Auswuchsstellen der Kotyledonen (Abb. 5A). Diese Maxima werden durch gerichteten Auxinfluss über PIN Proteine erreicht (Benkova et al., 2003), welche wiederum in ihrer Lokalisierung über Phosphorylierung durch die Kinase PINOID (PID) beeinflusst werden (Friml, J. et al., 2004; Huang et al., 2010). Mutanten in Genen für Auxintransport und nachgeschaltete Auxinantwort - unter anderen beispielsweise *mp* und *bdl* (Hamann et al., 1999) - haben Probleme in der vollständigen Ausbildung der Kotyledonen und Mehrfachmutanten wie *pid wag1 wag2*, *pin1 pid* oder auch die MP Zielgene *drn drnl1*

(*dornroeschen-like 1*) führen zum vollständigen Verlust der beiden Keimblätter (Furutani et al., 2004; Chandler et al., 2008; Cheng et al., 2008). Wichtig für die Keimblattentstehung ist ausserdem das Zusammenspiel mit Faktoren, die für die Sprossmeristembildung und dessen Aufrechterhaltung entscheidend sind (Abb. 5B). Im Kern des Sprossmeristems liegt die Expressionsdomäne des Transkriptionsfaktorgens *WUSCHEL* (*WUS*), welche eine Art organisierendes Zentrum für die umliegenden Stammzellen darstellt (Lenhard et al., 2002). Wie jedoch dieses Zentrum initiert wird und operiert, ist nicht bekannt. Drei weitere Transkriptionsfaktoren, nämlich *SHOOT MERISTEMLESS* (*STM*) sowie *CUP-SHAPED COTYLEDON 1* und *2* (*CUC*), regulieren die Entstehung des Sprossmeristems. Dabei fungiert *STM* zusätzlich als Repressor der Zelldifferenzierung, hauptsächlich als Antagonist zu *ASYMMETRIC LEAVES 1* und *2* (Byrne et al., 2000; Byrne et al., 2002), und die CUC Genprodukte zusammen mit STM sind entscheidend für die räumliche Trennung der Kotyledonen (Aida et al., 1997; Aida et al., 1999; Lenhard et al., 2002). *STM* ist in einerseits seiner Genaktivität abhängig von *CUC1*/*2*, auf der anderen Seite reguliert *STM* auch indirekt die Genexpression der beiden anderen Transkriptionsfaktoren (Aida et al., 1999; Takada et al., 2001; Spinelli et al., 2011). Zwar sind Sprossmeristem und Kotyledonen nicht zwingend aufeinander angewiesen, da Mutanten existieren, welche jeweils nur Sprossmeristem oder Kotyledonen besitzen (Barton and Poethig, 1993; Laux et al., 1996; Furutani et al., 2004). Jedoch sind die Expressionsdomänen der *CUC* Gene sowie von *STM* stark erweitert in *pin1 pid* Doppelmutanten und interessanterweise können die beiden Keimblätter durch ein Entfernen von *CUC1*/*2* oder *STM* zumindest teilweise wieder hergestellt werden (Furutani et al., 2004; Treml et al., 2005). Für die Aufrechterhaltung des Sprossmeristems sind in erster Linie *class III HOMEODOMAIN-LEUCINE ZIPPER* (*HD-ZIP III*) Transkriptionsfaktoren und *ZLL* verantwortlich. *ZLL* codiert für ein Argonautprotein und wirkt negativ auf gegen *HD-ZIP III* gerichtete micro-RNAs, wodurch die *HD-ZIP III* Expression im Bereich des Sprossmeristems ermöglicht wird (Zhu et al., 2011). Verlust von HD-ZIP III Proteinen in Mehrfachmutanten führt zum Verlust des Sprossmeristems und möglicherweise wirken diese Proteine auch direkt auf *STM* (Grigg et al., 2009). Noch immer sind viele Musterbildungsprozesse unverstanden und daher sind Transkriptomanalysen spezifischer Zell- und Gewebetypen notwendig.

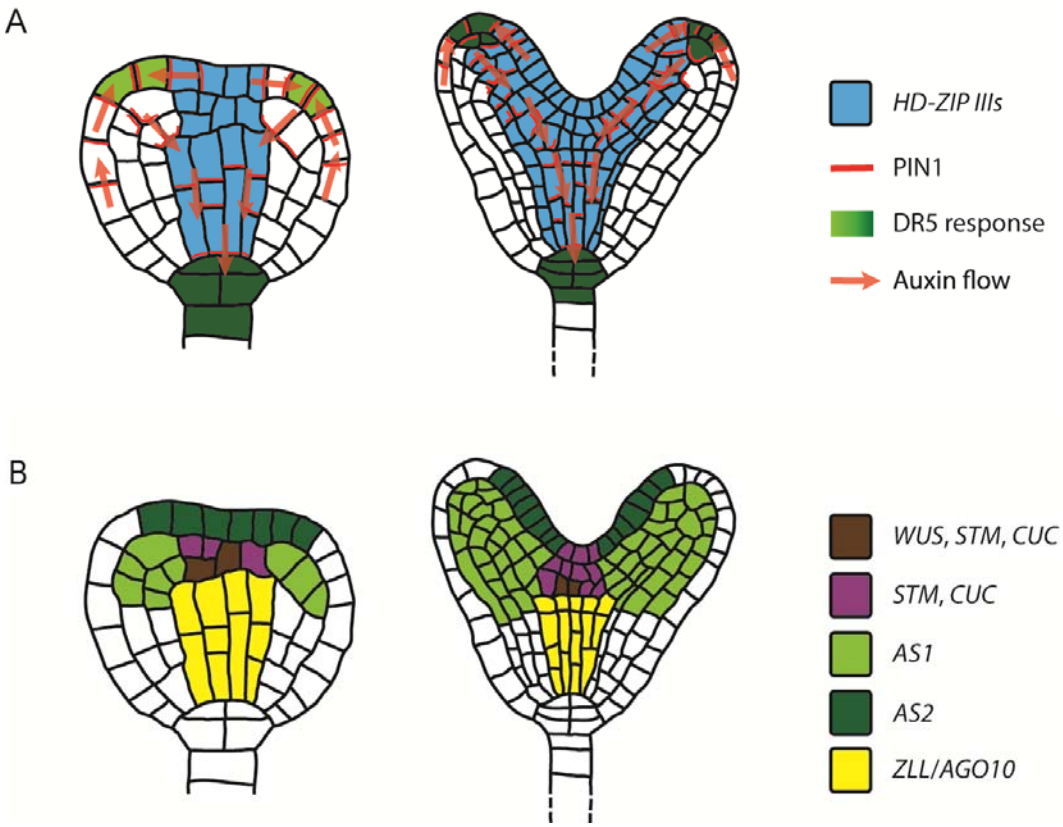

Abbildung 5: Aussbildung der bilateralen Symmetriebene. A) Auxinfluss und Expression der *HD-ZIP III* Gene während der Keimblattentstehung. B) Expressionsdomänen entscheidender Gene für die Entstehung von Sprossmeristem und Keimblättern (modifiziert nach Lau et al., 2012).

3.4. Embryonale Transkriptomanalysen

Wie bereits erwähnt wurde, markieren die Expressionsmuster einiger, bereits charakterisierter Gene spezifische Bereiche während der frühen Embryogenese. Oftmals sind diese Gene auch essentiell für die Etablierung von Zellidentitäten und ihrer räumlichen sowie zeitlichen Differenzierung, welche die Grundlage für eine korrekte Ausbildung von Zellverbänden, Geweben oder ganzen Organen darstellt (Lau et al., 2012; Wendrich and Weijers, 2013). Trotz dieser Erkenntnisse über die frühe Embryogenese in *Arabidopsis* fehlt ein Grossteil an Wissen über Genregulationsnetzwerke, welche diesen zellulären Entstehungs- und Differenzierungsprozessen unter anderem zugrunde liegen. Deswegen ist es von grundlegender Bedeutung, tiefere Einblicke in diese Netzwerke zu erhalten, um die für die Pflanze essentielle, embryonale Musterbildung verstehen zu können. Aufgrund der geringen Größe und limitierten Zellzahl früher Embryonalstadien sowie

der tiefen Verankerung des Embryos in der Samenanlage, sind Studien an spezifischen Zelltypen des Embryos jedoch stark erschwert. Die bisherigen Transkriptomergebnisse für *Arabidopsis* Embryonen wurden daher aus vollständigen Samenanlagen, durch manuelle Präparierung ganzer Embryonen aus diesen oder mit Hilfe von Laser-Mikrodissektion erzielt (Emmert-Buck et al., 1996; Girke et al., 2000; Kerk et al., 2003; Casson et al., 2005; Schmid et al., 2005; Spencer et al., 2007; Le et al., 2010; Autran et al., 2011; Xiang et al., 2011; Nodine and Bartel, 2012; Belmonte et al., 2013). Eine dieser Studien beschreibt die Laser-Mikrodissektion und anschliessende Analyse von frühen Stadien des *Arabidopsis* Proembryos und des Suspensors auf Ebene der Genexpression (Belmonte et al., 2013). Obwohl Laser-Mikrodissektion den Vorteil hat, dass keine Transgenen benötigt werden und das Gewebe durch Fixierung intakt bleibt, muss ein grosser Aufwand betrieben werden um genügend RNA aus den verwendeten Dünnschnitten extrahieren zu können. Ausserdem ist es praktisch nicht möglich, einzelne Zellen z.B. aus den Stammzellnischen im frühen Embryo zu präparieren und zusätzlich besteht stets die Gefahr, die isolierten Gewebe von Interesse durch Anschneiden von umliegenden Zellen zu kontaminieren. Zusätzlich zur Laser-Mikrodissektion stehen drei weitere Methoden zur Verfügung, und zwar fluorescent activated cell sorting (FACS, zu Deutsch fluoreszenzbasierte Durchflusszytometrie), translating ribosome activity profiling (TRAP) und isolation of nuclei tagged in specific cell types (INTACT) (Bonner et al., 1972; Zong et al., 1999; Deal and Henikoff, 2010). TRAP und INTACT haben grosses Potential, wurden bisher aber noch nicht in schwer zugänglichem Gewebe getestet und es besteht in Pflanzen im Moment noch Optimierungsbedarf (Zanetti et al., 2005; Mustroph et al., 2009; Deal and Henikoff, 2011; Steiner et al., 2012; Palovaara et al., 2013). Im Gegensatz dazu ist FACS eine schnelle und in *Arabidopsis* etablierte Technik (Birnbaum et al., 2003; Birnbaum et al., 2005), die unter anderem dazu benutzt wurde, eine der bisher organismusübergreifend aufwendigsten Genexpressionsstudien zu erstellen (Brady et al., 2007). Ausserdem konnten beispielsweise neue molekulare Marker (Yadav et al., 2009), zelltypspezifische Stressantworten (Dinneny et al., 2008) oder interzelluläre Kommunikations - und Regulationswege gefunden werden (De Smet et al., 2008; Slotkin et al., 2009). Fast alle Studien basieren jedoch auf Protoplasten - Zellen ohne Zellwand - aus Wurzeln oder anderen leicht zugänglichen Geweben. Dies macht die Anwendung auf Embryonen nicht sehr praktikabel, da die dafür benötigten,

zellwandverdauenden Enzyme womöglich entweder nicht tief genug in die Samenanlagen eindringen oder die Embryonen vorher manuell aus der Samenanlage präpariert werden müssten. Interessanterweise wurde in Wurzeln gezeigt, dass anstelle ganzer Zellen auch Zellkerne durchflusszytometrisch aufgereinigt werden können und dass die Transkriptome von Zellen und Zellkernen ähnlich sind (Barthelson et al., 2007; Jacob et al., 2007; Zhang et al., 2008; Deal and Henikoff, 2010). Aus diesem Grund ist die Kombination aus Extraktion von fluorophormarkierten Zellkernen aus ganzen Geweben oder Organen in Kombination mit Durchflusszytometrie eine geeignete Alternative, um Genexpressionsprofile unterschiedlichster, auch schwer zugänglicher Zellpopulationen wie z.B. den Stammzellnischen im frühen Embryo herzustellen.

4. Zielsetzung

Die Kenntnis über die Transkriptmengen möglichst aller Gene einzelner Zellen oder gewisser Zelltypen ist grundlegend für das Verständnis von Zellfunktionen im Verlaufe der Entwicklung eines Organismus. Da vor allem in Pflanzen diese Kenntnis über Zellen von Interesse aus tieferliegenden Gewebeschichten nur schwierig zu erlangen ist, sollte zunächst eine Technik etabliert werden, mit Hilfe derer Transkriptionsprofile von solchen Zellen oder Zelltypen erstellt werden können. Dies sollte anhand früher Embryonen in Samenanlagen von *Arabidopsis* mittels fluoreszensbasierter Durchflusszytometrie geschehen, wobei entweder alle Zellen des frühen Embryos oder nur diejenigen des Proembryos bzw. des Suspensors markiert sein sollten. Daraufhin sollten jeweils entsprechende Transkriptomprofile eines Grossteils der im *Arabidopsis* Genom vorhandenen Gene erstellt werden. Um zu gewährleisten, dass die gewonnenen Daten die tatsächliche *in vivo* Situation widerspiegelten, sollten die Daten mit Expressionsdaten bekannter Gene verglichen und die Expressionsmuster zufällig ausgewählter, zwischen Proembryo und Suspensor differenziell exprimierter Gene in pflanzlichen Embryonen mit den Genexpressionsdaten verglichen werden.

5. Publikationsübersicht

5.1. Forschungsartikel

In der Studie „Cell type-specific transcriptome analysis in the early *Arabidopsis thaliana* embryo“ wird die Etablierung sowie Auswertung einer Methode dargestellt, um Transkriptomprofile von unzugänglichem Gewebe in *Arabidopsis* zu erstellen. Da die beiden Tochterzellen der Zygote in den folgenden Stadien der Embryonalentwicklung völlig unterschiedliche Richtungen einschlagen und der Embryo in *Arabidopsis* tief im maternalen Gewebe verankert ist, erschienen diese Gewebetypen als geeignetes Testfeld. Zunächst wurde eine Technik ausgearbeitet, mit deren Hilfe fixierte, mit fluoreszierenden Proteinen gekennzeichnete Zellkerne aus diesen Geweben des frühen Embryos isoliert werden können. Die extrahierte RNA wies dabei gute RNA Integritätswerte auf und wurde aufgrund relativ geringer Ausbeute amplifiziert bevor die spezifischen Transkriptmengen über DNA-Microarrays bestimmt wurden. Überdies zeigte der Vergleich mit RNA aus intakten, embryonalen Zellen ein hohes Maß an Übereinstimmung. Die statistische Analyse zeigte, dass ca. 500 Transkripte zwischen Proembryo und Suspensor differentiell exprimiert sind. Unter diesen lassen sich die meisten bisher beschriebenen Gene finden, welche für die apikal-basale Musterbildung während der frühen *Arabidopsis* Embryogenese von Bedeutung sind wie beispielsweise *WOX2*, *PIN1* oder *HAN* (siehe Einleitung). Die Ergebnisse der *in vivo* Experimente anhand von Promoter-Reporter Fusionskonstrukten sowie *in situ* Hybridisierung zeigten eine hohe Korrelation mit den Microarray Ergebnissen für signifikant differentiell exprimierte Transkripte in Proembryo oder Suspensor. Auch konnte anhand mehrerer Analysen aufgezeigt werden, dass die hier beschriebene Methode Vorteile gegenüber der etablierten Laser-Mikrodissektion für embryonale Gewebe bietet, da diese anfälliger ist, Kontaminationen duch Embryo angrenzende Zellen aufzuweisen. Durch Vergleich mit Expressionsdaten aus nicht-embryonalem Gewebe der Samenanlage konnten zudem ca. 100 putative, embryospezifische Gene gefunden werden, unter denen wiederum bekannte Faktoren der Embryonalentwicklung als auch mehrere unserer eigenen, zufällig für die *in vivo* Analysen gewählten Gene waren. Zusammengenommen lässt sich sagen, dass mit dieser Arbeit eindeutig gewebe-spezifische Expressionsdaten aufgestellt werden können.

5.2. Übersichtsartikel

Im Übersichtsartikel „Early Embryogenesis in Flowering plants: Setting Up the Basic Body Pattern“ werden die entwicklungsgenetischen Erkenntnisse in der frühen Embryogenese von *Arabidopsis thaliana* behandelt, aber auch diejenigen der dafür verantwortlichen, orthologen Verwandten anderer Pflanzenspezies wie beispielsweise Reis (*Oryza sativa*), Mais (*Zea mays*) oder Tabak (*Nicotiana tabacum*). Dabei liegt das Hauptaugenmerk auf der Polarisierung, Streckung und asymmetrischen Teilung der Zygote, der asymmetrischen Teilung der Hypophyse und Ausbildung des Wurzelpols, der Entstehung des Protoderms sowie der Festlegung des Sprossmeristems in Zusammenhang mit der Bildung der Keimblätter.

6. Schlussbetrachtung

Im Forschungsteil dieser Arbeit konnten spezifische Gene gefunden werden, die vorwiegend im Proembryo oder Suspensor aktiv sind. Dies wurde mit herkömmlichen DNA-Microarrays bewerkstelligt. Da jedoch nicht alle Gene auf den Microarray Chips vertreten sind und die Sensitivität in den unteren Expressionbereichen nicht ausreichend ist, wäre es interessant, die gewebe-spezifischen Transkriptome zusätzlich mittels RNA-Sequenzierung zu untersuchen. Zukünftig wäre überdies zu finden, welche dieser Gene funktional entscheidend sind für die Entwicklung der beiden Gewebe. Dies könnte mit Hilfe von T-DNA Insertionslinien, RNAi (RNA interference) oder mit der neuartigen CRISPR/CAS9 Methode (Cong et al., 2013) bewerkstelligt werden. Es ist zu vermuten, dass aufgrund hoher Redundanzen mehrere Gene ausgeschaltet werden müssten, um eindeutige Genfunktionen zu klären. Die vorliegende Arbeit befasst sich mit den Unterschieden zwischen Geweben auf der Ebene der Genexpression bzw. Transkriptmengen. Das Zustandekommen dieser Unterschiede wird auch beeinflusst durch epigenetische Faktoren wie DNA-Methylierungen oder Histonmodifikationen (Mosher and Melnyk, 2010). Da hier jedoch ausschliesslich Kerne aufgereinigt werden, ist es auch möglich, Modifikationen an der DNA selbst und an Histonen zu untersuchen. Mit der vorgestellten Methode sollte es möglich sein, nicht nur bis dato schwer zugängliche Gewebe in *Arabidopsis* charakterisieren zu können, sondern auch jegliche Gewebe aus anderen genetisch veränderbaren Organismen.

7. Publikationen

7.1. Cell type-specific transcriptome analysis in the early *Arabidopsis thaliana* embryo

Daniel Slane[1*], Jixiang Kong[1,2*], Kenneth W. Berendzen[3], Joachim Kilian[3], Agnes Henschen[1], Martina Kolb[1], Markus Schmid[4], Klaus Harter[3], Ulrike Mayer[5], Ive De Smet[6,7,8], Martin Bayer[1] and Gerd Jürgens[1,2‡]

[1]Department of Cell Biology, Max Planck Institute for Developmental Biology, 72076 Tübingen, Germany.

[2]Department of Developmental Genetics, Center for Plant Molecular Biology, University of Tübingen, 72076 Tübingen, Germany.

[3]Department of Plant Physiology, Center for Plant Molecular Biology, University of Tübingen, 72076 Tübingen, Germany.

[4]Department of Molecular Biology, Max Planck Institute for Developmental Biology, 72076 Tübingen, Germany.

[5]Microscopy facility, Center for Plant Molecular Biology, University of Tübingen, 72076 Tübingen, Germany.

[6]Department of Plant Systems Biology, VIB, Technologiepark 927, B-9052 Ghent, Belgium.

[7]Department of Plant Biotechnology and Bioinformatics, Ghent University, Technologiepark 927, B-9052 Ghent, Belgium.

[8]Division of Plant and Crop Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, UK.

[*]These authors contributed equally to this work

[‡]Author for correspondence (gerd.juergens@zmbp.uni-tuebingen.de)

Running title: Arabidopsis early embryo profiling

(Manuskript angenommen zur Publikation bei Development am 13.10.2014 - DEVELOP/2014/116459)

ABSTRACT

In multicellular organisms, cellular differences in gene activity are a prerequisite for differentiation and establishment of cell types. In order to study transcriptome profiles, specific cell types have to be isolated from a given tissue or even the whole organism. However, whole-transcriptome analysis of early embryos in flowering plants has been hampered by their size and inaccessibility. Here we describe the purification of nuclear RNA from early stage *Arabidopsis thaliana* embryos using fluorescence-activated nuclear sorting (FANS) to generate expression profiles of early stages of the whole embryo, the proembryo, and the suspensor. We validated our datasets of differentially expressed candidate genes by promoter-reporter gene fusions and *in situ* hybridization. Our study revealed that different classes of genes with respect to biological processes and molecular functions are preferentially expressed either in the proembryo or in the suspensor. This method especially can be used for tissues with a limited cell population and inaccessible tissue types. Furthermore, we provide a valuable resource for research on Arabidopsis early embryogenesis.

KEY WORDS: Fluorescence-activated nuclear sorting, Proembryo, Suspensor, Transcriptome analysis

Abbreviation	**Full name**
FACS	Fluorescence-activated cell sorting
FANS	Fluorescence-activated nuclear sorting
LCM	Laser capture microdissection
TRAP	Translating ribosome affinity purification
INTACT	Isolation of nuclei tagged in specific cell types
PE	Proembryo
SUS	Suspensor
EMB	Whole embryo
nEMB	Nuclei from whole embryo
nPE	Nuclei from proembryo
nSUS	Nuclei from suspensor
cgPE	Cellular globular-stage proembryo
cgSUS	Cellular globular-stage suspensor
cgSEED	Cellular globular-stage entire seed excluding embryo
cEMB	Cellular whole embryo
cKAN1	Cellular KANADI 1 expression domain adult shoot

INTRODUCTION

Multicellular organisms are made up of various cell and tissue types consisting of differentiated cells which all derive from pluripotent, undifferentiated progenitor cells. Since these cell and tissue types fulfill a plethora of different functions during the life cycle, progenitor cells have to undergo coordinated changes in spatial and temporal gene expression programs during differentiation. Comprehensive characterization of transcriptional profiles is therefore of great importance to understand the establishment and maintenance of specific cell types. In the case of embryogenesis in flowering plants with the embryos often being deeply embedded in the maternal seed tissue, however, the isolation of cells from specific cell types is already a very challenging task. In general, several existing methods have been employed to overcome such difficulties for different tissues and organisms, such as laser capture microdissection (LCM), fluorescence-activated cell sorting (FACS), translating ribosome affinity purification (TRAP), and isolation of nuclei tagged in specific cell types (INTACT) (Bonner et al., 1972; Emmert-Buck et al., 1996; Heiman et al., 2008; Deal and Henikoff, 2010). At present TRAP and INTACT are still under optimization in order to be widely used for special tissues such as those in plant embryos (Palovaara et al., 2013). LCM has been used in different studies to isolate tissues from sectioned material without the need of generating transgenic plants (Kerk et al., 2003). Recently, parts of different tissues inside the *Arabidopsis thaliana* seed including the embryo were isolated by LCM and the different expression profiles were analyzed (Spencer et al., 2007; Le et al., 2010). Nonetheless, LCM requires high precision during tissue excision in order to avoid contamination from adjoining cells. Additionally, since the used material originates from tissue sections, only parts of the cell can be effectively collected. Consequently, precise isolation of certain cell types, such as shoot apical meristem cells, which are deeply embedded within the embryo, is a considerable challenge. Evidently, FACS in combination with gene expression analysis has been broadly employed for many studies, such as purification of *Drosophila melanogaster* embryonic cell populations (Cumberledge and Krasnow, 1994; Shigenobu et al., 2006), clinical applications (Jayasinghe et al., 2006; Jaye et al., 2012), and isolation of different cell types in Arabidopsis root and shoot tissue (Birnbaum et al., 2003; De Smet et al., 2008; Yadav et al., 2014). Most of the FACS studies in plants were based on the generation of protoplasts from easily accessible tissues and therefore this method is very difficult to apply to Arabidopsis embryos, in

particular in large amount. In contrast, fluorescently labeled nuclei from the companion cells of phloem root tissue were isolated by fluorescence-activated nuclear sorting (FANS) for further transcriptome analysis (Zhang et al., 2008). Importantly, reports showed that the diversity of nuclear and total cellular RNA is overall comparable (Barthelson et al., 2007; Zhang et al., 2008).

In light of specific advantages and disadvantages of the different techniques mentioned above, we combined fluorescent-activated sorting of nuclei (FANS) with linear RNA amplification and microarray analysis to characterize the transcriptomes of two cell types – the proembryo (PE) and suspensor (SUS) – in the early Arabidopsis embryo originating from a single cell – the zygote – as well as the whole embryo (EMB). Our strategy was to label nuclei with nuclear localized GFP (nGFP) driven by cell-type specific promoters only active either in the cells of the proembryo or the suspensor, or uniformly active in the whole embryo. GFP-positive nuclei were sorted by flow cytometry and afterwards standard ATH1 microarray chips were used for transcriptome analysis. Our analysis demonstrated that specific transcripts are differentially expressed between the proembryo and suspensor at early stages of embryogenesis, including genes that were previously reported to be differentially expressed *in vivo* (Lau et al., 2012). The datasets were further validated by promoter-reporter fusion analysis and *in situ* hybridization for a subset of genes that were preferentially expressed in one or the other cell type. Additionally, we also compared our nuclear whole embryo transcriptional profile with that of manually isolated, early-stage whole embryos as well as with publicly available data. In summary, we developed a robust method in order to generate comprehensive expression profiles of specific cell types in Arabidopsis early embryos. In particular, this method can be widely used for characterizing gene expression of deeply embedded cell types with a limited number of cells. In addition, we provide a comprehensive resource for the earliest stages and tissues of Arabidopsis development.

RESULTS

Identification of embryo-specific marker lines

In order to obtain marker lines that show specific expression during the early stages of Arabidopsis embryogenesis in the proembryo, suspensor, or whole embryo, we first screened the GAL4-GFP enhancer-trap collection from the Haseloff lab (Haseloff, 1999). Tracing back expression from microscopic analysis of seedling

roots, one of the Haseloff lines (N9322) showed specific suspensor expression and the insertion locus was identified by TAIL-PCR to position 610 bp upstream of the *AT5G42203* coding sequence (supplementary material Fig. S1). We then cloned about 2kb upstream region including 5' untranslated region (5' UTR) sequences for both the neighboring *AT5G42200* and *AT5G42203* genes fused to n3xGFP in order to check whether one or the other of the two promoters could recapitulate the expression pattern of the enhancer trap line. Regarding the expression pattern of the different transgenic lines, the promoter containing the upstream region of the *AT5G42200* gene showed specific expression only in the suspensor from the embryonic 2-cell stage onward (Fig. 1A).

Second, according to published data, the *DORNROESCHEN (DRN)* gene (*AT1G12980*) was shown to be expressed exclusively in the proembryo until early globular stage (Chandler et al., 2007; Cole et al., 2009). Therefore, we cloned the upstream region of *DRN* together with its 3'UTR as was described before (Chandler et al., 2007). Indeed, the expression pattern for this construct in transgenic embryos fit the published data for *DRN* (Fig. 1B).

Finally, as a whole embryo marker we used a marker line available in the lab driving GFP expression from the upstream region of the *AT3G10010* locus (Fig. 1C).

FANS analysis and microarray results

The individual fluorescent marker lines showing specific expression in proembryo, suspensor, or whole embryo nuclei were subsequently used to generate cell type-specific nuclear transcription profiles of the early Arabidopsis embryo. Since we were not able to recover protoplasts from early embryonic stages due to the embryonic cell wall and cuticle being recalcitrant to enzymatic digestion, we developed a workflow that enabled us to efficiently extract nuclei from ovule tissue. For nuclear extraction, we isolated ovules from self-pollinated, young siliques. The embryos from those ovules ranged from 1- to 16-cell embryonic stages which we microscopically checked from a number of siliques of the plants used prior to the start of the workflow. Afterwards, we fixed the ovules with 0.1% paraformaldehyde in order to maintain nuclear integrity. Additionally, by fixing the cellular contents we made sure that the transcriptional status of the nuclei did not change during the subsequent extraction and separation steps. After nuclear extraction, approximately 1000 GFP-positive nuclei from ovules of about 100 siliques were purified for the different marker lines on

average by flow cytometry (supplementary material Fig. S2). Pools of approximately 3000 GFP-positive nuclei were used for RNA extraction representing one biological replicate.

After RNA amplification and biotinylation, the transcriptome analyses were carried out in biological triplicates with a standard Affymetrix ATH1 genome array, which covers roughly 71% of the to date presumed 33602 total Arabidopsis genes (Lamesch et al., 2012). For our analyses, we used MAS5 normalized probe set signals (supplementary material Table S1) as well as gcRMA (gene chip robust multi-array average) normalized and log2 transformed (supplementary material Table S2) values. When we compared microarray probe sets only detected as present (P) in the MAS5 normalization algorithm for raw values across all three replicates, they showed a chip coverage of 34, 32, and 25% for nEMB (nuclei from whole embryo), nPE (nuclei from proembryo), and nSUS (nuclei from suspensor), respectively. The lower coverage for the nSUS is due to the lower concordance in present (P), marginal (M), and absent (A) MAS5 calls between all three nSUS replicates compared to nPE or nEMB values (supplementary material Fig. S3). Nevertheless, there is substantial overlap of expressed genes designated as three times present (3xP) in the MAS5 calls between the three samples (supplementary material Fig. S4). The gcRMA values were used for correlation analysis of the biological replicates for nuclear transcriptomes from the whole embryo, proembryo, and suspensor as well as from data recently acquired from the shoot apex in adult plants (Yadav et al., 2014). This analysis showed high similarity between nuclear embryo replicates with Pearson correlation coefficients (PCC) ranging from 0.962 to 0.984. Interestingly, correlation was also high between nuclear replicates of the different embryonic tissues, whereas the correlation was low compared to the cellular shoot apex transcriptomes (supplementary material Table S3). Taken together, we detected a substantial number of genes that are active in the proembryo and/or the suspensor, as well as the whole embryo during Arabidopsis early embryogenesis.

Differentially expressed candidate genes

In order to find significantly differentially expressed candidate genes between the nPE and nSUS samples, a rank product analysis was conducted with a percentage of false positives smaller than 0.1 and a change of greater than two-fold. A total of 307 and 180 array elements corresponding to 335 and 181 locus identifiers were

enriched for nPE and nSUS, respectively (supplementary material Tables S4 and S5). To gain insight into the function and complexity of both cell types during early embryogenesis, we analyzed Gene Ontology (GO) terms for differentially expressed genes between the proembryo and the suspensor. Our analysis showed that overrepresented GO terms for proembryo-enriched genes are ‘DNA or RNA metabolism’/‘cell organization and biogenesis’ and ‘structural molecule activity’/‘protein binding’ in the categories “Biological processes” and “Molecular function”, respectively (Fig. 2A, supplemental material Table S6). The multidimensional proembryo often changes its plane of cell division in contrast to the stereotypic suspensor division type and undergoes cellular differentiation during early embryogenesis, which requires dynamic cytoskeleton reorganization and the coordinated change of gene expression (Webb and Gunning, 1991; Lau et al., 2012). In the suspensor, preferentially expressed genes were associated with ‘response to stress’/‘transport’ and ‘receptor binding or activity’/‘hydrolase’ in the categories “Biological processes” and “Molecular function”, respectively (Fig. 2B, supplemental material Table S7). Evidently, the suspensor has also been implicated in providing the proembryo with nutrients and plant hormones to be delivered by transporter proteins and it undergoes programmed cell death during late development which might share similar mechanisms with stress response signaling (Bozhkov et al., 2005; Kawashima and Goldberg, 2010). Overall, our results revealed that additionally to the morphological differences, the proembryo and the suspensor also appear distinct in gene expression profile during early embryogenesis. Furthermore, our GO analysis indicated a distinct function and an increased complexity of cellular activities in the proembryo compared to the suspensor during embryogenesis.

When we had a closer look into the two gene lists we could find genes that were previously shown to be differentially expressed and important for patterning and specification processes during embryogenesis e.g. *PIN-FORMED 1* (*PIN1*, *AT1G73590*), *WUSCHEL-RELATED HOMEOBOX 2* (*WOX2*, *AT5G59340*), *HANABU TARANU* (*HAN*, *AT3G50870*), *OBF BINDING PROTEIN 1* (*OBP1*, *AT3G50410*), or *FUSCA3* (*FUS3*, *AT3G26790*) (Aida et al., 2002; Friml et al., 2003; Kroj et al., 2003; Haecker et al., 2004; Skirycz et al., 2008; Nawy et al., 2010). Since *DRN* is not represented on the ATH1 chip, we tested mRNA levels by qRT-PCR. Indeed, *DRN* transcripts are highly abundant in the proembryo when compared to the suspensor (supplementary material Fig. S5A). Several other genes previously reported as being

preferentially expressed in the suspensor (Friml et al., 2003; Haecker et al., 2004; Breuninger et al., 2008) did either not pass the stringent statistical analysis (*WOX9*/*AT2G33880*), or were not detectable with the microarray (*PIN7*/*AT1G23080*, *WOX8*/*AT5G45980*). However, even though, for example, *PIN7* was under the microarray detection limit, we were able to detect its mRNA slightly more abundant in the suspensor by qRT-PCR (supplementary material Fig. S5B) which in conclusion indicates the existence of false negatives in our data set due to sensitivity thresholds.

Auxin was indirectly shown to accumulate in the cells of the proembryo at early embryonic stages (Friml et al., 2003). In addition to the already mentioned auxin efflux carrier PIN1, we were also able to detect the auxin biosynthesis genes *TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS1 (TAA1, AT1G70560)* and *YUCCA4* (*YUC4*, *AT5G11320*) as well as *SHORT INTERNODES* (*SHI*, *AT5G66350*) and *MYB77* (*AT3G50060*) as being proembryo-enriched, which are involved in auxin biosynthesis and signal transduction, respectively (Cheng et al., 2007; Shin et al., 2007; Stepanova et al., 2008; Baylis et al., 2013). Interestingly, suspensor-specific promoter-reporter expression of *YUC4* was recently shown at 16-cell stage of embryogenesis (Robert et al., 2013). However, since previous results showed transcript accumulation at later stages only in the proembryo (Cheng et al., 2007) and LCM-derived data do not show expression in the suspensor at globular stage (supplementary material Table S1), the observed suspensor-specific expression of *YUC4* might be due to the promoter-reporter construct likely not reflecting the *in vivo* mRNA distribution. All this is in accordance with the evidence demonstrating the importance of auxin signaling in cell division and differentiation during early embryogenesis (Lau et al., 2012).

Microarray data validation by promoter expression analysis and *in situ* hybridization

To further validate the microarray results, we randomly selected twelve genes statistically enriched for the proembryo and nine for the suspensor for global expression analysis. Promoters of differing lengths including the 5’UTR region were constructed to drive expression of n3xGFP or n3xRFP. In most cases, the expression patterns of the promoter fusion constructs were in concordance with the microarray results (Table 1, Fig. 3). In the one- or two-cell stage embryo, there was no exclusive expression in either the suspensor or the proembryo detectable but rather a broad

expression in all cells of the whole embryo with differences in expression strength between proembryo and suspensor was visible. Interestingly, some genes (e.g. *AT3G62480* and *AT3G52780*) showed expression in the suspensor but not the proembryo (Table 1, Fig. 3A,B). One gene (*AT5G46230*) showed global expression in the whole embryo at earliest stages but expression was later predominantly visible in the suspensor (Fig. 3H). Moreover, reporter expression for several candidate genes (e.g. *AT2G32100*, *AT5G05940*, and *AT3G17290*) remained universal in the whole embryo, which only later appeared stronger in one cell lineage and weaker in the other (Table 1, Fig. 3C,E,F). Three promoter fusion constructs did not confer any visible GFP expression in the embryo, which might be due to missing elements important for proper expression or a false-positive signal from the microarray (not shown). Taken together, the expression patterns of the promoter fusions are overall in concordance with the differences found in the statistical analysis of the microarray data. Minor discrepancies between the promoter fusion and the microarray data can most likely be attributed to the stability and low turnover rate of GFP protein inside the plant cell. In total, we tested 21 promoters fused to n3xGFP or n3xRFP of which only 3 were not embryo-expressed. Of the 18 embryo-expressed genes, 16 recapitulated the microarray results of differentially expressed transcripts (Table 1).

As promoter fusion constructs in some cases may not fully recapitulate true gene expression due to the possible lack of critical regulatory elements, we performed *in situ* hybridization for some of the proembryo- and suspensor-enriched transcripts. Overall, the *in situ* hybridization results for the selected, differentially expressed candidate genes (*AT1G04645*, *AT1G28300*, *AT5G46230*, *AT5G61030*, and *AT3G44750*) were consistent with the microarray analysis (Fig. 3D,G-J). However, we could not detect any signal in the early embryo for *AT2G46690* (data not shown). Since the promoter-reporter lines also did not give any signal, this is probably a false-positive signal on the microarray. Moreover, the promoter fusion analysis for two proembryo-enriched genes (*AT5G61030* and *AT3G44750*) did not correlate with our microarray analysis since the corresponding reporter-gene constructs indicated ubiquitous expression in all cells of the embryo (Fig. 3I,J). The *in situ* hybridization for these two genes, however, showed stronger signals in the proembryo at early stages of embryogenesis (Fig. 3I,J), indicating a possible lack of certain regulatory elements in the respective promoter regions cloned or post-transcriptional regulation of the endogenous gene. Furthermore, the validation of the

differentially expressed genes by *in situ* hybridization was not only additive to, but also complementary with the promoter fusion analysis. In summary, the promoter fusion studies and *in situ* hybridization results for in total 23 genes strongly correlated with the results of the microarray analysis which emphasizes the high quality of the whole dataset.

Nuclear transcriptomic data as proxy for gene expression profiling

For comparability reasons and to demonstrate that the nuclear results are indeed useful for detection of tissue-specific transcripts, we manually isolated intact whole embryos at 16-32 cell stages and directly extracted RNA without prior fixation. After amplification and microarray hybridization, samples were analyzed as mentioned above (cEMB, supplementary material Tables S1 and S2). When we compared MAS5 calls 3xP between nEMB and cEMB, we observed a strong 70% overlap (Fig. 4A). Additionally, the 30% genes not overlapping in the analysis showed weaker expression across the replicates on average (nEMB average value 239, cEMB 203) compared to the average expression of the overlapping 70% of 934 (supplementary material Tables S8 – S10), indicating that the differences in detection calls might be due to sample/microarray noise.

Recently, LCM was used in combination with microarrays to generate a very elaborate expression atlas of various seed compartments including the embryo at different developmental stages of the ovule (Le et al., 2010; Belmonte et al., 2013). Among other tissue types, cellular expression profiles were created for the proembryo and suspensor at the globular-embryo stage which we here term cellular globular proembryo (cgPE) and cellular globular suspensor (cgSUS), respectively (Le et al., 2010). We both MAS5-normalized and log2-transformed the raw values from the cgPE and cgSUS replicates as described above (supplementary material Tables S1 and S2). To see if the corresponding nuclear and cellular datasets were comparable, we first checked the overlap of MAS5 calls 3xP. However, unlike the higher overlap between nuclear samples (nPE/nSUS 64.5%, supplementary material Fig. S4), there are substantially fewer array elements shared between nPE/cgPE (47.2%) and nSUS/cgSUS (38.7%, Fig. 4B,C) and furthermore, these percentages are very similar for nPE/cgSUS or nSUS/cgPE (data not shown).

Additionally, after testing the normalized and transformed values of all replicates for comparability by box plot analysis (supplementary material Fig. S6A),

we performed hierarchical cluster analysis to group the different expression profiles. In summary, all replicates of one specific experiment group together and there are two main clusters consisting of 1) all nuclear (nPE, nSUS, nEMB) plus the cellular embryo sample (cEMB) and 2) the cellular globular-embryo samples (cgPE, cgSUS). In cluster 1) there are subgroups of nuclear samples and the cellular embryo sample (supplementary material Fig. S6B). These differences are further corroborated in a principal component analysis (PCA) plot where cgPE clusters with cgSUS, nPE with nSUS, and nEMB with cEMB (Fig. 4D). Published data of a KAN1 (KANADI 1) expression domain was used as an outgroup (Yadav et al., 2014). However, the cgPE and cgSUS cluster is farther away from the two other embryonic clusters. We can conclude that since the influence of fixation, nuclear RNA, and age of embryos on the observed expression profiles seems to be subtle, the main factor for these discrepancies between the LCM-derived and our datasets must be the different extraction techniques and RNA amplification protocols.

To compare the nuclear FANS and cellular LCM datasets beyond *in silico*, we compared the expression values of the LCM data for genes we tested with the promoter fusion constructs and *in situ* hybridization (Table 1). For three constructs not showing expression in the embryo, the LCM values were consistent with our microarray results indicating the same false-positive results (*AT1G31400*, *AT2G46690*, *AT1G48470*). The LCM array element values for seventeen genes showing expression in the embryo were consistent with our results (*AT1G77580*, *AT5G05940*, *AT2G35605*, *AT5G61030*, *AT1G64220*, *AT1G28300*, *AT5G22650*, *AT5G66940*, *AT3G44750*, *AT3G55660*, *AT3G62480*, *AT1G04645*, *AT1G54160*, *AT3G52780*, *AT5G07440*, *AT1G74190*, *AT2G32100*). Four genes (*AT5G26270*, *AT3G17290*, *AT5G43510*, *AT5G46230*) appeared as false negatives in the LCM dataset as the expression values were very low and often the MAS5 call was absent for both the proembryo and the suspensor replicates.

Recently, a report described the expression patterns of multiple auxin response factors (ARF) using promoter-reporter constructs during early Arabidopsis embryogenesis (Rademacher et al., 2011). Four of the tested ARF promoters (*ARF12*/*AT1G34310*, *ARF17*/*AT1G77850*, *ARF21*/*AT1G34410*, *ARF23*/*AT1G43950*) were designated as only being expressed in the endosperm but not the embryo itself (Rademacher et al., 2011) and these genes were designated as absent and in essence not expressed in our dataset (supplementary material Table S11). In the

LCM dataset however, all four genes were called present and at least weakly expressed within the suspensor. We also compared the different suspensor data sets for presence of previously described endosperm-specific genes (Kinoshita et al., 1999; Luo et al., 2000; Kinoshita et al., 2004; Kang et al., 2008; Li et al., 2013; Barthole et al., 2014). The LCM results show all six genes tested as present, whereas our data indicate that only two out of six are also at least weakly expressed in the suspensor (supplementary material Table S11) and those two were also detected in a RNA-seq transcriptome analysis (Nodine and Bartel, 2012). This suggests contamination of at least the suspensor samples with surrounding endosperm in the LCM dataset. Since it is essentially impossible to accurately dissect tissue with LCM in the third dimension, the list of apparent suspensor genes is likely contaminated by endosperm-expressed genes. On the contrary, with our methodology, we could minimize contamination with cellular or nuclear material from embryo-surrounding cells. To substantiate this notion, we generated a list of putative embryo-specific genes for the Arabidopsis seed by comparing our statistically enriched candidates with publicly available seed transcriptome data. For this purpose, we combined our enriched nPE and nSUS gene lists and differentiated it to the combined LCM data 3xP for different seed compartments at the globular stage excluding the globular proembryo and suspensor (cgSEED). In total, we detected 95 genes (supplementary material Tables S12 and S13) which contained known genes like the aforementioned *HAN*/*MNP*, *WOX2*, *OBP1*, and *YUC4* as well as the embryonic identity regulators *LEAFY COTYLEDON 2* (*LEC2*) and *BABYBOOM* (*BBM*) (Stone et al., 2001; Boutilier et al., 2002). Interestingly, 11 of our 23 *in vivo* tested candidate genes were also present in this putative embryo-specific list (Table 1). In summary, this strongly indicates that with our approach we can detect tissue-specific genes.

DISCUSSION

In this study we described and validated a nuclear extraction and purification protocol for expression analysis of inaccessible cell types in the *Arabidopsis thaliana* seed. Given that the unequal distribution of some transcripts in the early embryo leads to distinguishable cell types and likewise the unequal distribution of specific transcripts was reported in the apical and basal cells of tobacco embryos (Breuninger et al., 2008; Hu et al., 2010; Ueda et al., 2011), we reasoned that these cell types might be

a well suited test field for our method and that the generation of expression profiles for the proembryo and suspensor of early Arabidopsis embryos will provide insights into better understanding of early embryo development. Several of the statistically-enriched candidate genes for the proembryo were previously described to have important functions during early embryogenesis, some of which were shown as proembryo-enriched expressed genes in our dataset. For example, *HANABA TANARU* (*HAN*) was shown to be expressed in the apical cell of the embryo and plays a role in setting up the boundary between proembryo and suspensor (Nawy et al., 2010). *PIN-FORMED 1* (*PIN1*), known as an auxin efflux facilitator, is expressed in the proembryo cells, mediating auxin flow from apical cells to the hypophysis (Friml et al., 2003), which is in turn critical for root initiation. Another apically expressed gene is the homeobox transcription factor *WUSCHEL RELATED HOMEOBOX 2* (*WOX2*) plays a fundamental role in the establishment of the apical domain (Haecker et al., 2004). Moreover, the suspensor-expressed gene *FUSCA3* (*FUS3*) lacks the apical expression due to the repression by *DICER-LIKE1* (*DCL1*), and early matured embryos in the *dcl1* mutant show ectopic expression of *FUS3* in the proembryo (Willmann et al., 2011). All these examples initially substantiated our results as these genes were not only present in one or the other dataset but were among the statistically most significant ones.

The *in vivo* expression analyses using promoter-GFP fusion constructs as well as *in situ* hybridization strongly correlated with the microarray results for the candidate genes tested. This demonstrated the validity of the microarray results after stringent statistical analysis from expression data generated for specific tissues in the Arabidopsis embryo at the earliest developmental stages.

The high correlation of nuclear and cellular embryonic transcriptome data generated is very encouraging for the use of this method also in other studies. However, comparison with published expression data generated from respective cellular embryonic tissues by laser capture microdissection (Le et al., 2010) apart from certain similarities revealed major differences in types of genes expressed in the given tissues. Potentially there are many factors influencing the final transcriptomic data. These include 1) the plant accession used, 2) the developmental stage of the tissue studied, 3) the RNA composition (cellular, cytoplasmic, nuclear), 4) different fixation approaches, 5) RNA extraction, and 6) RNA amplification method. By comparison of our nuclear RNA transcriptome results with those from cellular RNA of

non-fixed embryos – assuming the influence of accession and RNA extraction method as marginal – we conclude that the RNA amplification or probably tissue isolation approaches has the greatest impact. This notion seems reasonable because we used a commercial kit and a polyT primer whereas the LCM RNA was amplified with a polyT/random primer mixture (Le et al., 2010).

Crosschecking *in vivo* expression results, we did not see any disadvantages of our transcriptomic data except a certain proportion of false-negatives in detection of low-expressed genes. On the contrary, we propose that our approach has certain advantages, most importantly one being the possibility to study any tissue of interest and the other being a decreased risk of contamination with embryo-surrounding cells compare to LCM. Furthermore, we demonstrate that this approach is able to detect tissue-specific genes with a very small expression domain. Even though there is the necessity for transgenics in order to use our approach, it is nevertheless applicable to any other transformable plant or animal tissue to generate expression data from a given cell type. Importantly, the method described here not only enables expression studies to be performed but also has the potential to study DNA and histone modifications.

MATERIALS AND METHODS

Plant materials and growth conditions

All *Arabidopsis thaliana* lines used are *Col-0*. The GAL4-GFP enhancer-trap lines generated by the Haseloff lab were obtained from the Nottingham Arabidopsis Stock Centre (NASC). For growth under sterile conditions, seeds were surface-sterilized with 25% bleach, washed three times, and grown on half-strength Murashige and Skoog (MS) containing 0.8% agar plates containing 10 g/l sucrose. Seedlings were transferred to soil and grown at 22°C to 24°C in a growth chamber under a 16 h/8 h light/dark cycle.

Molecular cloning and genotyping

TAIL-PCR was performed as previously described (Liu and Chen, 2007). All genomic fragments (626 bp-2615 bp upstream of ATG) for the promoter-GFP fusions were PCR-amplified and sub-cloned into *pGEM-T* vector (Promega). The n3xRFP was assembled from PCR-amplified monomers in *pGII Kan* vector. All fragments were finally introduced into *pGII Kan:n3×GFP* (Takada and Jürgens, 2007) or *pGII*

Kan:n3×RFP. A *pAT3G10100* fragment was introduced into *pGII Kan:n3xGFP* which resulted in *pAT3G10100::nGFP*.

The n2xGFP was amplified from *pGII Kan:n3xGFP* and introduced into *pGII Kan*. For generating *pDRN:n2×GFP:DRN 3'UTR*, a 1378 bp *DRN 3'UTR* fragment was PCR-amplified and sub-cloned into *pGEM-T.* Then the *DRN* 3'UTR was introduced into *pGII Kan:n2×GFP* generating *pGII Kan:n2×GFP:DRN 3'UTR.* A 4145 bp DRN promoter upstream of the start codon was PCR-amplified and sub-cloned into *pGEM-T. pDRN* was finally introduced into *pGII Kan:n2×GFP:DRN 3'UTR* generating *pDRN:n2×GFP:DRN 3'UTR*. Oligonucleotides used for cloning can be found in supplementary material Table S14.

Nuclear isolation

Fresh Arabidopsis ovules were collected in RNAlater buffer (QIAGEN) and kept in fixation buffer (0.1% Paraformaldehyde in RNAlater) for 5-10 min and ground thoroughly using the pestle in the 1.5 ml tube. The CelLytic^{TM} PN kit (Sigma) was used for the following procedures.

FANS

Fluorescently labeled nuclei were identified by plotting peak GFP fluorescence (513/17) against autofluorescence (575/25) using a MoFlo Legacy (Beckman Coulter) FACS fitted with a 488 nm laser (100 mW) triggering off the FSC (forward scatter channel). Tests with co-staining with propidium iodide to label free nuclei identified the same GFP population therefore staining was deemed unnecessary. Flow cytometric analyses were carried out as follows: 1x PBS pH 7.0, 70 µM stream, ~60.5 / ~60.0 psi, ~95 kHz, 1-2 single drop envelope.

Manual isolation of embryos

Isolation was performed essentially as previously described (Nodine and Bartel, 2012). In brief, early-globular stage embryos were squeezed out from the ovules on a microscope slide and washed three times in water and subsequently collected in RNAlater. 40-50 embryos were pooled per biological replicate.

RNA extraction and amplification

The sorted positive nuclei were collected in RNA extraction buffer [10 mM Tris-HCl pH 7.9, 50 mM EDTA pH 7.9, 0.2 M NaCl, 0.5% SDS, 0.5 mg/ml RNase inhibitor (Fermentas), 600 µg/ml proteinase K] (Khodosevich et al., 2007). The buffer containing the GFP-positive nuclei was incubated at 55°C with vigorous shaking for 10-15 min. The total volume was adjusted to 600 µl RNase-free water and an equal volume of phenol pH 4.2 was added. The solution was vortexed thoroughly and kept on ice for 5 min and afterwards centrifuged at 14,000 *g* for 10 min at 4°C. The aqueous phase was transferred into a new tube and an equal volume of phenol:chloroform (1:1) was added. The solution was mixed thoroughly and kept on ice for 5 min and centrifuged at 14,000 *g* for 10 min at 4°C. The aqueous phase was transferred into a new tube and an equal volume of isopropanol and 20 µg glycogen were added. Then the solution was mixed thoroughly and kept at -20°C overnight and centrifuged at 16,100 g for 45 min at 4°C. Following the centrifugation, the resulting pellet was washed with 70% cold ethanol and dried at room temperature. The pellet was eventually dissolved in RNase-free water. For DNase treatment, a commercial kit (DNase I, Fermentas) was used and afterwards the RNeasy Micro Kit (QIAGEN) was used for RNA cleanup.

One to three ng of total RNA was used for cDNA synthesis and amplification (Arcturus® RiboAmp® HS PLUS RNA Amplification Kit) and the resulting cDNA was fragmented and labeled using the ENZO BioArray™ Single-round RNA amplification and biotin labeling system. 12.5 µg of fragmented cDNA was hybridized on Affymetrix GeneChip ATH1 Arabidopsis Genome Array.

Microarray data analysis

Microarray datasets as .CEL files for LCM and shoot KAN1 were downloaded from the GEO DataSets on the NCBI (National Center for Biotechnology) website (https://www.ncbi.nlm.nih.gov/). The globular-stage seed gene list excluding the embryo and suspensor was downloaded from Gene Networks in Seed Development website (http://seedgenenetwork.net/). Microarray data analyses were performed using diverse packages implemented in “R” (v2.14.2; http://www.r-project.org). Log2-based expression estimates were obtained from .CEL files using “gcRMA” (v2.26.0) (Wu et al., 2004). Differentially expressed genes were identified by “RankProducts” (v2.26.0) using 100 permutations and a percentage false positive (pfp) cut-off of 0.05

(Breitling et al., 2004). Present, marginal, and absent calls were calculated using MAS5 as implemented in the “affy” package (v1.32.1). Pearson correlation coefficients of gcRMA values were calculated with Microsoft Office Excel 2007. GO classification of proembryo- and suspensor-enriched genes was created with the Classification SuperViewer Tool from BAR (The Bio-Analytical Resource for Plant Biology (http://bar.utoronto.ca/ntools/cgi-bin/ntools_classification_superviewer.cgi). All Venn diagrams were generated with a combination of BioVenn (http://www.cmbi.ru.nl/cdd/biovenn/), Venn diagram plotter (http://omics.pnl.gov/software/venn-diagram-plotter), and Adobe Illustrator. Quality control analyses (Box plot, hierarchical clustering of samples, principal component analysis) of all biological replicates were performed with CLC Main Workbench software (version 6.6.2).

Quantitative real-time PCR

Due to limitations in RNA quantity, amplified cRNA was used for cDNA synthesis (RevertAid First Strand cDNA Synthesis Kit, Fermentas). Quantitative PCR was performed on a Chromo4 Real-Time Detector (Bio-Rad) with Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen). PCRs were done in triplicates with specific primer pairs (supplementary material Table S14) and transcript levels were normalized to *ACTIN2*.

RNA *in situ* hybridization

The primers for probe synthesis are listed in supplementary material Table S14. The fragments for the sense and antisense probes were PCR-amplified and inserted into *pBSK*$^{-}$ or *pGEM-T* vectors. *In vitro* transcription was performed with T7 or SP6 primers and with Fermentas in vitro transcription kit. Both ends of young siliques were cut off and the middle part was fixed in cold fixation solution (4% Paraformaldehyde in DEPC-treated water, 0.1% Tween-20). A conventional plastic syringe was used for vacuum infiltration and the samples were kept overnight in the fixation solution at 4°C. Following 1×PBS incubation for 2x30 min, the samples were dehydrated through a graded ethanol series (30%, 40%, 50%, 60%, 80%, 90%, 95%) for 1 hour each and finally embedded in paraffin. Paraffin-embedded samples were microtome-sectioned to 6 µm thickness. The procedures of hybridization and staining were performed as described (Schlereth et al., 2010).

Microscopy

For differential interference contrast (DIC) microscopy and fluorescence analysis, ovules were mounted on slides containing clearing solution [chloral hydrate, water, and glycerol (ratio w/v/v: 8:3:1)]. For fluorescence analysis, embryos were gently squeezed out from ovules and mounted in 10% glycerol (v/v). An Olympus IX81 confocal laser scanning microscope (image acquisition software: FV10-ASW; objectives: UPlanSApo *x*40) was used for confocal microscopy analysis. Images were further processed using Adobe Photoshop software. Zeiss Axio Imager (image acquisition software: AxioVision; camera: AxioCam HRc; objectives: Plan-APOCHROMAT ×20 and ×40) was used for wide-field and DIC images and images were further processed with AxioVision SE64 Rel. 4.9.1 software.

Acknowledgements

We thank Steffen Lau for critical reading of the manuscript and Arvid Herrmann and Ole Herud for assistance. We thank the Nottingham Arabidopsis Stock Center (NASC) for providing enhancer-trap lines.

Competing interests

The authors declare no competing financial interests.

Author contributions

D.S., J.Kong, U.M., I.D.S., M.B., and G.J. were involved in the conception and design of the experiments. D.S. and J.Kong co-wrote the manuscript and performed most of the experiments. K.H., K.W.B. and J.K. performed the flow cytometry analysis. A.H. and M.B. generated the transcriptome datasets for manually isolated embryos. M.S. performed microarray analysis for MAS5, gcRMA, and RankProducts. M.K. performed the qRT-PCR analysis.

Funding

This work was funded by the SIREN network (European Commission under FP7-PEOPLE-2007-1-1-ITN), the European Molecular Biology Organization (EMBO-ALTF 108-2006 to I.D.S.), the Marie Curie Intra-European Fellowship scheme (postdoctoral fellowship FP6 MEIF-CT-2007-041375 to I.D.S.), the DFG (Deutsche Forschungsgemeinschaft, BA3356/2-1 to M.B.), and the Max Planck Society.

References

Aida, M., Vernoux, T., Furutani, M., Traas, J. and Tasaka, M. (2002). Roles of PIN-FORMED1 and MONOPTEROS in pattern formation of the apical region of the Arabidopsis embryo. *Development* **129**, 3965-3974.

Barthelson, R. A., Lambert, G. M., Vanier, C., Lynch, R. M. and Galbraith, D. W. (2007). Comparison of the contributions of the nuclear and cytoplasmic compartments to global gene expression in human cells. *BMC Genomics* **8**, 340.

Barthole, G., To, A., Marchive, C., Brunaud, V., Soubigou-Taconnat, L., Berger, N., Dubreucq, B., Lepiniec, L. and Baud, S. (2014). MYB118 Represses Endosperm Maturation in Seeds of Arabidopsis. *Plant Cell*.

Baylis, T., Cierlik, I., Sundberg, E. and Mattsson, J. (2013). SHORT INTERNODES/STYLISH genes, regulators of auxin biosynthesis, are involved in leaf vein development in Arabidopsis thaliana. *New Phytol* **197**, 737-750.

Belmonte, M. F., Kirkbride, R. C., Stone, S. L., Pelletier, J. M., Bui, A. Q., Yeung, E. C., Hashimoto, M., Fei, J., Harada, C. M., Munoz, M. D. et al. (2013). Comprehensive developmental profiles of gene activity in regions and subregions of the Arabidopsis seed. *Proc Natl Acad Sci U S A* **110**, E435-444.

Birnbaum, K., Shasha, D. E., Wang, J. Y., Jung, J. W., Lambert, G. M., Galbraith, D. W. and Benfey, P. N. (2003). A gene expression map of the Arabidopsis root. *Science* **302**, 1956-1960.

Bonner, W. A., Hulett, H. R., Sweet, R. G. and Herzenberg, L. A. (1972). Fluorescence activated cell sorting. *Rev Sci Instrum* **43**, 404-409.

Boutilier, K., Offringa, R., Sharma, V. K., Kieft, H., Ouellet, T., Zhang, L., Hattori, J., Liu, C. M., van Lammeren, A. A., Miki, B. L. et al. (2002). Ectopic expression of BABY BOOM triggers a conversion from vegetative to embryonic growth. *Plant Cell* **14**, 1737-1749.

Bozhkov, P. V., Filonova, L. H. and Suarez, M. F. (2005). Programmed cell death in plant embryogenesis. *Curr Top Dev Biol* **67**, 135-179.

Breitling, R., Armengaud, P., Amtmann, A. and Herzyk, P. (2004). Rank products: a simple, yet powerful, new method to detect differentially regulated genes in replicated microarray experiments. *FEBS Lett* **573**, 83-92.

Breuninger, H., Rikirsch, E., Hermann, M., Ueda, M. and Laux, T. (2008). Differential expression of WOX genes mediates apical-basal axis formation in the Arabidopsis embryo. *Dev Cell* **14**, 867-876.

Chandler, J. W., Cole, M., Flier, A., Grewe, B. and Werr, W. (2007). The AP2 transcription factors DORNRÖSCHEN and DORNRÖSCHEN-LIKE redundantly control *Arabidopsis* embryo patterning via interaction with PHAVOLUTA. *Development* **134**, 1653-1662.

Cheng, Y., Dai, X. and Zhao, Y. (2007). Auxin synthesized by the YUCCA flavin monooxygenases is essential for embryogenesis and leaf formation in Arabidopsis. *Plant Cell* **19**, 2430-2439.

Cole, M., Chandler, J., Weijers, D., Jacobs, B., Comelli, P. and Werr, W. (2009). *DORNRÖSCHEN* is a direct target of the auxin response factor MONOPTEROS in the *Arabidopsis* embryo. *Development* **136**, 1643-1651.

Cumberledge, S. and Krasnow, M. A. (1994). Preparation and analysis of pure cell populations from Drosophila. *Methods Cell Biol* **44**, 143-159.

De Smet, I., Vassileva, V., De Rybel, B., Levesque, M. P., Grunewald, W., Van Damme, D., Van Noorden, G., Naudts, M., Van Isterdael, G., De Clercq, R. et al. (2008). Receptor-like kinase ACR4 restricts formative cell divisions in the Arabidopsis root. *Science* **322**, 594-597.

Deal, R. B. and Henikoff, S. (2010). A simple method for gene expression and chromatin profiling of individual cell types within a tissue. *Dev Cell* **18**, 1030-1040.
Emmert-Buck, M. R., Bonner, R. F., Smith, P. D., Chuaqui, R. F., Zhuang, Z., Goldstein, S. R., Weiss, R. A. and Liotta, L. A. (1996). Laser capture microdissection. *Science* **274**, 998-1001.
Friml, J., Vieten, A., Sauer, M., Weijers, D., Schwarz, H., Hamann, T., Offringa, R. and Jürgens, G. (2003). Efflux-dependent auxin gradients establish the apical-basal axis of Arabidopsis. *Nature* **426**, 147-153.
Haecker, A., Gross-Hardt, R., Geiges, B., Sarkar, A., Breuninger, H., Herrmann, M. and Laux, T. (2004). Expression dynamics of WOX genes mark cell fate decisions during early embryonic patterning in Arabidopsis thaliana. *Development* **131**, 657-668.
Haseloff, J. (1999). GFP variants for multispectral imaging of living cells. *Methods Cell Biol* **58**, 139-151.
Heiman, M., Schaefer, A., Gong, S., Peterson, J. D., Day, M., Ramsey, K. E., Suarez-Farinas, M., Schwarz, C., Stephan, D. A., Surmeier, D. J. et al. (2008). A translational profiling approach for the molecular characterization of CNS cell types. *Cell* **135**, 738-748.
Hu, T. X., Yu, M. and Zhao, J. (2010). Comparative transcriptional profiling analysis of the two daughter cells from tobacco zygote reveals the transcriptome differences in the apical and basal cells. *BMC Plant Biol* **10**, 167.
Jayasinghe, S. M., Wunderlich, J., McKee, A., Newkirk, H., Pope, S., Zhang, J., Staehling-Hampton, K., Li, L. and Haug, J. S. (2006). Sterile and disposable fluidic subsystem suitable for clinical high speed fluorescence-activated cell sorting. *Cytometry B Clin Cytom* **70**, 344-354.
Jaye, D. L., Bray, R. A., Gebel, H. M., Harris, W. A. and Waller, E. K. (2012). Translational applications of flow cytometry in clinical practice. *J Immunol* **188**, 4715-4719.
Kang, I. H., Steffen, J. G., Portereiko, M. F., Lloyd, A. and Drews, G. N. (2008). The AGL62 MADS domain protein regulates cellularization during endosperm development in Arabidopsis. *Plant Cell* **20**, 635-647.
Kawashima, T. and Goldberg, R. B. (2010). The suspensor: not just suspending the embryo. *Trends Plant Sci* **15**, 23-30.
Kerk, N. M., Ceserani, T., Tausta, S. L., Sussex, I. M. and Nelson, T. M. (2003). Laser capture microdissection of cells from plant tissues. *Plant Physiol* **132**, 27-35.
Khodosevich, K., Inta, D., Seeburg, P. H. and Monyer, H. (2007). Gene expression analysis of in vivo fluorescent cells. *PLoS One* **2**, e1151.
Kinoshita, T., Yadegari, R., Harada, J. J., Goldberg, R. B. and Fischer, R. L. (1999). Imprinting of the MEDEA polycomb gene in the Arabidopsis endosperm. *Plant Cell* **11**, 1945-1952.
Kinoshita, T., Miura, A., Choi, Y., Kinoshita, Y., Cao, X., Jacobsen, S. E., Fischer, R. L. and Kakutani, T. (2004). One-way control of FWA imprinting in Arabidopsis endosperm by DNA methylation. *Science* **303**, 521-523.
Kroj, T., Savino, G., Valon, C., Giraudat, J. and Parcy, F. (2003). Regulation of storage protein gene expression in Arabidopsis. *Development* **130**, 6065-6073.
Lamesch, P., Berardini, T. Z., Li, D., Swarbreck, D., Wilks, C., Sasidharan, R., Muller, R., Dreher, K., Alexander, D. L., Garcia-Hernandez, M. et al. (2012). The Arabidopsis Information Resource (TAIR): improved gene annotation and new tools. *Nucleic Acids Res* **40**, D1202-1210.

Lau, S., Slane, D., Herud, O., Kong, J. and Jürgens, G. (2012). Early embryogenesis in flowering plants: setting up the basic body pattern. *Annu Rev Plant Biol* **63**, 483-506.
Le, B. H., Cheng, C., Bui, A. Q., Wagmaister, J. A., Henry, K. F., Pelletier, J., Kwong, L., Belmonte, M., Kirkbride, R., Horvath, S. et al. (2010). Global analysis of gene activity during Arabidopsis seed development and identification of seed-specific transcription factors. *Proc Natl Acad Sci U S A* **107**, 8063-8070.
Li, J., Nie, X., Tan, J. L. and Berger, F. (2013). Integration of epigenetic and genetic controls of seed size by cytokinin in Arabidopsis. *Proc Natl Acad Sci U S A* **110**, 15479-15484.
Liu, Y. G. and Chen, Y. (2007). High-efficiency thermal asymmetric interlaced PCR for amplification of unknown flanking sequences. *Biotechniques* **43**, 649-650, 652, 654 passim.
Luo, M., Bilodeau, P., Dennis, E. S., Peacock, W. J. and Chaudhury, A. (2000). Expression and parent-of-origin effects for FIS2, MEA, and FIE in the endosperm and embryo of developing Arabidopsis seeds. *Proc Natl Acad Sci U S A* **97**, 10637-10642.
Nawy, T., Bayer, M., Mravec, J., Friml, J., Birnbaum, K. D. and Lukowitz, W. (2010). The GATA factor HANABA TARANU is required to position the proembryo boundary in the early Arabidopsis embryo. *Dev Cell* **19**, 103-113.
Nodine, M. D. and Bartel, D. P. (2012). Maternal and paternal genomes contribute equally to the transcriptome of early plant embryos. *Nature* **482**, 94-97.
Palovaara, J., Saiga, S. and Weijers, D. (2013). Transcriptomics approaches in the early Arabidopsis embryo. *Trends Plant Sci* **18**, 514-521.
Rademacher, E. H., Moller, B., Lokerse, A. S., Llavata-Peris, C. I., van den Berg, W. and Weijers, D. (2011). A cellular expression map of the Arabidopsis AUXIN RESPONSE FACTOR gene family. *Plant J* **68**, 597-606.
Robert, H. S., Grones, P., Stepanova, A. N., Robles, L. M., Lokerse, A. S., Alonso, J. M., Weijers, D. and Friml, J. (2013). Local auxin sources orient the apical-basal axis in Arabidopsis embryos. *Curr Biol* **23**, 2506-2512.
Schlereth, A., Möller, B., Liu, W., Kientz, M., Flipse, J., Rademacher, E. H., Schmid, M., Jürgens, G. and Weijers, D. (2010). MONOPTEROS controls embryonic root initiation by regulating a mobile transcription factor. *Nature* **464**, 913-916.
Shigenobu, S., Arita, K., Kitadate, Y., Noda, C. and Kobayashi, S. (2006). Isolation of germline cells from Drosophila embryos by flow cytometry. *Dev Growth Differ* **48**, 49-57.
Shin, R., Burch, A. Y., Huppert, K. A., Tiwari, S. B., Murphy, A. S., Guilfoyle, T. J. and Schachtman, D. P. (2007). The Arabidopsis transcription factor MYB77 modulates auxin signal transduction. *Plant Cell* **19**, 2440-2453.
Skirycz, A., Radziejwoski, A., Busch, W., Hannah, M. A., Czeszejko, J., Kwasniewski, M., Zanor, M. I., Lohmann, J. U., De Veylder, L., Witt, I. et al. (2008). The DOF transcription factor OBP1 is involved in cell cycle regulation in Arabidopsis thaliana. *Plant J* **56**, 779-792.
Spencer, M. W., Casson, S. A. and Lindsey, K. (2007). Transcriptional profiling of the Arabidopsis embryo. *Plant Physiol* **143**, 924-940.
Stepanova, A. N., Robertson-Hoyt, J., Yun, J., Benavente, L. M., Xie, D. Y., Dolezal, K., Schlereth, A., Jürgens, G. and Alonso, J. M. (2008). TAA1-mediated auxin biosynthesis is essential for hormone crosstalk and plant development. *Cell* **133**, 177-191.
Stone, S. L., Kwong, L. W., Yee, K. M., Pelletier, J., Lepiniec, L., Fischer, R. L., Goldberg, R. B. and Harada, J. J. (2001). LEAFY COTYLEDON2 encodes a B3

domain transcription factor that induces embryo development. *Proc Natl Acad Sci U S A* **98**, 11806-11811.
Takada, S. and Jürgens, G. (2007). Transcriptional regulation of epidermal cell fate in the *Arabidopsis* embryo. *Development* **134**, 1141-1150.
Ueda, M., Zhang, Z. and Laux, T. (2011). Transcriptional activation of Arabidopsis axis patterning genes WOX8/9 links zygote polarity to embryo development. *Dev Cell* **20**, 264-270.
Webb, M. C. and Gunning, B. E. (1991). The microtubular cytoskeleton during development of the zygote, proemhryo and free-nuclear endosperm in Arabidopsis thaliana (L.) Heynh. *Planta* **184**, 187-195.
Willmann, M. R., Mehalick, A. J., Packer, R. L. and Jenik, P. D. (2011). MicroRNAs regulate the timing of embryo maturation in Arabidopsis. *Plant Physiol* **155**, 1871-1884.
Wu, Z., A., I. R., Robert, G., Martinez-Murillo, F. and Spencer, F. (2004). A Model-Based Background Adjustment for Oligonucleotide Expression Arrays. *Journal of the American Statistical Association* **99**, 909-917.
Yadav, R. K., Tavakkoli, M., Xie, M., Girke, T. and Reddy, G. V. (2014). A high-resolution gene expression map of the Arabidopsis shoot meristem stem cell niche. *Development* **141**, 2735-2744.
Zhang, C., Barthelson, R. A., Lambert, G. M. and Galbraith, D. W. (2008). Global characterization of cell-specific gene expression through fluorescence-activated sorting of nuclei. *Plant Physiol* **147**, 30-40.

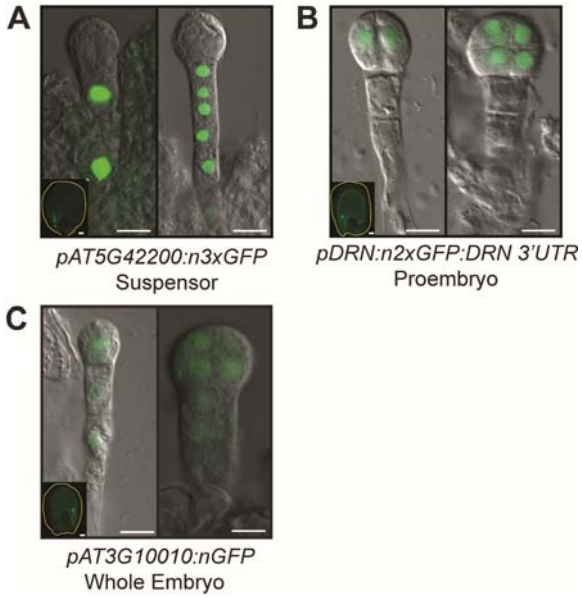

Figure 1. Specific marker lines used for FANS. (A) Suspensor marker line at 2-cell and early globular stages. (B) Proembryo marker line at 2-cell and 8-cell stages. (C) Whole embryo marker line at 1-cell and 4-cell stages. Insets show overview of seed with embryo-specific GFP expression for each marker line. Inset scale bars, 20 µm. All other scale bars, 10 µm.

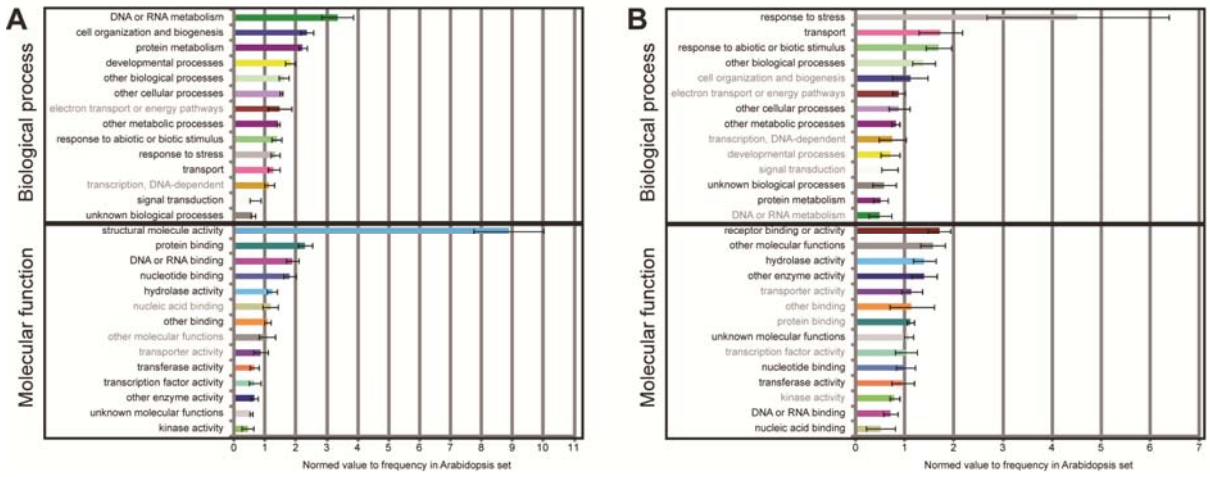

Figure 2. Bar graph representation of GO analysis for nuclear proembryo- and suspensor-enriched genes. (A) Enrichment of GO terms in categories "biological process" and "molecular function" for proembryo. (B) Enrichment of GO terms in categories "biological process" and "molecular function" for suspensor. ± Bootstrap standard deviation is given for each bar. GO terms depicted in grey letters are not statistically significant (p-value > 0.05).

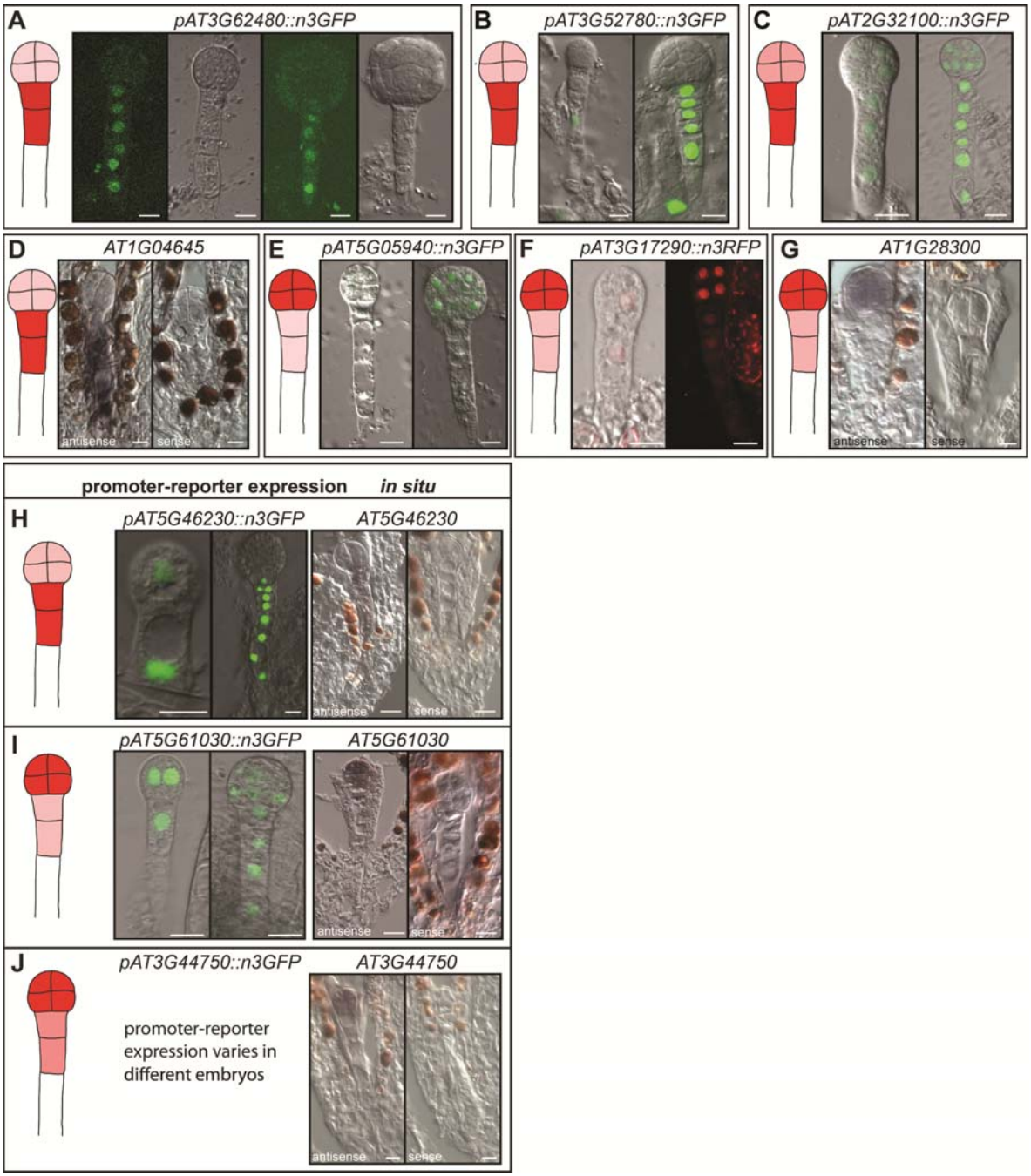

Figure 3. Promoter fusion analysis and *in situ* hybridizations for selected differentially expressed candidate genes in early embryos. (A-G) Temporal promoter-reporter expression and *in situ* hybridization of suspensor-enriched genes (A-D) and proembryo-enriched genes (E-G) during early embryogenesis. (H-J) Comparison of promoter-reporter expression and *in situ* hybridization for the same genes enriched in suspensor (H) and proembryo (I and J). Color shading in the schematic representation of Arabidopsis embryo indicates the expression levels

according to the microarray dataset (dark red: stronger expression; light red: weaker expression). Scale bars, 10 µm.

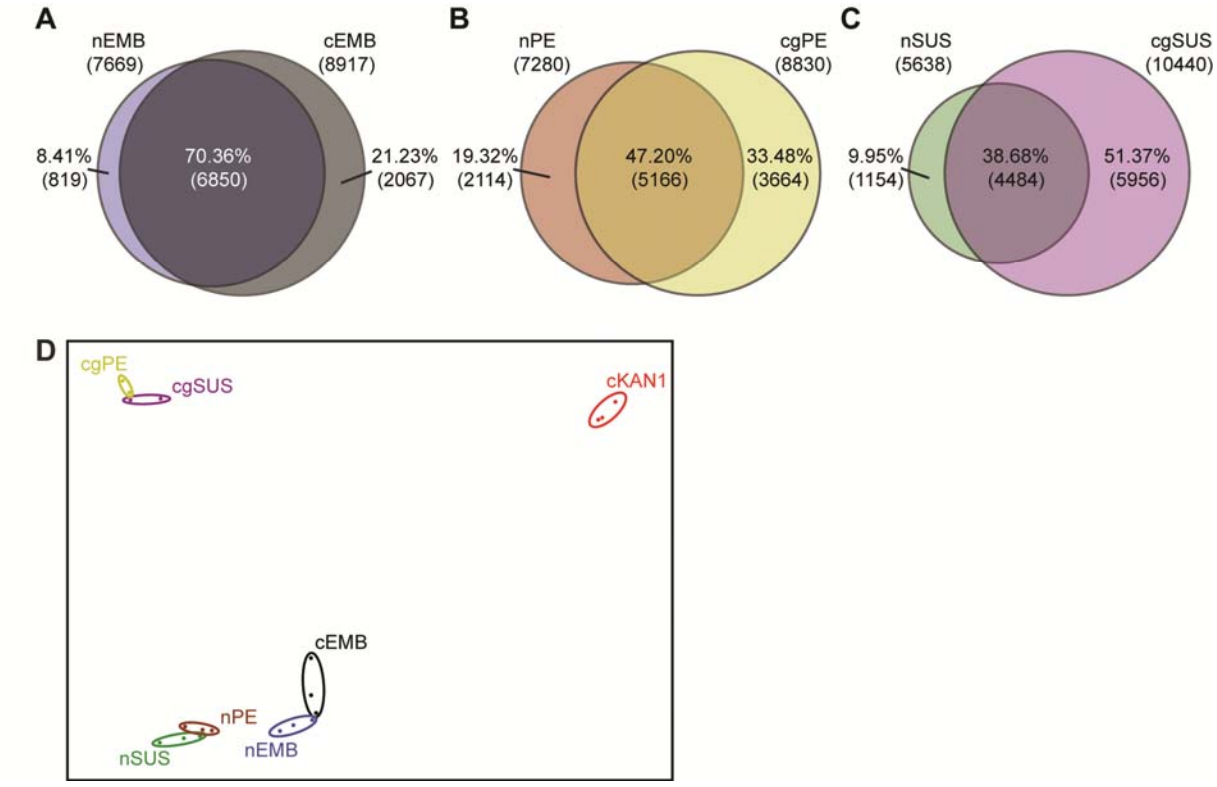

Figure 4. Comparison of nuclear and cellular transcriptome data from different tissue types. (A-C) Venn diagrams showing overlap of MAS5 3x present calls between nEMB and cEMB (A), nPE and cgPE (B), and nSUS and cgSUS (C). (D) Principal component analysis of biological replicates from the different nuclear and cellular tissue types. nPE = nuclei from proembryo, nSUS = nuclei from suspensor, nEMB = nuclei from whole embryo, cEMB = cells from whole embryo, cgPE = cellular globular-stage proembryo, cgSUS = cellular globular-stage suspensor, cKAN1 = cellular KANADI 1 expression domain in the shoot.

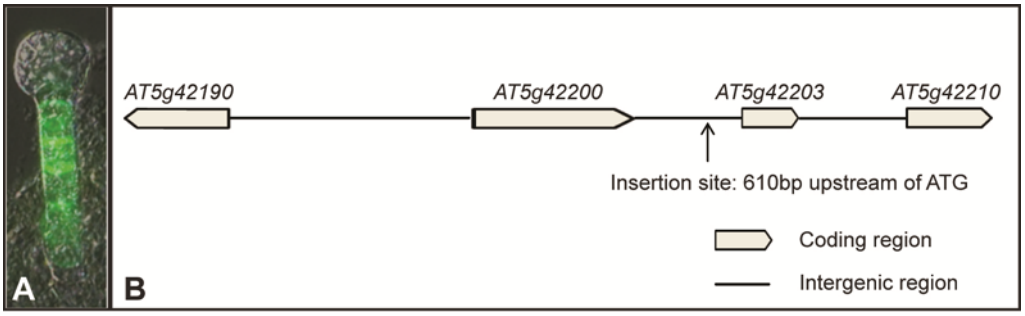

Figure S1. Enhancer-trap line N9322 and identification of genomic insertion site. (A) Suspensor and hypophysis expression at globular stage. (B) Insertion site of T-DNA determined by TAIL-PCR.

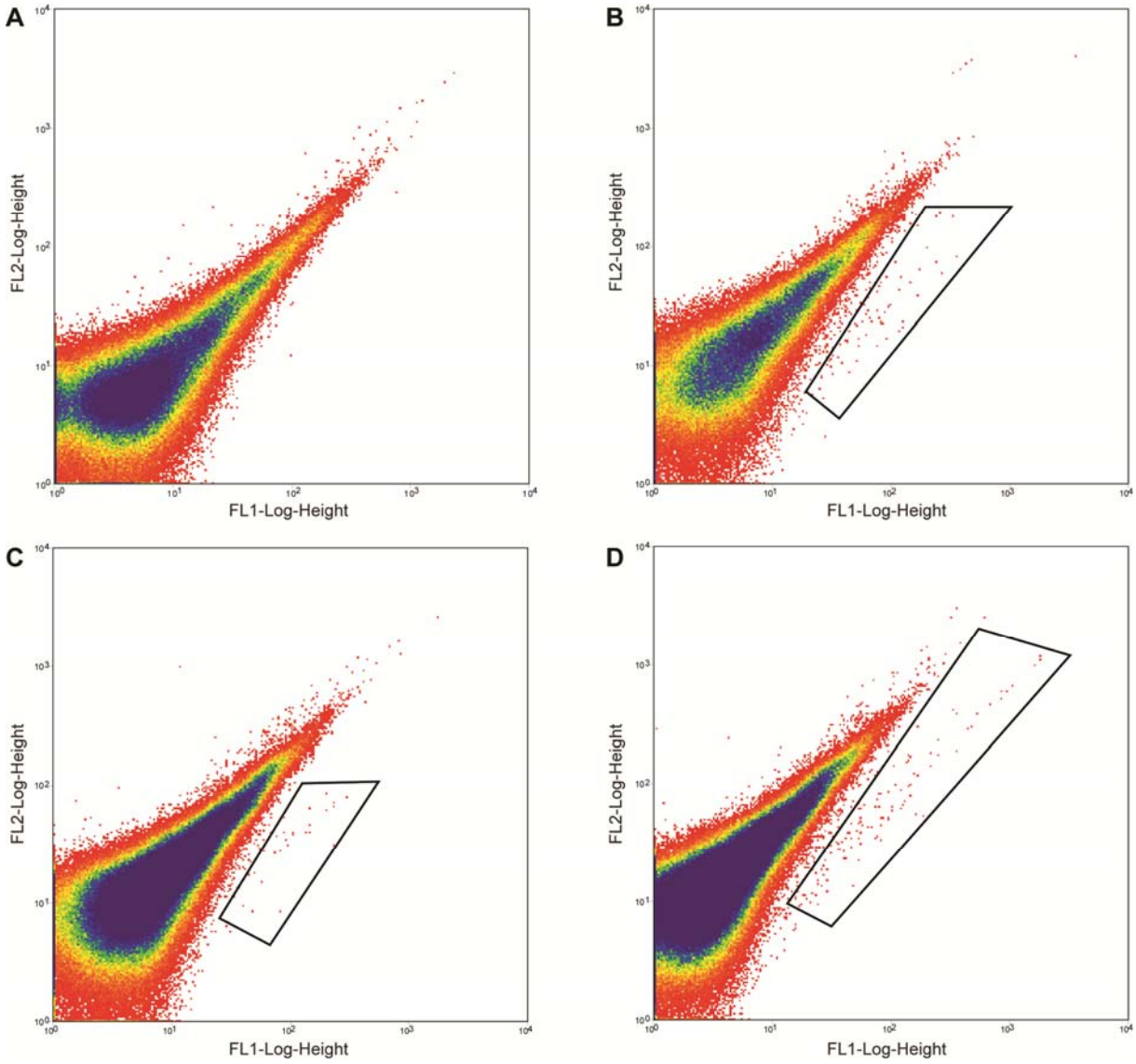

Figure S2. Scatter plots of FANS for GFP-tagged nuclear samples. (A) Mock sample. (B) Suspensor marker line *pAT5G42200:n3xGFP*. (C) Proembryo marker line *pDRN:n2xGFP:DRN 3'UTR*. (D) Whole embryo marker line *pAT3G10010:nGFP*. Fluorescent nuclei were detected by plotting the GFP channel (FL1, log, 513/17, x-axis) against auto-fluorescence (FL2, log, 575/25, y-axis) and drawing a gate around the GFP-positive events.

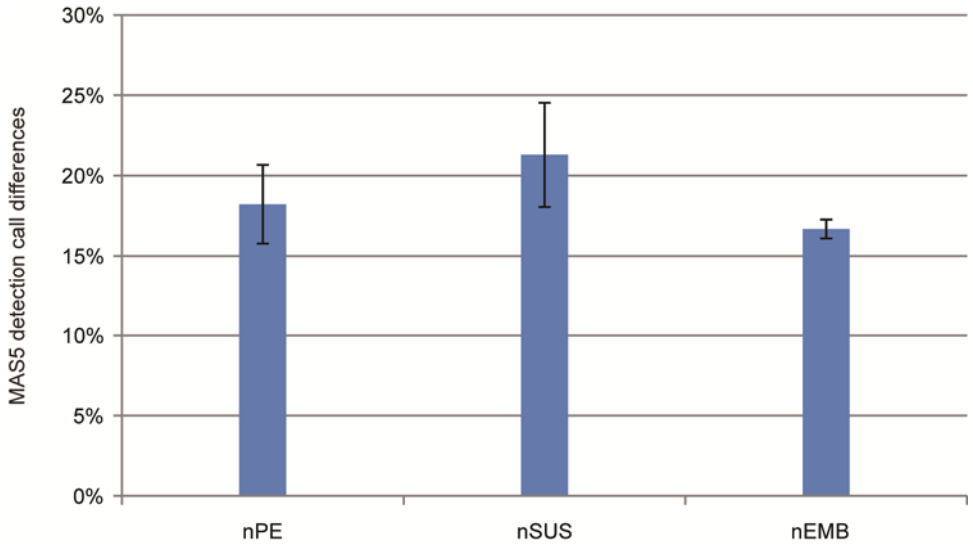

Figure S3. Percentage and standard deviation of MAS5 calls not correlating across three biological replicates. Replicates were compared to each other and the average percentage of calls not matching was calculated.

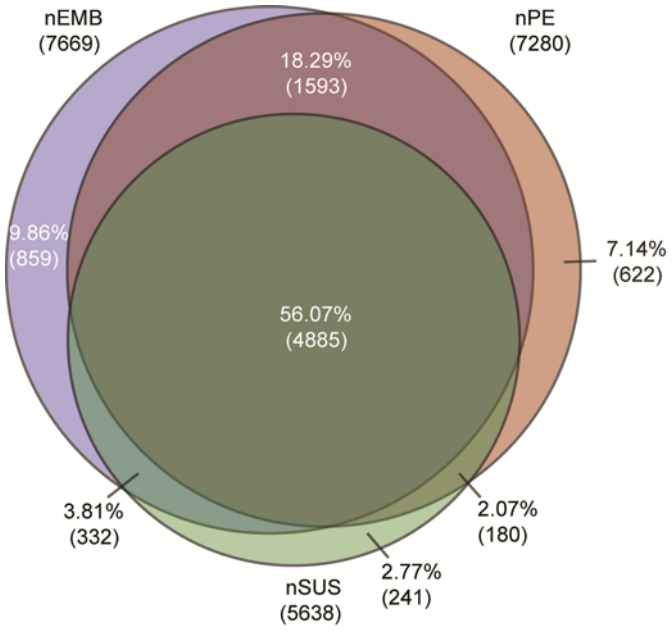

Figure S4. Venn diagram showing overlap of genes expressed in nuclei of the proembryo, suspensor, and whole embryo. For the analysis, only array elements with calls of 3x present (P) across all three biological replicates were used

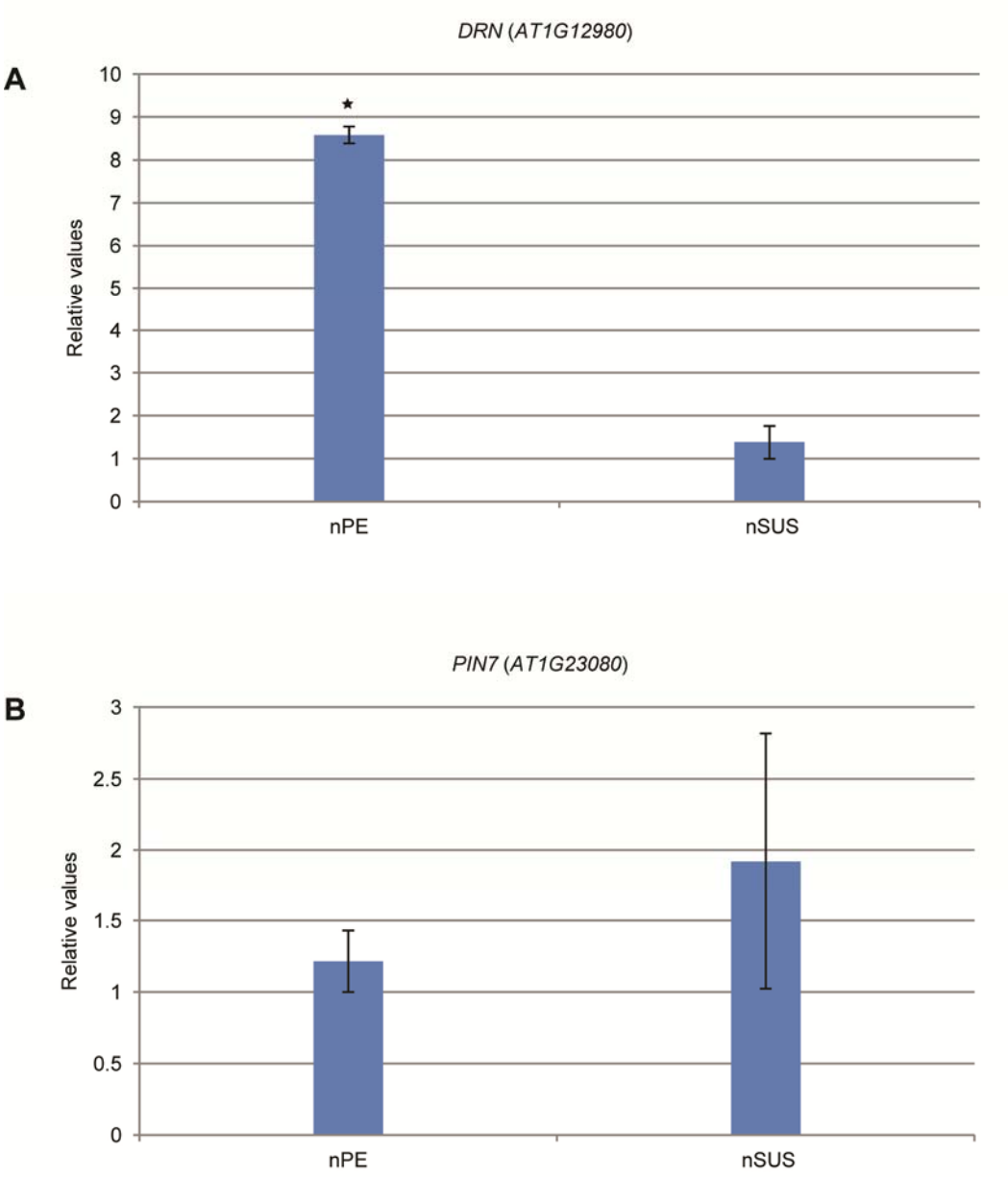

Figure S5. Relative mRNA levels detected by qRT-PCR analysis. (A) *PIN7* relative transcript levels are more abundant in nSUS compared to nPE. (B) *DRN* relative transcript levels are more abundant in nPE compared to nSUS. Average values and standard error are given for two biological replicates for nuclear RNA from both proembryo (nPE) and suspensor (nSUS). ***P*<0.01 (Student's *t*-test).

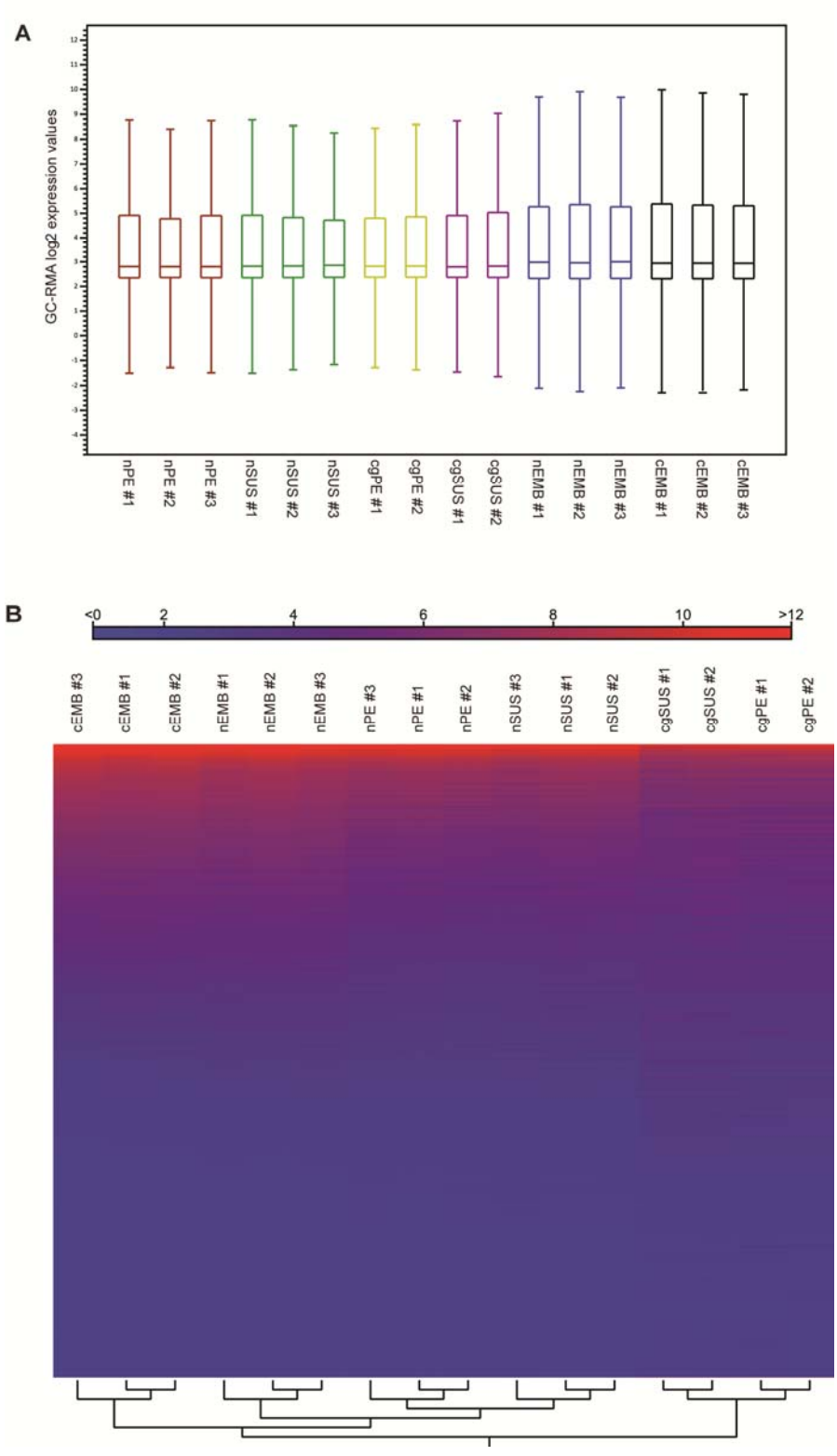

Figure S6. Qualitiy analysis of biological replicates from different nuclear and cellular tissue types. (A) Box-plot analysis. (B) Hierarchical clustering analysis (Pearson correlation, complete linkage).

Proembryo enriched transcripts tested

Locus	Probe set ID	Promoter expression analysis *in vivo*	*In situ*	FC:(cl1/cl2)	average MAS5 nPE	average MAS5 nSUS	embryo-specific
AT5G26270	246888_at	EMB, stronger PE		35.21	5141.74	155.85	*
AT1G77580	259760_at	globular stage PE		7.07	315.51	85.40	*
AT5G05940	250756_at	EMB, at globular/early heart stage stronger PE		6.50	326.50	65.65	*
AT3G17290	258459_at	EMB, at 8/16-cell stage stronger PE	X	4.44	324.18	90.10	*
AT2G35605	266641_at	EMB, stronger PE		4.41	2616.68	850.30	
AT5G61030	247575_at	EMB, stronger PE	X	3.92	1181.90	365.75	
AT1G31400	262555_at	no expression		3.70	464.74	202.79	*
AT1G64220	262336_at	globular stage PE		3.44	555.46	235.96	
AT1G28300	245669_at	not available	X	3.41	448.10	244.37	*
AT5G22650	249901_at	inconsistant expression		3.41	5074.09	2203.15	
AT5G43510	249157_at	PE early heart stage		3.15	362.94	159.05	*
AT3G44750	252625_at	inconsistant expression	X	2.31	1970.12	885.23	
AT3G55660	251778_at	late globular stage hypophysis/lower tier		2.22	584.78	366.01	*

Suspensor enriched transcripts tested

Locus	Probe set ID	Promoter expression analysis *in vivo*	*In situ*	FC:(cl2/cl1)	average MAS5 nPE	average MAS5 nSUS	embryo-specific
AT2G46690	266322_at	no expression	X	8.60	119.51	618.06	
AT3G62480	251212_at	SUS		5.98	100.65	683.83	
AT1G48470	261305_at	no expression		5.67	139.09	603.22	*
AT1G04645	264610_at	not available	X	4.92	2343.15	10066.28	
AT1G54160	263158_at	EMB, stronger SUS		4.39	210.41	588.35	
AT3G52780	252004_at	SUS		4.17	366.63	1419.88	
AT5G46230	248889_at	EMB, stronger SUS	X	3.87	166.79	413.70	
AT5G07440	250580_at	EMB, stronger SUS		3.51	451.98	1162.80	
AT1G74190	260253_at	SUS		3.30	27.33	166.46	*
AT2G32100	265724_at	EMB, stronger SUS		2.84	252.65	613.39	*

Table 1. Overview of differentially expressed candidate genes used for *in vivo* validation of microarray results. For all constructs a short description of the expression patterns in transgenic embryos is given. Gene expression tested by *in situ* hybridizations is indicated with an X. Results of the RankProduct analysis for fold change (FC) are indicated. Additionally, average MAS5 expression values of the three replicates are given for nPE and nSUS samples (decreasing values from red to blue) and the genes overlapping with the embryo-specific analysis results are designated with asterisks. PE = Proembryo, SUS = Suspensor, EMB = Whole embryo, FC = fold change.

Supplementary tables can be downloaded online.

7.2. Early Embryogenesis in Flowering plants: Setting Up the Basic Body Pattern

Lau, S., Slane, D., Herud, O., Kong, J. and Jurgens, G. (2012). Early embryogenesis in flowering plants: setting up the basic body pattern. *Annu Rev Plant Biol* **63**, 483-506.

Early Embryogenesis in Flowering Plants: Setting Up the Basic Body Pattern

Steffen Lau,[1] Daniel Slane,[1] Ole Herud,[1] Jixiang Kong,[1,2] and Gerd Jürgens[1,2]

[1]Department of Cell Biology, Max Planck Institute for Developmental Biology, D-72076 Tübingen, Germany

[2]Center for Plant Molecular Biology, University of Tübingen, D-72076 Tübingen, Germany; email: gerd.juergens@zmbp.uni-tuebingen.de

Annu. Rev. Plant Biol. 2012. 63:483–506

First published online as a Review in Advance on January 3, 2012

The *Annual Review of Plant Biology* is online at plant.annualreviews.org

This article's doi: 10.1146/annurev-arplant-042811-105507

1543-5008/12/0602-0483$20.00

Keywords

zygote, apical-basal axis, root pole initiation, radial pattern, shoot meristem initiation, cotyledon initiation

Abstract

Early embryogenesis is the critical developmental phase during which the basic features of the plant body are established: the apical-basal axis of polarity, different tissue layers, and both the root pole and the shoot pole. Polarization of the zygote correlates with the generation of apical and basal (embryonic and extraembryonic) cell fates. Whereas mechanisms of zygote polarization are still largely unknown, distinct expression domains of WOX family transcription factors as well as directional auxin transport and local auxin response are known to be involved in early apical-basal patterning. Radial patterning of tissue layers appears to be mediated by cell-cell communication involving both peptide signaling and transcription factor movement. Although the initiation of the shoot pole is still unclear, the apical organization of the embryo depends on both the proper establishment of transcription factor expression domains and, for cotyledon initiation, upward auxin flow in the protoderm. Here we focus on the essential patterning processes, drawing mainly on data from *Arabidopsis thaliana* and also including relevant data from other species if available.

Contents

INTRODUCTION

Zygote: fertilization product of egg and sperm cell

The basic body pattern of a multicellular organism is established from the zygote—the fertilized egg cell—during embryogenesis. In flowering plants, embryogenesis lays down the basis for a stereotyped seedling displaying a simple body organization of two superimposed patterns. Along the main apical-basal axis of polarity, the apically located shoot meristem, which is usually flanked by one or two cotyledons, is linked with the basally located root meristem via the hypocotyl and seedling root. The perpendicular radial pattern comprises a series of concentrically arranged tissue layers, from the outermost epidermal tissue via the ground tissue to the centrally located vascular tissue. Although the body organization of the seedling looks similar in different flowering plant species, its developmental origin can vary between species. For example, members of the Brassicaceae family (such as *Arabidopsis thaliana*) display distinct, nearly stereotypic cell-division patterns in early embryogenesis, whereas embryos of other flowering plant species grow by seemingly random cell divisions (62, 63, 66, 94). In the former group of species, the origin of seedling tissues and organs can thus be easily traced back to specific cells or groups of cells in the early embryo (**Figure 1**). Although this correlation might suggest a causal link between the spatial regulation of cell divisions and pattern formation in the early embryo, *A. thaliana* mutants such as *fass* (*fs*) displaying altered cell-division planes nonetheless generate a normal body organization, whereas morphogenesis is compromised (147). Thus, the stereotypic cell-division pattern seen in *A. thaliana* embryos expresses, but is not instrumental to, developmental decisions and might facilitate such decisions in the early embryo comprising very few cells.

This review covers recent studies that address molecular mechanisms underlying the origin of the apical-basal axis of polarity, the initiation of both the root meristem and the shoot meristem as well as the cotyledons, and radial patterning. It also discusses the parental contributions to gene activity in early embryogenesis in regard to their potential role in early patterning events. For ease of reference, **Table 1** lists the gene abbreviations and full names referred to in this review.

ZYGOTE POLARITY AND ELONGATION

Zygote Polarity

In flowering plants, the zygote is formed by the fusion of the egg cell with one of the two sperm cells delivered by the pollen tube (reviewed in 25). Like the egg cell, the zygote is usually polarized with respect to the relative position of nucleus and vacuole. However, egg cell polarity and zygote polarity are different in some species, suggesting that the latter might be established independently of the former.

In many species, the egg cell has its nucleus located toward the chalazal end of the ovule (i.e., apically) and usually has a large vacuole located toward the micropylar end (i.e., basally). This is, for example, the case in *A. thaliana*, *Capsella bursa-pastoris*, and *Nicotiana tabacum* (tobacco), in all of which zygote organization resembles egg cell organization (94, 95, 103, 131, 170); polarity—as inferred from nucleus and vacuole position—appears thus to be maintained after fertilization. However, this was shown not to be the case in *A. thaliana* and probably *N. tabacum*. A transient symmetric stage, in which the nucleus is located centrally and smaller vacuoles are distributed rather evenly within the cell, developmentally separates the polarized egg cell from the similarly polarized zygote (29, 103, 151, 170). In *A. thaliana*, the transcription factor WRKY DNA-BINDING PROTEIN 2 (WRKY2) is involved in the polarization of the zygote by transcriptionally activating *WUSCHEL RELATED HOMEOBOX 8* (*WOX8*) and possibly *WOX9* (151). *WRKY2* is dispensable for the establishment or maintenance of egg cell polarity, which corroborates the notion that egg cell and zygote polarity are not intimately linked (151). Even stronger effects of fertilization on zygote polarity are, for example, observed in *Oryza sativa* (rice), *Zea mays* (maize), and *Papaver nudicaule*, in all of which egg cell polarity is reversed after fertilization. Whereas the nucleus localizes to the micropylar/basal end of the egg cell and the large vacuole to the chalazal/apical end, the opposite is the case in the zygote (25, 114, 123).

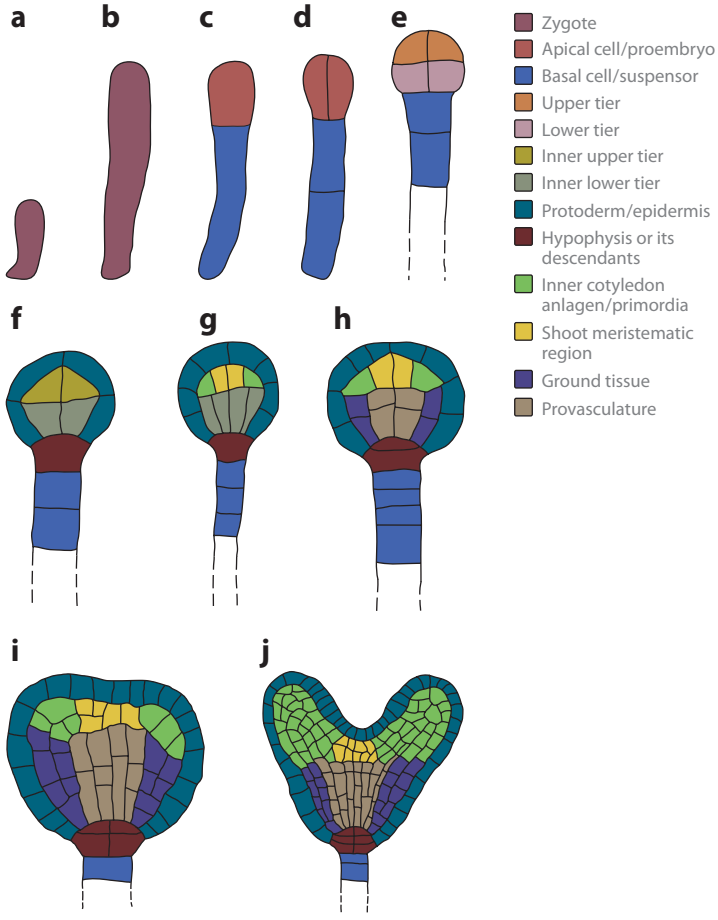

Figure 1

Early embryogenesis in *Arabidopsis thaliana*. Panels show longitudinal sections of embryos during consecutive developmental stages: (*a*) zygote, (*b*) elongated zygote, (*c*) one-cell stage, (*d*) two- or four-cell stage, (*e*) octant stage, (*f*) dermatogen stage, (*g*) early-globular stage, (*h*) mid-globular stage, (*i*) transition stage, and (*j*) heart stage. Groups of developmentally related cells are color-coded. Embryos not drawn to scale.

Zygote Elongation

The *A. thaliana* zygote not only becomes polarized but also elongates approximately threefold along its apical-basal axis before it divides. This elongation depends on the GDP/GTP exchange factor for small G proteins of the ARF class (ARF-GEF) GNOM (GN). If *GN* is knocked out, elongation and asymmetric division are compromised, but GN targets in the zygote are not known (98, 132). Zygote elongation or its asymmetric division also depends

Shoot meristem: group of self-replenishing cells at the shoot apex that sustain shoot growth and the formation of lateral organs such as leaves and flowers

Table 1 Gene abbreviations and full names used in this review

Abbreviation	Full name
ACR4	*ARABIDOPSIS CRINKLY 4*
AGO1	*ARGONAUTE 1*
ALE1/2	*ABNORMAL LEAF-SHAPE 1/2*
ARR7/15	*ARABIDOPSIS RESPONSE REGULATOR 7/15*
AS1/2	*ASYMMETRIC LEAVES 1/2*
ATDEK1	*ARABIDOPSIS THALIANA DEFECTIVE KERNEL 1*
ATH1	*ARABIDOPSIS THALIANA HOMEOBOX 1*
ATHB8/15	*ARABIDOPSIS THALIANA HOMEOBOX 8/15*
ATML1	*ARABIDOPSIS THALIANA MERISTEM LAYER 1*
BBM/PLT4	*BABY BOOM/PLETHORA 4*
BDL/IAA12	*BODENLOS/INDOLE-3-ACETIC-ACID 12*
BIM1	*BES INTERACTING MYC-LIKE PROTEIN 1*
BOP1/2	*BLADE-ON-PETIOLE 1/2*
CLE40	*CLV3/ESR-RELATED 40*
CLV3	*CLAVATA 3*
CUC1/2/3	*CUP-SHAPED COTYLEDON 1/2/3*
CUP	*CUPULIFORMIS*
DCL1	*DICER-LIKE 1*
DRN	*DORNRÖSCHEN*
DRNL	*DORNRÖSCHEN-LIKE*
ENP/MAB4	*ENHANCER OF PINOID/MACCHI-BOU 4*
FDH	*FIDDLEHEAD*
FS	*FASS*
GN	*GNOM*
GRN/RKD4	*GROUNDED/RWP-RK DOMAIN 4*
HAN	*HANABA TARANU*
KAN1	*KANADI 1*
KN1	*KNOTTED 1*
KNAT1/BP	*KNOTTED-LIKE FROM ARABIDOPSIS THALIANA 1/BREVIPEDICELLUS*
LOG	*LONELY GUY*
LTP1	*LIPID TRANSFER PROTEIN 1*
MKK4/5	*MITOGEN-ACTIVATED PROTEIN KINASE KINASE 4/5*
MP/ARF5	*MONOPTEROS/AUXIN RESPONSE FACTOR 5*
MPK3/6	*MITOGEN-ACTIVATED PROTEIN KINASE 3/6*
NAM	*NO APICAL MERISTEM*
NPH4/ARF7	*NONPHOTOTROPIC HYPOCOTYL 4/AUXIN RESPONSE FACTOR 7*
OSH1	*Oryza sativa homeobox 1*
OSTF1	*Oryza sativa transcription factor 1*
PDF1/2	*PROTODERMAL FACTOR 1/2*
PHB	*PHABULOSA*
PHV	*PHAVOLUTA*

(*Continued*)

Table 1 (*Continued*)

Abbreviation	Full name
PID	*PINOID*
PID2	*PINOID 2*
PIN1/3/4/7	*PIN-FORMED 1/3/4/7*
PLT1/2/3	*PLETHORA 1/2/3*
PNF	*POUND-FOOLISH*
PNY	*PENNYWISE*
QHB	*quiescent-center-specific homeobox*
REV	*REVOLUTA*
RPK1	*RECEPTOR-LIKE PROTEIN KINASE 1*
SCR	*SCARECROW*
SHR	*SHORT-ROOT*
SSP	*SHORT SUSPENSOR*
STM	*SHOOT MERISTEMLESS*
TAA1	*TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS 1*
TAR1/2	*TRYPTOPHAN AMINOTRANSFERASE RELATED 1/2*
TMO7	*TARGET OF MONOPTEROS 7*
TOAD2	*TOADSTOOL 2*
TPL	*TOPLESS*
WAG1/2	*WAG 1/2*
WOX1/2/3/5/8/9	*WUSCHEL RELATED HOMEOBOX 1/2/3/5/8/9*
WRKY2/33	*WRKY DNA-BINDING PROTEIN 2/33*
WUS	*WUSCHEL*
YDA	*YODA*
YUC1/4/10/11	*YUCCA 1/4/10/11*
ZLL/AGO10	*ZWILLE/ARGONAUTE 10*
ZMCUC3	*Zea mays CUP-SHAPED COTYLEDON 3*
ZMNAM1/2	*Zea mays NO APICAL MERISTEM 1/2*

on the interleukin-1 receptor-associated kinase (IRAK)/Pelle-like kinase SHORT SUSPENSOR (SSP), the MAPKK kinase YODA (YDA), MITOGEN-ACTIVATED PROTEIN KINASE 3 (MPK3), MPK6, and the RWP-RK family protein GROUNDED (GRN)/RWP-RK domain 4 (RKD4), which functions as a transcriptional regulator (7, 58, 89, 154, 155). There is evidence that *SSP*, *YDA*, *MPK3*, and *MPK6* as well as MITOGEN-ACTIVATED PROTEIN KINASE KINASE 4 (MKK4) and MKK5 act in the same pathway (7, 155), but the direct targets of this hypothetical kinase pathway in the zygote remain unknown. However, it might be meaningful that a close homolog of WRKY2, WRKY33, is phosphorylated by MPK3 and MPK6 (96, 162).

ZYGOTIC GENOME ACTIVATION

Zygotic genome activation already occurs in the zygote in flowering plants. For *N. tabacum*, evidence has been presented that deposited maternal transcripts are not sufficient for zygote elongation and division, but that this process requires zygotic de novo transcription (170). In *Z. mays* and *N. tabacum*, transcripts not present in egg and sperm cells accumulate in the zygote, which indicates that these transcripts

Cotyledon: leaf formed in the developing embryo

Root meristem: group of self-replenishing cells at the root tip that sustain root growth

Ground tissue: primordium that will give rise to two tissue layers, endodermis and cortex

are made de novo in the zygote (110, 125, 170). Comparable experiments have not been done in *A. thaliana*. However, in both *A. thaliana* and *Z. mays*, genes whose expression has not been detected in pollen are expressed in the zygote from the paternal allele (130, 151), implying zygotic genome activation at the zygote stage in these species.

This de novo expression of paternal genes in the zygote also indicates that the paternal genome is not generally silenced in the zygote or early embryo. This idea has received support from other studies (120, 156, 165), although in these cases it cannot be clearly distinguished between transcripts delivered by the pollen and de novo transcription from the paternal alleles in the zygote. However, whereas *Z. mays* displays an equivalent parental contribution in the zygote and during early embryo development (101), in *A. thaliana* maternal transcripts appear to predominate during early embryogenesis (5). This maternal predominance is thought to result from the downregulation of the paternal alleles by the maternal chromatin small interfering RNA (siRNA) pathway, whereas the activation of the paternal alleles during the course of embryogenesis is thought to be mediated by maternal histone chaperone complex CAF1 (5). However, it cannot be excluded that the maternal predominance during early *A. thaliana* embryogenesis is mainly or also due to transcript carryover from the egg cell rather than specific downregulation of the paternal alleles. Hence, the two aforementioned mechanisms (the chromatin siRNA pathway and activity of the CAF1 complex) could generally be involved in zygotic genome activation. In conjunction with a supposed stronger transcript contribution of the egg cell as compared with the sperm cell, mechanisms delaying the zygotic genome activation would prolong the predominance of transcripts derived from the maternal alleles.

Some observations argue against general differences between paternal and maternal alleles in *A. thaliana*. For example, both paternal and maternal histone H3 variants are replaced by de novo synthesized H3 variants in the zygote (50, 51). And although imprinting is quite common in the angiosperm endosperm, only a few genes imprinted in the embryo have been reported so far (56, 90, 118). The maternal-to-zygotic transition thus appears to already commence in the zygote. In contrast to animals, however, because there is pronounced postmeiotic gene expression in both female and male gametophytes followed by postfertilization gene expression, the maternal-to-zygotic transition might more appropriately be called the gametophytic-to-sporophytic transition. This transition might be completed sooner or later, presumably depending mainly on species-specific velocities of development during early embryogenesis. In this view, the longer it takes for the zygote and its progeny to divide, the earlier in developmental time the gametophytic-to-sporophytic transition might occur.

ZYGOTE DIVISION AND SEPARATION OF APICAL AND BASAL CELL FATE

In the vast majority of flowering plant species, the zygote divides transversely, generating an apical daughter cell and a basal daughter cell, whereas in some species oblique or longitudinal divisions occur (62, 133). When the zygote divides transversely, the two daughter cells may be quite different in size, depending on the position of the plane of cell division. In *Ricinus communis* and *Triticum aestivum* (wheat), for example, the zygote divides "symmetrically," generating two daughter cells of equal size (74, 133). In other species, zygotes divide asymmetrically. Whereas in *Coriaria nepalensis* and *Anethum graveolens*, for example, the apical daughter cell is larger than the basal one, in *A. thaliana* the apical daughter cell of the zygote is smaller than the basal one (94, 133). There seems to be no general rule regarding the size ratio of the apical daughter cell and the basal daughter cell of angiosperm zygotes (133).

Nonetheless, the division of the zygote might still—directly or indirectly—separate apical and basal cell fate and hence might also consolidate or establish the apical-basal axis of

polarity, which is then maintained throughout plant life. Some evidence supports this view. In both *Z. mays* and *N. tabacum*, the apical daughter cell of the zygote exhibits a transcriptional profile distinct from the basal counterpart (48, 113). In *A. thaliana*, two developmental pathways, in addition to the YDA pathway mentioned above, have been linked to apical-basal axis establishment after zygote division: One involves the transcription factors WOX8, WOX9, and WOX2, whereas the other is auxin dependent, involving the auxin efflux regulator PIN-FORMED 7 (PIN7) as well as the transcriptional regulators MONOPTEROS (MP)/AUXIN RESPONSE FACTOR 5 (ARF5) and BODENLOS (BDL)/INDOLE-3-ACETIC-ACID 12 (IAA12) (see below) (**Figure 2**).

Besides *WOX8*, whose expression in the zygote is induced by WRKY2, *WOX2* is also expressed in the zygote (40, 151). After zygote division, though, these two genes are not coexpressed anymore; *WOX2* is expressed in the apical daughter cell of the zygote, and *WOX8* (together with *WOX9*) is expressed in the basal (40). *WOX9*, which is assumed to be

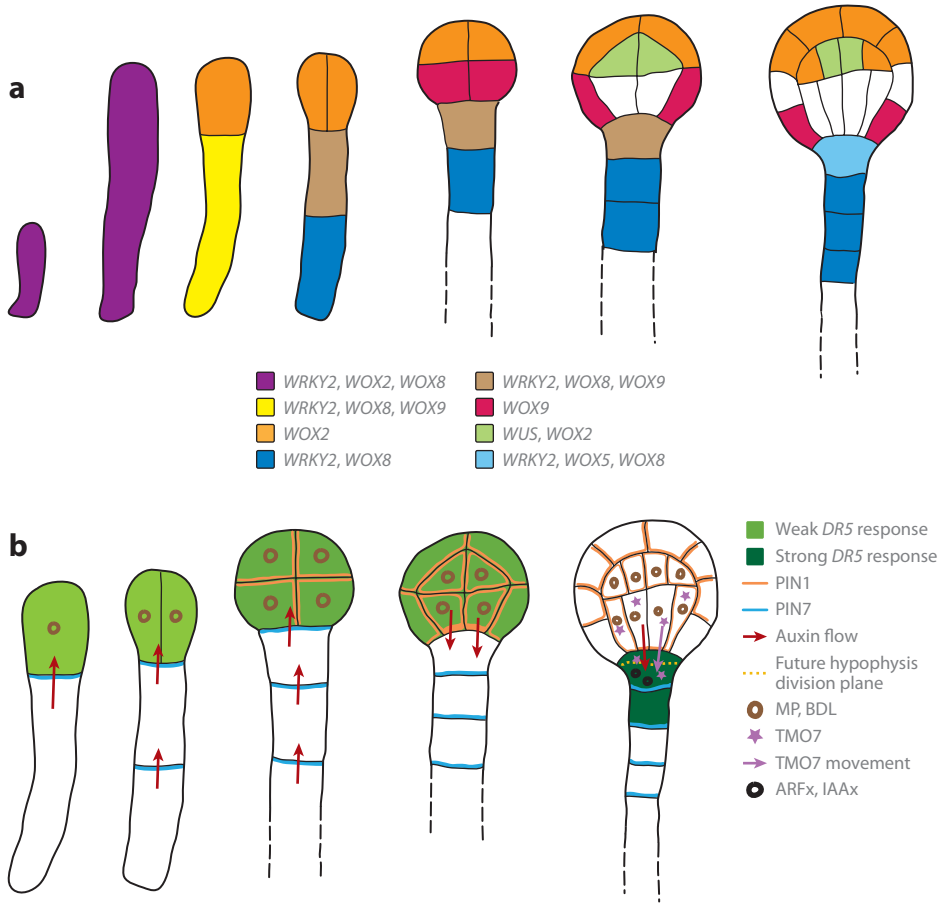

Figure 2

Apical-basal patterning and hypophysis specification in early embryogenesis of *Arabidopsis thaliana*. (*a*) Expression patterns of *WRKY2* and early-expressed *WOX* genes. (*b*) Auxin signaling and hypophysis specification. Embryos not drawn to scale.

Embryo proper: cells forming the embryo

Suspensor: extraembryonic, often filamentous structure anchoring the embryo proper to the ovule wall

Hypophysis: in *Arabidopsis thaliana*, a cell basally adjacent to the embryo proper and involved in root pole formation

a target of WRKY2 as well, might already be expressed in the zygote and possibly also in the apical daughter cell of the zygote (40, 151, 163). WOX8 and WOX9 are supposed to signal from the basal to the apical daughter cell for proper *WOX2* expression to occur (10). However, because there are stronger defects in *wox8 wox9* or *wox9* alone than there are in *wox2* mutant embryos, WOX8 and WOX9 appear to have additional, WOX2-independent functions in early development (10, 40, 163). *WRKY2* is coexpressed with *WOX8* and partially with *WOX9* during the earliest stages of embryogenesis (40, 151) (**Figure 2*a***), which could account for the early expression of these two *WOX* genes in the basal lineage. The problem of the separation of apical and basal cell fate, however, would not be solved with this extension of the WOX pathway; instead, the problem would be shifted from understanding *WOX2*, *WOX8*, and *WOX9* transcript distribution to understanding *WRKY2* transcript distribution.

The auxin-dependent pathway implicated in apical-basal axis establishment during *A. thaliana* embryogenesis becomes relevant immediately after zygote division, when auxin is transported from the basal to the apical daughter cell via PIN7 (30) (**Figure 2*b***). The auxin response in the apical descendant of the zygote triggered by this directional auxin transport might be important for its proper specification, as evidenced by its transverse instead of longitudinal division in *bdl*, *mp*, *mp bdl*, and *pin7* mutant embryos (30, 42). *MP* encodes an ARF, *BDL* encodes an AUXIN (AUX)/IAA inhibitor, and both are expressed in the apical cell lineage (41, 43); *MP* and *BDL* form a system of two interconnected feedback loops that can be modulated by auxin via the degradation of BDL protein (76). The initial transport of auxin to the apical cell(s) might thus be sufficient to establish expression of these two important developmental regulators. But, comparable to the WOX/WRKY case, the next step on the hierarchy ladder has to be taken now, and how PIN7-mediated basal-to-apical auxin transport is set up must be determined.

HYPOPHYSIS SPECIFICATION AND ROOT POLE FORMATION

Importance of Auxin in Hypophysis Specification and Root Pole Formation

The root pole is the basal end of the angiosperm embryo. In *A. thaliana*, the specification of the founder cell of the root meristem is not the result of a (spatially) isolated developmental program, but the consequence of developmental events that take place in the apically adjoining cells (157).

One of these events is the overall reversal of the above-mentioned basal-to-apical auxin flow from the dermatogen stage onward. The PIN1 auxin efflux regulator formerly nonpolarly distributed in the cells of the embryo proper starts to become localized predominantly to the basal side of the lower inner cells, and the formerly apically localized PIN7 becomes localized to the basal side of the suspensor cells. In consequence, auxin accumulates in the hypophysis and the subhypophyseal cell as indicated by the auxin response reporter *DR5* (30) (**Figure 2*b***).

This accumulation of auxin in the hypophysis appears to be crucial for its specification and subsequent root pole formation, as suggested by the fact that impairment of auxin biosynthesis and transport as well as auxin signaling interfere with these processes. The auxin-biosynthesis multiple mutants *yucca 1* (*yuc1*) *yuc4 yuc10 yuc11* and *tryptophan aminotransferase of arabidopsis 1* (*taa1*) *tryptophan aminotransferase related 1* (*tar1*) *tar2* as well as the auxin transport quadruple mutant *pin1 pin3 pin4 pin7* are rootless, just like seedlings in which the phosphorylation status-dependent polar PIN1 localization is reversed from the basal to the apical side in the inner cells of the embryo proper by the misexpression of the PIN1-phosphorylating serine/threonine kinase PINOID (PID) (19, 30, 31, 102, 139). Moreover, the regulation of *PIN1* expression involves MP and its inhibitor BDL (157). This might explain why the knockout of *MP*, or mutations causing the stabilization of BDL, lead to the non- or misspecification of the hypophysis and subsequent failure to form

a root (157). Thus, MP-BDL-dependent auxin signaling in the cells of the embryo proper indirectly ensures the accumulation of auxin in the hypophysis, where signaling through another ARF-AUX/IAA pair presumably mediates the actual specification process (157) (**Figure 2*b***). Recently, detailed expression analysis revealed several *ARF* candidates expressed in the hypophysis (117).

Additional Factors Involved in Hypophysis Specification and Root Pole Formation

In addition to auxin, other molecules likewise serve as mobile signaling cues for hypophysis specification. TARGET OF MONOPTEROS 7 (TMO7), a small transcriptional regulator whose expression is regulated by MP and BDL, also moves from the provascular cells into the hypophysis and contributes to its specification (128) (**Figure 2*b***). SHORTROOT (SHR) might also move there, as inferred from the expression of *SCARECROW* (*SCR*) in the hypophysis (106, 164). Although SCR does not appear to be necessary for hypophysis specification itself—as indicated by the apparently normal hypophysis division in the *scr* mutant—SCR is subsequently required for proper root pole formation (164). Similar considerations apply to the *PLETHORA* (*PLT*) genes *PLT1*, *PLT2*, *PLT3*, and *BABY BOOM* (*BBM*)/*PLT4* and to *WOX5*. The expression of some of them depends on *MP* and its close homolog *NONPHOTOTROPIC HYPOCOTYL 4* (*NPH4*)/*ARF7* or is initiated in the hypophysis in an *MP-BDL*-dependent fashion, but at least *WOX5* is mainly required for root organization of later developmental stages and root stem cell maintenance (3, 34, 40, 122).

Although auxin signaling is of central importance for root pole initiation, it is not the only plant hormone signaling pathway involved. The brassinosteroid signaling component *BES INTERACTING MYC-LIKE PROTEIN 1* (*BIM1*) and the AP2 transcription factors DORNRÖSCHEN (DRN) and DORNRÖSCHEN-LIKE (DRNL), which interact with BIM1, are required for proper hypophysis division and root formation, suggesting that auxin-brassinosteroid crosstalk is involved in root pole initiation (16, 17, 169). In addition, the requirement of two feedback repressors of cytokinin signaling, *ARABIDOPSIS RESPONSE REGULATOR 7* (*ARR7*) and *ARR15*, for the same process indicates the necessity to dampen cytokinin signaling (105). This dampening happens specifically in the lower derivative of the hypophysis via *ARR7* and *ARR15*, whose expression depends on auxin (105) and hence possibly also indirectly on MP-BDL-dependent signaling.

Provasculature: cells that will give rise to the vasculature (the conductive tissue)

Positional Information During Root Initiation

The fate of the hypophysis thus appears to be determined by its position at the basal end of the early embryo rather than its descent from the basal daughter cell of the zygote. Indeed, the clonal origin of the hypophysis might not be relevant for root pole initiation. In the *hanaba taranu* (*han*) mutant, expression domains of genes are shifted apically so that genes normally expressed only in the suspensor replace "apical" genes in the lower half of the embryo proper. As a consequence, it is not the histologically still-discernable hypophysis that becomes the founder cell of the future root pole, but rather cell(s) from the lower-tier descendants (108). As in the wild type, the cell(s) to be recruited for root pole formation appear to be those closest to cells with an apical cell fate.

In an even more extraordinary case of atypical embryonic root initiation, which occurs in the *topless-1* (*tpl-1*) mutant, a root is initiated not only basally but also apically and, interestingly, like in *han*, in an MP-independent fashion (87, 108). TPL, a cosuppressor that binds to BDL and probably other AUX/IAAs as well as indirectly to jasmonate ZIM-domain (JAZ) repressor proteins and directly to WUSCHEL (WUS), might recruit histone deacetylases to repress gene expression (70, 86, 115, 141; reviewed in 73). The *tpl-1* mutation is a dominant negative mutation relieving the repression

Protoderm: outermost cell layer of the embryo proper that differentiates into the epidermis

of TPL targets; especially derepression of the TPL targets *PLT1* and *PLT2* leads to the formation of a secondary root pole (135).

Many angiosperm species—including various monocots and, e.g., *Pisum sativum* (pea)—do not exhibit a cell that clearly corresponds to the *A. thaliana* hypophysis, i.e., a single uppermost derivative of the basal daughter cell of the zygote that invariably divides into a smaller upper lens-shaped and a larger lower cell to give rise to the quiescent center and the columella of the root meristem, respectively (reviewed in 59). Nevertheless, these species of course also form a root, and they may do so by employing signaling pathways similar to those in *A. thaliana*, which specify the hypophysis in a position-dependent manner. In *O. sativa*, the *WUS*-type homeobox gene *quiescent-center-specific homeobox* (*QHB*) is—similar to *WOX5* in *A. thaliana*—expressed in a few cells at the basal pole of the embryo; in *Z. mays* and *O. sativa*, an *SCR* homolog might play a role in root patterning (40, 67, 68, 82, 83). The developmental significance of the singular hypophysis in *A. thaliana* might thus mainly relate to the minimal number of cells that constitute the embryo at the very early stage when the root pole is initiated.

RADIAL PATTERNING AND PROTODERM SPECIFICATION

Separation of Inner and Outer Fate in the Early Proembryo

In *A. thaliana*, the beginning of radial patterning is marked by the tangential divisions of the cells of the embryo proper in the octant-stage embryo. The eight outer cells thus formed are the founder cells of the protoderm, and during embryogenesis the eight inner cells will give rise to, e.g., the provasculature and the ground tissue (66, 94, 126) (**Figure 1**). Like apical-basal axis establishment, these tangential divisions have been linked to the action of *WOX* genes and *MP*. In *wox2* and, with a higher penetrance, in *wox2 mp*, *wox2 wox8*, and *wox1 wox2 wox3*, some cells of the octant-stage embryo proper do not divide tangentially, so that a "continuous" protodermal layer is not formed (10, 40). How *WOX* genes and MP-dependent auxin signaling mediate the proper orientation of these cell-division planes is not known.

An early difference between protodermal and inner cells is the divergence of transcriptional activities. The GLABRA 2 (GL2) family homeodomain transcription factors *ARABIDOPSIS THALIANA MERISTEM LAYER 1* (*ATML1*) and *PROTODERMAL FACTOR 2* (*PDF2*) are initially expressed throughout the early embryo proper, but immediately after the tangential divisions have occurred their expression becomes confined to the protodermal cells (1, 88) (**Figure 3*a*,*b***). Conversely, the expression of *ZWILLE* [*ZLL*, also called *ARGONAUTE 10* (*AGO10*)], which is expressed in the apical cells from the four-cell stage on and is involved in shoot meristem maintenance, becomes confined to the inner cells (91, 104) (**Figure 3*a*,*b***). Remarkably, in *Z. mays* and *O. sativa*, where the cell-division planes after the zygotic division appear randomly oriented, the expression of *ATML1* homologs also becomes confined to the protoderm, and these homologs might serve a similar function during protoderm development as their *A. thaliana* counterparts (52–54, 167).

In *atml1 pdf2* double-mutant seedlings, cotyledons seem devoid of an epidermis and the shoot apex lacks distinct cell layers (1). The *ATML1* promoter and the *PDF2* promoter each contain a potential binding site for WUS, the founding member of the WOX family (1, 40, 143), and thus the expression of *ATML1* and *PDF2* could be directly regulated by WOX transcription factors, including those involved in the tangential divisions of the octant-stage embryo (**Figures 2*a*** and **3*c***). Furthermore, both the *ATML1* promoter and the *PDF2* promoter contain an eight-nucleotide sequence termed the L1 box, which is also present in the promoters of other epidermally expressed genes such as *PDF1*, *FIDDLEHEAD* (*FDH*), *LIPID TRANSFER PROTEIN 1* (*LTP1*), and—almost perfectly matching—the *O. sativa* *ATML1* homolog *Oryza sativa transcription*

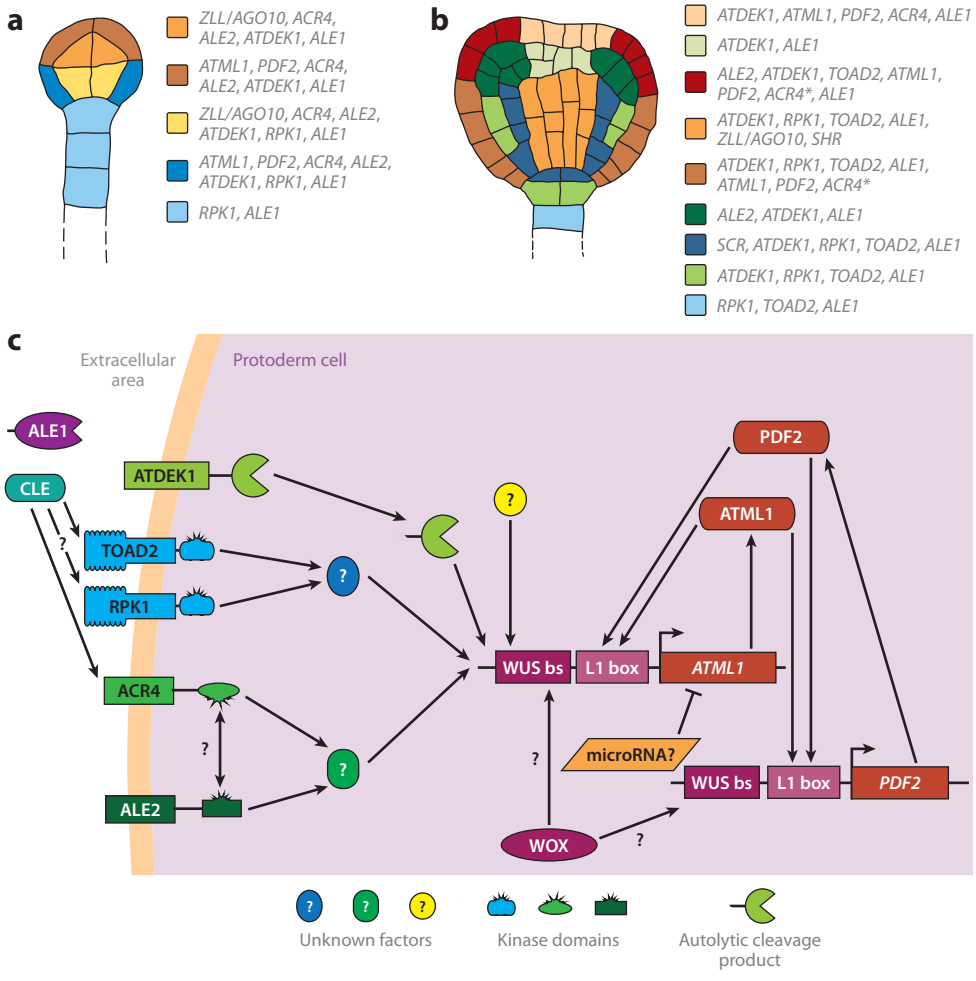

Figure 3

Radial patterning in early embryogenesis of *Arabidopsis thaliana*. (*a*,*b*) Expression patterns of genes important for radial patterning. Panel *a* shows the dermatogen stage; panel *b* shows the transition stage. Asterisk indicates that weak *ACR4* expression was detected ubiquitously in embryos. (*c*) Signaling pathways maintaining protoderm identity. Abbreviation: WUS bs, WUS binding site. Embryos not drawn to scale.

factor 1 (*OSTF1)* (1, 2, 167). Because ATML1 and PDF2 bind to the L1 box in vitro, it is conceivable that these two transcription factors establish a positive feedback loop that regulates the transcription of genes expressed in the epidermis (1, 2) (**Figure 3*c***). In the case of *ATML1*, however, the L1 box and the WUS binding site do not appear to be the only important regulatory regions. Although the L1 box is essential for the expression of *PDF1*, this is not the case for *ATML1* (2, 143). Rather, the L1 box controls expression redundantly with the WUS binding site in the *ATML1* promoter, but even when both elements are deleted, a hexameric copy of an *ATML1* promoter fragment still confers weak expression (143). In addition, *ATML1* is still expressed in the *atml1 pdf2* and *wox8 wox9* double-mutant backgrounds (10, 143). Thus, although these two "pathways" might converge on *ATML1* expression, other factors are probably involved in the regulation of this gene. Because the *ATML1* promoter

confers expression in the suspensor but the messenger RNA (mRNA) is detected there only in the *dicer-like 1* (*dcl1*) mutant, a microRNA might regulate the *ATML1* expression in the suspensor (111, 143) (**Figure 3*c***).

The inner cells of the *A. thaliana* embryo give rise to the various concentric tissue layers that have been described in the root and are laid down during embryogenesis (126, 127). The GRAS transcription factor SHR is one of the best-described players involved in radial patterning. It is expressed in the provasculature and moves out to the neighboring cell layer, where it activates the transcription of another GRAS transcription factor gene, *SCR* (46, 106). *SCR* is expressed in the ground tissue and the hypophysis at the globular stage of embryogenesis. When the cells of the ground tissue of the hypocotyl and the embryonic root pole divide periclinally between the triangular stage and the heart stage to generate the inner layer of endodermis and the outer layer of cortex cells, *SCR* continues to be expressed in the inner layer (164) (**Figure 3*b***). These periclinal cell divisions depend on both *SHR* and *SCR* (46, 164). SHR and SCR activate microRNA165/166 in the endodermis of the mature root, from where the microRNAs feed back onto the vasculature to control its patterning. Because the two microRNAs are already expressed during embryogenesis, they might contribute to embryonic patterning as well (14).

Maintenance of Radial Patterning

RECEPTOR-LIKE PROTEIN KINASE 1 (RPK1) and TOADSTOOL 2 (TOAD2), two closely related leucine-rich-repeat receptor-like kinases (LRR-RLKs), are redundantly required for the maintenance of radial patterning (112) (**Figure 3*c***). The protoderm marker *ATML1* as well as the central domain markers *ZLL/AGO10* and *SHR* are correctly expressed only initially in *rpk1 toad2* embryos, which have enlarged protoderm cells (112). At the late-globular stage of embryogenesis, the expression of *ATML1* is (almost) lost, and the expression of *ZLL/AGO10* and *SHR* extends over the entire basal domain in *rpk1 toad2*, suggesting that *RPK1* and *TOAD2* play an essential role in the maintenance but not the establishment of the radial pattern in *A. thaliana* (112).

The ligands binding to RPK1 and TOAD2 during embryogenesis are unknown, although it was recently suggested that the signaling peptide derived from CLAVATA 3 (CLV3) binds to TOAD2 (71). Because this signaling peptide is functionally similar to other signaling peptides of the CLV3/ESR-RELATED (CLE) family (109), any of these might be the endogenous ligand for RPK1 and TOAD2 (**Figure 3*c***). Hence, at least some of these signaling peptides might play a role during early embryogenesis, an assumption that receives support from the analysis of the RLK ARABIDOPSIS CRINKLY 4 (ACR4). ACR4 might bind the signaling peptide CLE40, which is the closest homolog of CLV3, and is involved in protoderm specification, where it acts together with ABNORMAL LEAF-SHAPE 2 (ALE2), another RLK (138, 145) (**Figure 3*c***). Although neither the single mutants nor the double mutant appear to show severe protodermal defects during embryo development, in mutant combinations with *ale1* the protoderm is misspecified (36, 145). Accordingly, *ale1 ale2* and *ale1 acr4* double mutants do not properly express *ATML1* (145). *ALE1* encodes a protease that is predominantly expressed in the endosperm, and thus ALE2 and ACR4 might perceive a signal from the endosperm to ensure proper protoderm specification (144, 145) (**Figure 3*c***). However, toxin-dependent endosperm ablation rather suggests that the endosperm is not involved in embryo patterning, and the feasibility of somatic embryogenesis also argues against essential peptide signals from the endosperm (158; reviewed in 168). In addition to its expression in the endosperm, *ALE1* is weakly expressed in the early embryo itself (144), and this might be relevant for embryogenesis.

Protoderm formation and *ATML1* expression are prevented in *arabidopsis thaliana defective kernel 1* (*atdek1*) mutant embryos, which arrest at the globular stage (60, 81, 150).

ATDEK1 encodes a calpain protease that undergoes autolytic cleavage (**Figure 3*c***) and is expressed in the embryo (60, 61, 81). In *ATDEK1* knockdown lines, seedlings show a transformation of epidermal to mesophyll-like cell fate in the cotyledons, similar to what has been observed in *atml1 pdf2* double mutants (1, 60). In conclusion, although a number of key players have been analyzed, the overall genetic program of setting up the radial pattern or only the protoderm is still largely unexplored.

SHOOT MERISTEM SPECIFICATION AND COTYLEDON INITIATION

The Organizing Center

The *A. thaliana* shoot meristem can be morphologically delineated for the first time during embryogenesis at the late-torpedo stage (6, 78). In the mature embryo, the shoot meristem consists of a few small cells with big nuclei and small vacuoles, and its first molecular mark is the onset of *WUS* expression in the four inner cells of the apical embryo region at the dermatogen stage (78, 97) (**Figure 2*a***). *WUS* encodes a homeodomain transcription factor, and its expression remains confined to a subset of cells close to the shoot apex during later stages of development (**Figure 4*a***), defining an organizing center that keeps the neighboring stem cells in a pluripotent state (97). The *wus* mutation results in the lack of a functional shoot meristem and the formation of a flat and enlarged shoot apex consisting of aberrant cells (78). *WUS* orthologs seem to play similar roles in dicots like *Petunia hybrida* and *Antirrhinum majus*, but possibly not in monocots like *O. sativa* and *Z. mays* (70, 107, 140).

Despite considerable efforts to identify regulators and downstream targets of this master regulator (11; reviewed in 24), our knowledge is scant about the mechanism(s) of initiation and early confinement of *WUS* expression and about the identity of the *WUS*-dependent non-cell-autonomous signal(s) maintaining stem cell fate in the shoot meristem. In postembryonic development, however, cytokinin signaling activates *WUS* expression (37). Because WUS inhibits the expression of several type-A *ARR*s that are negative regulators of cytokinin signaling, a positive feedback mechanism involving *WUS* and cytokinin signaling might thus operate in the shoot meristem to maintain

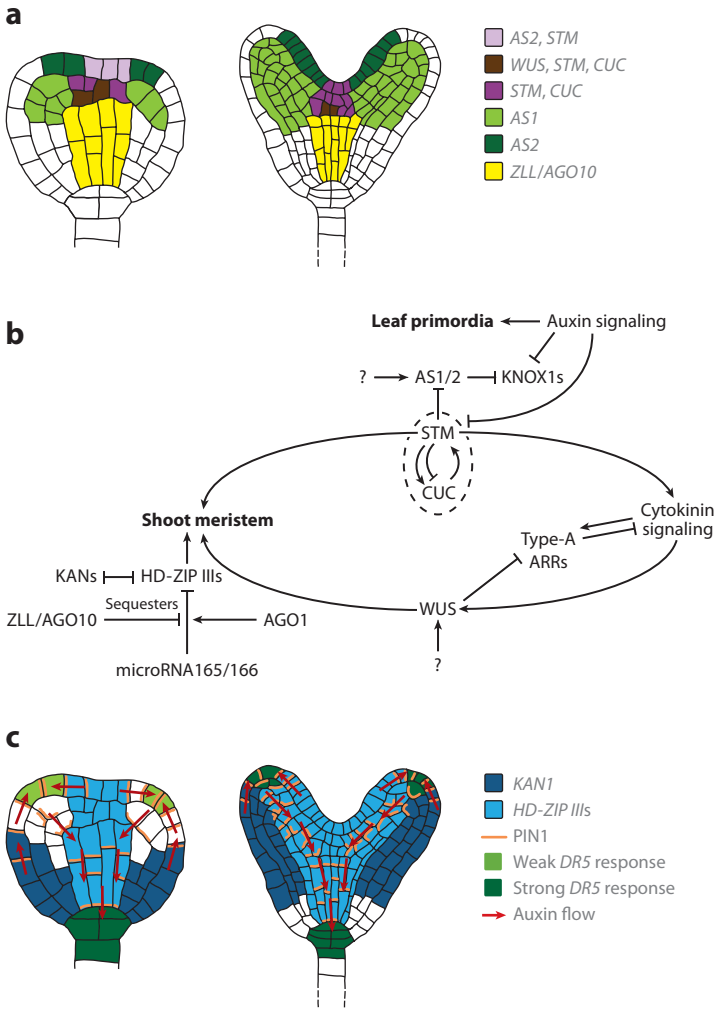

Figure 4

Shoot meristem and cotyledon initiation in *Arabidopsis thaliana*. (*a*) Expression patterns of genes important for establishment of the shoot meristem and initiation of cotyledons in *A. thaliana* during the transition stage and the heart stage. *CUC1–3* expression is generalized as *CUC*. (*b*) Pathways and hormonal regulation in shoot meristem and cotyledon initiation. (*c*) Expression patterns of *KAN1* and *HD-ZIP III* genes (exemplarily shown for *REV*, which includes domains of all other members), auxin flow mediated by PIN1 (idealized representation), and *DR5* response. Embryos not drawn to scale.

its integrity (37, 79) (**Figure 4*b***). This crosstalk may already operate during embryogenesis. In *O. sativa*, the *LONELY GUY* (*LOG*) gene, which encodes a cytokinin-activating enzyme and is specifically expressed in the shoot meristem region, is important for shoot meristem maintenance (75).

Shoot Meristem Indeterminacy and the Shoot Meristem–Cotyledon Boundary Region

The class I KNOTTED-like homeodomain transcription factor SHOOT MERISTEMLESS (STM) might indirectly activate *WUS* expression via its induction of cytokinin biosynthesis and signaling (37, 57, 85, 166) (**Figure 4*b***), and in addition to its cytokinin-related effects, it restricts gibberellic acid levels (45, 57). Similar to its *Z. mays* ortholog *KNOTTED 1* (*KN1*) and its *O. sativa* ortholog *Oryza sativa homeobox 1* (*OSH1*), *STM* is expressed in the presumptive shoot meristem from the globular stage onward (85, 124, 134) (**Figure 4*a***); in addition, in the oil palm *Elaeis guineensis* an *STM* ortholog is expressed in the shoot meristem, at least during vegetative development (64). Together with WUS, STM is required to maintain the shoot meristem: WUS acts as the instructor of the organizing center, and STM acts as a repressor of differentiation across the entire shoot meristem (80). In differentiated tissue, simultaneous expression of WUS and STM can induce meristematic activity, with WUS non-cell-autonomously triggering divisions in *STM*-expressing tissue (35).

Being a transcription factor, STM functions in the nucleus, and this localization depends on BEL1-like homeodomain transcription factors (22, 121). Shoot meristem initiation is consistently inhibited in the *stm* mutant and the *arabidopsis thaliana homeobox 1* (*ath1*) *pennywise* (*pny*) *pound-foolish* (*pnf*) triple mutant, and also in the *cup-shaped cotyledon 1* (*cuc1*) *cuc2* double mutant, which fails to express *STM* in the presumptive shoot meristem (4, 6, 121). The NAC transcription factors CUC1–3 are redundantly required for shoot meristem establishment as well as cotyledon separation. At early embryonic stages, their expression domains partially overlap with the *STM* expression domain (**Figure 4*a***), whereas *CUC1–3* expression domains in general surround the *STM* expression domain at later stages (4, 47, 142, 152). How this expression pattern evolves is not clear. However, there appears to be mutual regulation involving positive and negative feedback loops (**Figure 4*b***): Not only are the CUCs required for *STM* expression, but STM regulates the expression of *CUC1–3* and the expression of microRNA164, which in turn targets *CUC1* and *CUC2* transcripts for degradation (4, 77, 92, 137). The *P. hybrida* and *A. majus CUC* orthologs *NO APICAL MERISTEM* (*NAM*) and *CUPULIFORMIS* (*CUP*) are also expressed at organ boundaries, and they are important for both boundary establishment and shoot meristem development (136, 159). In *Z. mays*, the putative *CUC1/2* orthologs *Zea mays NO APICAL MERISTEM 1/2* (*ZmNAM1/2*) and the *CUC3* ortholog *Zea mays CUP-SHAPED COTYLEDON 3* (*ZmCUC3*) are in part initially coexpressed with a shoot meristem marker, and later in a ringlike pattern around the shoot meristem (173), hinting at a strong conservation of *CUC* gene function at least among flowering plants.

Meristem Establishment

A general prerequisite for shoot meristem identity seems to be the presence of class III HOMEODOMAIN-LEUCINE ZIPPER (HD-ZIP III) transcription factors. This family consists of PHABULOSA (PHB), PHAVOLUTA (PHV), REVOLUTA (REV), ARABIDOPSIS THALIANA HOMEOBOX 8 (ATHB8), and ATHB15. Expression of all but *ATHB8* is already detectable from early embryonic stages onward, and in part there is overlap with the future site of the shoot meristem, whereas especially *PHB*, *REV*, and *ATHB15* expression domains partially coincide with the *ZLL/AGO10* provascular expression domain as well; ATHB8 mRNA is detectable from the heart stage onward (26, 91, 100, 116). Conversely, expression domains of members of the *KANADI* (*KAN*)

gene family could be regarded as complementary to those of the *HD-ZIP III*s, which they are supposed to antagonize (26–28, 69) (**Figure 4*c***). The *phb rev* double, *phb phv rev* triple, and other loss-of-function mutant combinations involving *athb8* and *athb15* lack the embryonic shoot meristem and in severe cases fail to establish bilateral symmetry (26, 116). The dominant mutation *phb-1d* leads to ectopic meristems that express the shoot meristem marker *STM* on the lower side of leaves, and also causes an enlarged embryonic shoot meristem and partially suppresses the *stm* mutant phenotype (99). Two recent findings further support a pivotal role for HD-ZIP III transcription factors in shoot meristem formation. First, exclusion of HD-ZIP III proteins from the embryonic root pole is necessary for its proper establishment (38). Second, dominant *HD-ZIP III* mutants suppress the *tpl-1* double-root phenotype, possibly by excluding PLT1 and PLT2 from the future shoot meristem cells. Conversely, misexpression of dominant *HD-ZIP III*s can lead to (homeotic) root-pole-to-shoot-pole transformations during embryogenesis (135). It is not clear at present whether the HD-ZIP IIIs directly regulate *STM* and/or *WUS* in ectopic shoot meristem formation.

HD-ZIP III transcripts are targeted by microRNA165/166, and the dominant *HD-ZIP III* mutations reside in the microRNA pairing sites, rendering the HD-ZIP mRNAs resistant to degradation (93, 119, 146, 160, 171). The microRNA-dependent degradation involves the AGO proteins AGO1 and ZLL/AGO10, which both bind microRNA165/166 (172). It was suggested that ZLL/AGO10 and AGO1 act in an antagonistic fashion (**Figure 4*b***), with ZLL/AGO10 positively regulating HD-ZIP III transcript levels through competition with AGO1—possibly by sequestering microRNA165/166. Such a sequestration could ensure sufficiently high HD-ZIP III levels during shoot meristem establishment and maintenance (172). Given that *ZLL/AGO10* expression in the provasculature is necessary for embryonic shoot meristem maintenance, a non-cell-autonomous signal could, in principle, instruct the shoot meristem from the cells underneath (149). In this scenario, the two primary meristems of shoot and root would be initiated as WUS- and WOX5-positive cell groups, respectively, in response to inductive signals, at the opposite ends of the provasculature in early embryogenesis.

Initiation of Cotyledon Primordia

When the cotyledon primordia start to emerge in *A. thaliana*, the embryo organization shifts from radial to bilateral symmetry. The sites of cotyledon initiation correlate with auxin accumulation at subapical foci in the protoderm, as indicated by the auxin response reporter *DR5* (8) (**Figure 4*c***). Auxin might therefore directly cause cotyledon initiation in the apical margins of the globular embryo (8). In addition, *STM* and *CUC* expression have to be excluded from those sites (see below). Auxin transport toward the incipient primordia is mediated by PIN auxin efflux regulators, probably mainly by PIN1 (8) (**Figure 4*c***). PIN1 is apically localized in the protoderm, and the apical localization of PIN proteins is generally brought about by PID and its homologs PID2, WAG1, and WAG2, three of which have been shown to directly phosphorylate PINs (20, 23, 31, 49, 102). For example, the *pid wag1 wag2* triple mutant and the *pin1 pid* double mutant lack cotyledons (20, 33), as does the *pid enhancer of pinoid* (*enp*) double mutant (148). *ENP/MACCHI-BOU 4* (*MAB4*) encodes an NPH3-like protein that is involved in the regulation of PIN1 localization (32, 148). It is noteworthy that in both double mutants (*pin1 pid* and *pid enp*) the expression domains of *CUC* genes and *STM* are enlarged, and that cotyledon formation is partially restored when *CUC* genes or *STM* are knocked out in *pin1 pid* (33, 148); this highlights both the importance of directional auxin transport to the cotyledon initiation sites and the requirement to exclude specific transcripts/proteins from there. This view is supported by cotyledon formation defects in the auxin response mutants *mp* and *bdl* (9, 42). However, it might also be relevant in this context that MP directly activates the

expression of *DRN*—especially because *DRN* and *DRNL* redundantly act in cotyledon formation (16, 21). Additionally, DRN and DRNL are involved in the establishment and maintenance of boundary and shoot meristem gene expression domains, and they act together with PIN1 and PID (16, 18, 72). Auxin-related processes might be involved in cotyledon initiation in other flowering plant species as well, including monocots, but this has barely been investigated so far (reviewed in 15).

Another factor involved in cotyledon development, *ASYMMETRIC LEAVES 1* (*AS1*), which encodes a MYB domain protein and orthologs of which are present in *Z. mays* and *A. majus*, is initially expressed mainly subepidermally in the incipient cotyledon primordia, whereas *AS2*, which encodes a LATERAL ORGAN BOUNDARY (LOB) domain protein, is expressed protodermally before cotyledon outgrowth and later at the adaxial cotyledon side (12, 55, 84, 129, 153) (**Figure 4*a***). The loss of *AS1* or *AS2* makes *STM* dispensable for shoot meristem initiation and maintenance, suggesting that STM negatively regulates *AS1* and *AS2* (12, 13). Studies in primarily adult leaves suggest that *KNOX* genes are negatively regulated by AS1/2 and that AS1/2 possibly converge with auxin signaling to repress the *KNOX* member *KNOTTED-LIKE FROM ARABIDOPSIS THALIANA 1* (*KNAT1*)/*BREVIPEDICELLUS* (*BP*) (12, 39, 44) (**Figure 4*b***). The expression of *AS2* itself is negatively regulated by KAN1 and positively by BLADE-ON-PETIOLE 1/2 (BOP1/2), the expression of the latter in turn being directly or indirectly repressed by *STM* (65, 161). How exactly *AS1* and *AS2* are linked to auxin, however, has not been resolved.

PERSPECTIVES

Considerable progress has been made in the analysis of mechanisms underlying specific events in early embryogenesis, notably in *A. thaliana*. For example, we now have a clear conceptual framework for the initiation of the root meristem in the early embryo. However, although the main regulators have been identified and characterized, it is still rather obscure how these early events relate to the establishment of the molecular system for self-maintenance of the functional root meristem at the heart stage of embryogenesis. The initiation and establishment of the self-maintenance system are even less clear for the shoot meristem. Large-scale approaches combining expression profiling of specific embryo regions with functional characterization of putative developmental regulators might contribute to closing the gap.

Another unsolved problem is the origin of the apical-basal pattern. Although genes encoding developmental regulators are expressed in either the apical or the basal daughter cell of the zygote, it is not known how the expression of these regulators is ultimately established. This also relates to the mode of division of the zygote: Is it truly unequal, reflecting an intrinsic polarity of the zygote before division? Alternatively, the division might be equal, and only the two daughter cells would be exposed to different environments and thus might perceive different signals.

The contribution of the gametes to early embryogenesis still needs to be assessed. Although differentially regulated genes have been identified, their role in early patterning has not been clarified. And the significance of epigenetic regulation of patterning is still an open question.

Finally, most studies have focused on a few species, notably *A. thaliana*. Considering the differences in cell-division patterns between early embryos from different species, exploring orthologous developmental regulators might reveal to what extent their actions and regulatory networks are conserved among the flowering plant species when the cellular contexts of developmental events are not.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank Martin Bayer for critical reading of the manuscript.

LITERATURE CITED

1. Abe M, Katsumata H, Komeda Y, Takahashi T. 2003. Regulation of shoot epidermal cell differentiation by a pair of homeodomain proteins in *Arabidopsis*. *Development* 130:635–43
2. Abe M, Takahashi T, Komeda Y. 2001. Identification of a *cis*-regulatory element for L1 layer-specific gene expression, which is targeted by an L1-specific homeodomain protein. *Plant J.* 26:487–94
3. Aida M, Beis D, Heidstra R, Willemsen V, Blilou I, et al. 2004. The *PLETHORA* genes mediate patterning of the *Arabidopsis* root stem cell niche. *Cell* 119:109–20
4. Aida M, Ishida T, Tasaka M. 1999. Shoot apical meristem and cotyledon formation during *Arabidopsis* embryogenesis: interaction among the *CUP-SHAPED COTYLEDON* and *SHOOT MERISTEMLESS* genes. *Development* 126:1563–70
5. Autran D, Baroux C, Raissig MT, Lenormand T, Wittig M, et al. 2011. Maternal epigenetic pathways control parental contributions to *Arabidopsis* early embryogenesis. *Cell* 145:707–19
6. Barton MK, Poethig RS. 1993. Formation of the shoot apical meristem in *Arabidopsis thaliana*: an analysis of development in the wild type and in the *shoot meristemless* mutant. *Development* 119:823–31
7. Bayer M, Nawy T, Giglione C, Galli M, Meinnel T, Lukowitz W. 2009. Paternal control of embryonic patterning in *Arabidopsis thaliana*. *Science* 323:1485–88
8. Benková E, Michniewicz M, Sauer M, Teichmann T, Seifertová D, et al. 2003. Local, efflux-dependent auxin gradients as a common module for plant organ formation. *Cell* 115:591–602
9. Berleth T, Jürgens G. 1993. The role of the *monopteros* gene in organising the basal body region of the *Arabidopsis* embryo. *Development* 118:575–87
10. Breuninger H, Rikirsch E, Hermann M, Ueda M, Laux T. 2008. Differential expression of *WOX* genes mediates apical-basal axis formation in the *Arabidopsis* embryo. *Dev. Cell* 14:867–76
11. Busch W, Miotk A, Ariel FD, Zhao Z, Forner J, et al. 2010. Transcriptional control of a plant stem cell niche. *Dev. Cell* 18:849–61
12. Byrne ME, Barley R, Curtis M, Arroyo JM, Dunham M, et al. 2000. *Asymmetric leaves1* mediates leaf patterning and stem cell function in *Arabidopsis*. *Nature* 408:967–71
13. Byrne ME, Simorowski J, Martienssen RA. 2002. *ASYMMETRIC LEAVES1* reveals *knox* gene redundancy in *Arabidopsis*. *Development* 129:1957–65
14. Carlsbecker A, Lee JY, Roberts CJ, Dettmer J, Lehesranta S, et al. 2010. Cell signalling by microRNA165/6 directs gene dose-dependent root cell fate. *Nature* 465:316–21
15. Chandler JW. 2008. Cotyledon organogenesis. *J. Exp. Bot.* 59:2917–31
16. Chandler JW, Cole M, Flier A, Grewe B, Werr W. 2007. The AP2 transcription factors DORNRÖSCHEN and DORNRÖSCHEN-LIKE redundantly control *Arabidopsis* embryo patterning via interaction with PHAVOLUTA. *Development* 134:1653–62
17. Chandler JW, Cole M, Flier A, Werr W. 2009. BIM1, a bHLH protein involved in brassinosteroid signalling, controls *Arabidopsis* embryonic patterning via interaction with DORNRÖSCHEN and DORNRÖSCHEN-LIKE. *Plant Mol. Biol.* 69:57–68
18. Chandler JW, Cole M, Jacobs B, Comelli P, Werr W. 2011. Genetic integration of *DORNRÖSCHEN* and *DORNRÖSCHEN-LIKE* reveals hierarchical interactions in auxin signalling and patterning of the *Arabidopsis* apical embryo. *Plant Mol. Biol.* 75:223–36
19. Cheng Y, Dai X, Zhao Y. 2007. Auxin synthesized by the YUCCA flavin monooxygenases is essential for embryogenesis and leaf formation in *Arabidopsis*. *Plant Cell* 19:2430–39

20. Cheng Y, Qin G, Dai X, Zhao Y. 2008. *NPY* genes and AGC kinases define two key steps in auxin-mediated organogenesis in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* 105:21017–22
21. Cole M, Chandler J, Weijers D, Jacobs B, Comelli P, Werr W. 2009. *DORNRÖSCHEN* is a direct target of the auxin response factor MONOPTEROS in the *Arabidopsis* embryo. *Development* 136:1643–51
22. Cole M, Nolte C, Werr W. 2006. Nuclear import of the transcription factor SHOOT MERISTEMLESS depends on heterodimerization with BLH proteins expressed in discrete sub-domains of the shoot apical meristem of *Arabidopsis thaliana*. *Nucleic Acids Res.* 34:1281–92
23. Dhonukshe P, Huang F, Galvan-Ampudia CS, Mahonen AP, Kleine-Vehn J, et al. 2010. Plasma membrane-bound AGC3 kinases phosphorylate PIN auxin carriers at TPRXS(N/S) motifs to direct apical PIN recycling. *Development* 137:3245–55
24. Dodsworth S. 2009. A diverse and intricate signalling network regulates stem cell fate in the shoot apical meristem. *Dev. Biol.* 336:1–9
25. Dumas C, Rogowsky P. 2008. Fertilization and early seed formation. *C. R. Biol.* 331:715–25
26. Emery JF, Floyd SK, Alvarez J, Eshed Y, Hawker NP, et al. 2003. Radial patterning of *Arabidopsis* shoots by class III HD-ZIP and KANADI genes. *Curr. Biol.* 13:1768–74
27. Eshed Y, Baum SF, Perea JV, Bowman JL. 2001. Establishment of polarity in lateral organs of plants. *Curr. Biol.* 11:1251–60
28. Eshed Y, Izhaki A, Baum SF, Floyd SK, Bowman JL. 2004. Asymmetric leaf development and blade expansion in *Arabidopsis* are mediated by KANADI and YABBY activities. *Development* 131:2997–3006
29. Faure JE, Rotman N, Fortune P, Dumas C. 2002. Fertilization in *Arabidopsis thaliana* wild type: developmental stages and time course. *Plant J.* 30:481–88
30. Friml J, Vieten A, Sauer M, Weijers D, Schwarz H, et al. 2003. Efflux-dependent auxin gradients establish the apical-basal axis of *Arabidopsis*. *Nature* 426:147–53
31. Friml J, Yang X, Michniewicz M, Weijers D, Quint A, et al. 2004. A PINOID-dependent binary switch in apical-basal PIN polar targeting directs auxin efflux. *Science* 306:862–65
32. Furutani M, Kajiwara T, Kato T, Treml BS, Stockum C, et al. 2007. The gene *MACCHI-BOU 4/ENHANCER OF PINOID* encodes a NPH3-like protein and reveals similarities between organogenesis and phototropism at the molecular level. *Development* 134:3849–59
33. Furutani M, Vernoux T, Traas J, Kato T, Tasaka M, Aida M. 2004. *PIN-FORMED1* and *PINOID* regulate boundary formation and cotyledon development in *Arabidopsis* embryogenesis. *Development* 131:5021–30
34. Galinha C, Hofhuis H, Luijten M, Willemsen V, Blilou I, et al. 2007. PLETHORA proteins as dose-dependent master regulators of *Arabidopsis* root development. *Nature* 449:1053–57
35. Gallois JL, Woodward C, Reddy GV, Sablowski R. 2002. Combined SHOOT MERISTEMLESS and WUSCHEL trigger ectopic organogenesis in *Arabidopsis*. *Development* 129:3207–17
36. Gifford ML, Dean S, Ingram GC. 2003. The *Arabidopsis ACR4* gene plays a role in cell layer organisation during ovule integument and sepal margin development. *Development* 130:4249–58
37. Gordon SP, Chickarmane VS, Ohno C, Meyerowitz EM. 2009. Multiple feedback loops through cytokinin signaling control stem cell number within the *Arabidopsis* shoot meristem. *Proc. Natl. Acad. Sci. USA* 106:16529–34
38. Grigg SP, Galinha C, Kornet N, Canales C, Scheres B, Tsiantis M. 2009. Repression of apical homeobox genes is required for embryonic root development in *Arabidopsis*. *Curr. Biol.* 19:1485–90
39. Guo M, Thomas J, Collins G, Timmermans MC. 2008. Direct repression of *KNOX* loci by the ASYMMETRIC LEAVES1 complex of *Arabidopsis*. *Plant Cell* 20:48–58
40. Haecker A, Groß-Hardt R, Geiges B, Sarkar A, Breuninger H, et al. 2004. Expression dynamics of *WOX* genes mark cell fate decisions during early embryonic patterning in *Arabidopsis thaliana*. *Development* 131:657–68
41. Hamann T, Benkova E, Bäurle I, Kientz M, Jürgens G. 2002. The *Arabidopsis BODENLOS* gene encodes an auxin response protein inhibiting MONOPTEROS-mediated embryo patterning. *Genes Dev.* 16:1610–15
42. Hamann T, Mayer U, Jürgens G. 1999. The auxin-insensitive *bodenlos* mutation affects primary root formation and apical-basal patterning in the *Arabidopsis* embryo. *Development* 126:1387–95
43. Hardtke CS, Berleth T. 1998. The *Arabidopsis* gene *MONOPTEROS* encodes a transcription factor mediating embryo axis formation and vascular development. *EMBO J.* 17:1405–11

44. Hay A, Barkoulas M, Tsiantis M. 2006. ASYMMETRIC LEAVES1 and auxin activities converge to repress *BREVIPEDICELLUS* expression and promote leaf development in *Arabidopsis*. *Development* 133:3955–61
45. Hay A, Kaur H, Phillips A, Hedden P, Hake S, Tsiantis M. 2002. The gibberellin pathway mediates KNOTTED1-type homeobox function in plants with different body plans. *Curr. Biol.* 12:1557–65
46. Helariutta Y, Fukaki H, Wysocka-Diller J, Nakajima K, Jung J, et al. 2000. The *SHORT-ROOT* gene controls radial patterning of the *Arabidopsis* root through radial signaling. *Cell* 101:555–67
47. Hibara K, Karim MR, Takada S, Taoka K, Furutani M, et al. 2006. *Arabidopsis CUP-SHAPED COTYLEDON3* regulates postembryonic shoot meristem and organ boundary formation. *Plant Cell* 18:2946–57
48. Hu TX, Yu M, Zhao J. 2010. Comparative transcriptional profiling analysis of the two daughter cells from tobacco zygote reveals the transcriptome differences in the apical and basal cells. *BMC Plant Biol.* 10:167
49. Huang F, Zago MK, Abas L, van Marion A, Galvan-Ampudia CS, Offringa R. 2010. Phosphorylation of conserved PIN motifs directs *Arabidopsis* PIN1 polarity and auxin transport. *Plant Cell* 22:1129–42
50. Ingouff M, Hamamura Y, Gourgues M, Higashiyama T, Berger F. 2007. Distinct dynamics of HISTONE3 variants between the two fertilization products in plants. *Curr. Biol.* 17:1032–37
51. Ingouff M, Rademacher S, Holec S, Soljic L, Xin N, et al. 2010. Zygotic resetting of the HISTONE 3 variant repertoire participates in epigenetic reprogramming in *Arabidopsis*. *Curr. Biol.* 20:2137–43
52. Ingram GC, Boisnard-Lorig C, Dumas C, Rogowsky PM. 2000. Expression patterns of genes encoding HD-ZipIV homeo domain proteins define specific domains in maize embryos and meristems. *Plant J.* 22:401–14
53. Ingram GC, Magnard JL, Vergne P, Dumas C, Rogowsky PM. 1999. *ZmOCL1*, an HDGL2 family homeobox gene, is expressed in the outer cell layer throughout maize development. *Plant Mol. Biol.* 40:343–54
54. Ito M, Sentoku N, Nishimura A, Hong SK, Sato Y, Matsuoka M. 2002. Position dependent expression of *GL2*-type homeobox gene, *Roc1*: significance for protoderm differentiation and radial pattern formation in early rice embryogenesis. *Plant J.* 29:497–507
55. Iwakawa H, Ueno Y, Semiarti E, Onouchi H, Kojima S, et al. 2002. The *ASYMMETRIC LEAVES2* gene of *Arabidopsis thaliana*, required for formation of a symmetric flat leaf lamina, encodes a member of a novel family of proteins characterized by cysteine repeats and a leucine zipper. *Plant Cell Physiol.* 43:467–78
56. Jahnke S, Scholten S. 2009. Epigenetic resetting of a gene imprinted in plant embryos. *Curr. Biol.* 19:1677–81
57. Jasinski S, Piazza P, Craft J, Hay A, Woolley L, et al. 2005. KNOX action in *Arabidopsis* is mediated by coordinate regulation of cytokinin and gibberellin activities. *Curr. Biol.* 15:1560–65
58. Jeong S, Palmer TM, Lukowitz W. 2011. The RWP-RK factor *GROUNDED* promotes embryonic polarity by facilitating YODA MAP kinase signaling. *Curr. Biol.* 21:1268–76
59. Jiang K, Feldman LJ. 2005. Regulation of root apical meristem development. *Annu. Rev. Cell Dev. Biol.* 21:485–509
60. Johnson KL, Degnan KA, Ross Walker J, Ingram GC. 2005. *AtDEK1* is essential for specification of embryonic epidermal cell fate. *Plant J.* 44:114–27
61. Johnson KL, Faulkner C, Jeffree CE, Ingram GC. 2008. The phytocalpain Defective Kernel 1 is a novel *Arabidopsis* growth regulator whose activity is regulated by proteolytic processing. *Plant Cell* 20:2619–30
62. Johri BM. 1984. *Embryology of Angiosperms*. Berlin: Springer-Verlag
63. Johri BM, Ambegaokar KB, Srivastava PS. 1992. *Comparative Embryology of Angiosperms*. Berlin: Springer-Verlag
64. Jouannic S, Collin M, Vidal B, Verdeil JL, Tregear JW. 2007. A class I KNOX gene from the palm species *Elaeis guineensis* (Arecaceae) is associated with meristem function and a distinct mode of leaf dissection. *New Phytol.* 174:551–68
65. Jun JH, Ha CM, Fletcher JC. 2010. BLADE-ON-PETIOLE1 coordinates organ determinacy and axial polarity in *Arabidopsis* by directly activating ASYMMETRIC LEAVES2. *Plant Cell* 22:62–76
66. Jürgens G, Mayer U. 1994. *Arabidopsis*. In *A Colour Atlas of Developing Embryos*, ed. JBL Bard, pp. 7–21. London: Wolfe

67. Kamiya N, Itoh J-I, Morikami A, Nagato Y, Matsuoka M. 2003. The *SCARECROW* gene's role in asymmetric cell divisions in rice plants. *Plant J.* 36:45–54
68. Kamiya N, Nagasaki H, Morikami A, Sato Y, Matsuoka M. 2003. Isolation and characterization of a rice *WUSCHEL*-type homeobox gene that is specifically expressed in the central cells of a quiescent center in the root apical meristem. *Plant J.* 35:429–41
69. Kerstetter RA, Bollman K, Taylor RA, Bomblies K, Poethig RS. 2001. *KANADI* regulates organ polarity in *Arabidopsis*. *Nature* 411:706–9
70. Kieffer M, Stern Y, Cook H, Clerici E, Maulbetsch C, et al. 2006. Analysis of the transcription factor WUSCHEL and its functional homologue in *Antirrhinum* reveals a potential mechanism for their roles in meristem maintenance. *Plant Cell* 18:560–73
71. Kinoshita A, Betsuyaku S, Osakabe Y, Mizuno S, Nagawa S, et al. 2010. RPK2 is an essential receptor-like kinase that transmits the CLV3 signal in *Arabidopsis*. *Development* 137:3911–20
72. Kirch T, Simon R, Grünewald M, Werr W. 2003. The *DORNRÖSCHEN/ENHANCER OF SHOOT REGENERATION1* gene of Arabidopsis acts in the control of meristem cell fate and lateral organ development. *Plant Cell* 15:694–705
73. Krogan NT, Long JA. 2009. Why so repressed? Turning off transcription during plant growth and development. *Curr. Opin. Plant Biol.* 12:628–36
74. Kumlehn J, Lörz H, Kranz E. 1999. Monitoring individual development of isolated wheat zygotes: a novel approach to study early embryogenesis. *Protoplasma* 208:156–62
75. Kurakawa T, Ueda N, Maekawa M, Kobayashi K, Kojima M, et al. 2007. Direct control of shoot meristem activity by a cytokinin-activating enzyme. *Nature* 445:652–55
76. Lau S, De Smet I, Kolb M, Meinhardt H, Jürgens G. 2011. Auxin triggers a genetic switch. *Nat. Cell Biol.* 13:611–15
77. Laufs P, Peaucelle A, Morin H, Traas J. 2004. MicroRNA regulation of the CUC genes is required for boundary size control in *Arabidopsis* meristems. *Development* 131:4311–22
78. Laux T, Mayer KFX, Berger J, Jürgens G. 1996. The *WUSCHEL* gene is required for shoot and floral meristem integrity in *Arabidopsis*. *Development* 122:87–96
79. Leibfried A, To JP, Busch W, Stehling S, Kehle A, et al. 2005. WUSCHEL controls meristem function by direct regulation of cytokinin-inducible response regulators. *Nature* 438:1172–75
80. Lenhard M, Jürgens G, Laux T. 2002. The *WUSCHEL* and *SHOOTMERISTEMLESS* genes fulfil complementary roles in *Arabidopsis* shoot meristem regulation. *Development* 129:3195–206
81. Lid SE, Olsen L, Nestestog R, Aukerman M, Brown RC, et al. 2005. Mutation in the *Arabidopisis thaliana DEK1* calpain gene perturbs endosperm and embryo development while over-expression affects organ development globally. *Planta* 221:339–51
82. Lim J, Helariutta Y, Specht CD, Jung J, Sims L, et al. 2000. Molecular analysis of the *SCARECROW* gene in maize reveals a common basis for radial patterning in diverse meristems. *Plant Cell* 12:1307–18
83. Lim J, Jung JW, Lim CE, Lee M-H, Kim BJ, et al. 2005. Conservation and diversification of *SCARECROW* in maize. *Plant Mol. Biol.* 59:619–30
84. Lin WC, Shuai B, Springer PS. 2003. The Arabidopsis *LATERAL ORGAN BOUNDARIES*–domain gene *ASYMMETRIC LEAVES2* functions in the repression of *KNOX* gene expression and in adaxial-abaxial patterning. *Plant Cell* 15:2241–52
85. Long JA, Moan EI, Medford JI, Barton MK. 1996. A member of the KNOTTED class of homeodomain proteins encoded by the *STM* gene of *Arabidopsis*. *Nature* 379:66–69
86. Long JA, Ohno C, Smith ZR, Meyerowitz EM. 2006. TOPLESS regulates apical embryonic fate in *Arabidopsis*. *Science* 312:1520–23
87. Long JA, Woody S, Poethig S, Meyerowitz EM, Barton MK. 2002. Transformation of shoots into roots in *Arabidopsis* embryos mutant at the *TOPLESS* locus. *Development* 129:2797–806
88. Lu P, Porat R, Nadeau JA, O'Neill SD. 1996. Identification of a meristem L1 layer-specific gene in Arabidopsis that is expressed during embryonic pattern formation and defines a new class of homeobox genes. *Plant Cell* 8:2155–68
89. Lukowitz W, Roeder A, Parmenter D, Somerville C. 2004. A MAPKK kinase gene regulates extra-embryonic cell fate in *Arabidopsis*. *Cell* 116:109–19

90. Luo M, Taylor JM, Spriggs A, Zhang H, Wu X, et al. 2011. A genome-wide survey of imprinted genes in rice seeds reveals imprinting primarily occurs in the endosperm. *PLoS Genet.* 7:e1002125
91. Lynn K, Fernandez A, Aida M, Sedbrook J, Tasaka M, et al. 1999. The *PINHEAD/ZWILLE* gene acts pleiotropically in *Arabidopsis* development and has overlapping functions with the *ARGONAUTE1* gene. *Development* 126:469–81
92. Mallory AC, Dugas DV, Bartel DP, Bartel B. 2004. MicroRNA regulation of NAC-domain targets is required for proper formation and separation of adjacent embryonic, vegetative, and floral organs. *Curr. Biol.* 14:1035–46
93. Mallory AC, Reinhart BJ, Jones-Rhoades MW, Tang G, Zamore PD, et al. 2004. MicroRNA control of *PHABULOSA* in leaf development: importance of pairing to the microRNA 5′ region. *EMBO J.* 23:3356–64
94. Mansfield SG, Briarty LG. 1991. Early embryogenesis in *Arabidopsis thaliana*. II. The developing embryo. *Can. J. Bot.* 69:461–76
95. Mansfield SG, Briarty LG, Erni S. 1991. Early embryogenesis in *Arabidopsis thaliana*. I. The mature embryo sack. *Can. J. Bot.* 69:447–60
96. Mao G, Meng X, Liu Y, Zheng Z, Chen Z, Zhang S. 2011. Phosphorylation of a WRKY transcription factor by two pathogen-responsive MAPKs drives phytoalexin biosynthesis in *Arabidopsis*. *Plant Cell* 23:1639–53
97. Mayer KFX, Schoof H, Haecker A, Lenhard M, Jürgens G, Laux T. 1998. Role of *WUSCHEL* in regulating stem cell fate in the *Arabidopsis* shoot meristem. *Cell* 95:805–15
98. Mayer U, Büttner G, Jürgens G. 1993. Apical-basal pattern formation in the *Arabidopsis* embryo: studies on the role of the *gnom* gene. *Development* 117:149–62
99. McConnell JR, Barton MK. 1998. Leaf polarity and meristem formation in *Arabidopsis*. *Development* 125:2935–42
100. McConnell JR, Emery J, Eshed Y, Bao N, Bowman J, Barton MK. 2001. Role of *PHABULOSA* and *PHAVOLUTA* in determining radial patterning in shoots. *Nature* 411:709–13
101. Meyer S, Scholten S. 2007. Equivalent parental contribution to early plant zygotic development. *Curr. Biol.* 17:1686–91
102. Michniewicz M, Zago MK, Abas L, Weijers D, Schweighofer A, et al. 2007. Antagonistic regulation of PIN phosphorylation by PP2A and PINOID directs auxin flux. *Cell* 130:1044–56
103. Mogensen HL, Suthar HK. 1979. Ultrastructure of the egg apparatus of *Nicotiana tabacum* (Solanaceae) before and after fertilization. *Bot. Gaz.* 140:168–79
104. Moussian B, Schoof H, Haecker A, Jürgens G, Laux T. 1998. Role of the *ZWILLE* gene in the regulation of central shoot meristem cell fate during *Arabidopsis* embryogenesis. *EMBO J.* 17:1799–809
105. Müller B, Sheen J. 2008. Cytokinin and auxin interaction in root stem-cell specification during early embryogenesis. *Nature* 453:1094–97
106. Nakajima K, Sena G, Nawy T, Benfey PN. 2001. Intercellular movement of the putative transcription factor SHR in root patterning. *Nature* 413:307–11
107. Nardmann J, Werr W. 2006. The shoot stem cell niche in angiosperms: expression patterns of *WUS* orthologues in rice and maize imply major modifications in the course of mono- and dicot evolution. *Mol. Biol. Evol.* 23:2492–504
108. Nawy T, Bayer M, Mravec J, Friml J, Birnbaum KD, Lukowitz W. 2010. The GATA factor *HANABA TARANU* is required to position the proembryo boundary in the early *Arabidopsis* embryo. *Dev. Cell* 19:103–13
109. Ni J, Clark SE. 2006. Evidence for functional conservation, sufficiency, and proteolytic processing of the CLAVATA3 CLE domain. *Plant Physiol.* 140:726–33
110. Ning J, Peng XB, Qu LH, Xin HP, Yan TT, Sun M. 2006. Differential gene expression in egg cells and zygotes suggests that the transcriptome is restructed before the first zygotic division in tobacco. *FEBS Lett.* 580:1747–52
111. Nodine MD, Bartel DP. 2010. MicroRNAs prevent precocious gene expression and enable pattern formation during plant embryogenesis. *Genes Dev.* 24:2678–92
112. Nodine MD, Yadegari R, Tax FE. 2007. *RPK1* and *TOAD2* are two receptor-like kinases redundantly required for *Arabidopsis* embryonic pattern formation. *Dev. Cell* 12:943–56

113. Okamoto T, Scholten S, Lorz H, Kranz E. 2005. Identification of genes that are up- or down-regulated in the apical or basal cell of maize two-celled embryos and monitoring their expression during zygote development by a cell manipulation- and PCR-based approach. *Plant Cell Physiol.* 46:332–38
114. Olson AR, Cass DD. 1981. Changes in megagametophyte structure in *Papaver nudicaule* L. (Papaveraceae) following in vitro placental pollination. *Am. J. Bot.* 68:1333–41
115. Pauwels L, Barbero GF, Geerinck J, Tilleman S, Grunewald W, et al. 2010. NINJA connects the co-repressor TOPLESS to jasmonate signalling. *Nature* 464:788–91
116. Prigge MJ, Otsuga D, Alonso JM, Ecker JR, Drews GN, Clark SE. 2005. Class III homeodomain-leucine zipper gene family members have overlapping, antagonistic, and distinct roles in Arabidopsis development. *Plant Cell* 17:61–76
117. Rademacher E, Möller B, Lokerse AS, Llavata-Peris CI, van den Berg W, Weijers D. 2011. A cellular expression map of the *Arabidopsis AUXIN RESPONSE FACTOR* gene family. *Plant J.* 68:597–606
118. Raissig MT, Baroux C, Grossniklaus U. 2011. Regulation and flexibility of genomic imprinting during seed development. *Plant Cell* 23:16–26
119. Rhoades MW, Reinhart BJ, Lim LP, Burge CB, Bartel B, Bartel DP. 2002. Prediction of plant microRNA targets. *Cell* 110:513–20
120. Ronceret A, Gadea-Vacas J, Guilleminot J, Lincker F, Delorme V, et al. 2008. The first zygotic division in Arabidopsis requires *de novo* transcription of thymidylate kinase. *Plant J.* 53:776–89
121. Rutjens B, Bao D, van Eck-Stouten E, Brand M, Smeekens S, Proveniers M. 2009. Shoot apical meristem function in Arabidopsis requires the combined activities of three BEL1-like homeodomain proteins. *Plant J.* 58:641–54
122. Sarkar AK, Luijten M, Miyashima S, Lenhard M, Hashimoto T, et al. 2007. Conserved factors regulate signalling in *Arabidopsis thaliana* shoot and root stem cell organizers. *Nature* 446:811–14
123. Sato A, Toyooka K, Okamoto T. 2010. Asymmetric cell division of rice zygotes located in embryo sac and produced by in vitro fertilization. *Sex. Plant Reprod.* 23:211–17
124. Sato Y, Hong SK, Tagiri A, Kitano H, Yamamoto N, et al. 1996. A rice homeobox gene, *OSH1*, is expressed before organ differentiation in a specific region during early embryogenesis. *Proc. Natl. Acad. Sci. USA* 93:8117–22
125. Sauter M, von Wiegen P, Lörz H, Kranz E. 1998. Cell cycle regulatory genes from maize are differentially controlled during fertilization and first embryonic cell division. *Sex. Plant Reprod.* 11:41–48
126. Scheres B, Di Laurenzio L, Willemsen V, Hauser MT, Janmaat K, et al. 1995. Mutations affecting the radial organisation of the *Arabidopsis* root display specific defects throughout the embryonic axis. *Development* 121:53–62
127. Scheres B, Wolkenfelt H, Willemsen V, Terlouw M, Lawson E, et al. 1994. Embryonic origin of the *Arabidopsis* primary root and root meristem initials. *Development* 120:2475–87
128. Schlereth A, Möller B, Liu W, Kientz M, Flipse J, et al. 2010. MONOPTEROS controls embryonic root initiation by regulating a mobile transcription factor. *Nature* 464:913–16
129. Schneeberger R, Tsiantis M, Freeling M, Langdale JA. 1998. The *rough sheath2* gene negatively regulates homeobox gene expression during maize leaf development. *Development* 125:2857–65
130. Scholten S, Lorz H, Kranz E. 2002. Paternal mRNA and protein synthesis coincides with male chromatin decondensation in maize zygotes. *Plant J.* 32:221–31
131. Schulz R, Jensen WA. 1968. Capsella embryogenesis: the egg, zygote, and young embryo. *Am. J. Bot.* 55:807–19
132. Shevell DE, Leu WM, Gillmor CS, Xia G, Feldmann KA, Chua NH. 1994. *EMB30* is essential for normal cell division, cell expansion, and cell adhesion in Arabidopsis and encodes a protein that has similarity to Sec7. *Cell* 77:1051–62
133. Sivaramakrishna D. 1977. Size relationships of apical cell and basal cell in two-celled embryos in angiosperms. *Can. J. Bot.* 56:1434–38
134. Smith LG, Jackson D, Hake S. 1995. Expression of *knotted1* marks shoot meristem formation during maize embryogenesis. *Dev. Genet.* 16:344–48
135. Smith ZR, Long JA. 2010. Control of *Arabidopsis* apical-basal embryo polarity by antagonistic transcription factors. *Nature* 464:423–26

136. Souer E, van Houwelingen A, Kloos D, Mol J, Koes R. 1996. The *No Apical Meristem* gene of Petunia is required for pattern formation in embryos and flowers and is expressed at meristem and primordia boundaries. *Cell* 85:159–70
137. Spinelli SV, Martin AP, Viola IL, Gonzalez DH, Palatnik JF. 2011. A mechanistic link between *STM* and *CUC1* during *Arabidopsis* development. *Plant Physiol.* 156:1894–904
138. Stahl Y, Wink RH, Ingram GC, Simon R. 2009. A signaling module controlling the stem cell niche in *Arabidopsis* root meristems. *Curr. Biol.* 19:909–14
139. Stepanova AN, Robertson-Hoyt J, Yun J, Benavente LM, Xie DY, et al. 2008. *TAA1*-mediated auxin biosynthesis is essential for hormone crosstalk and plant development. *Cell* 133:177–91
140. Stuurman J, Jaggi F, Kuhlemeier C. 2002. Shoot meristem maintenance is controlled by a *GRAS*-gene mediated signal from differentiating cells. *Genes Dev.* 16:2213–18
141. Szemenyei H, Hannon M, Long JA. 2008. TOPLESS mediates auxin-dependent transcriptional repression during *Arabidopsis* embryogenesis. *Science* 319:1384–86
142. Takada S, Hibara K, Ishida T, Tasaka M. 2001. The *CUP-SHAPED COTYLEDON1* gene of *Arabidopsis* regulates shoot apical meristem formation. *Development* 128:1127–35
143. Takada S, Jürgens G. 2007. Transcriptional regulation of epidermal cell fate in the *Arabidopsis* embryo. *Development* 134:1141–50
144. Tanaka H, Onouchi H, Kondo M, Hara-Nishimura I, Nishimura M, et al. 2001. A subtilisin-like serine protease is required for epidermal surface formation in *Arabidopsis* embryos and juvenile plants. *Development* 128:4681–89
145. Tanaka H, Watanabe M, Sasabe M, Hiroe T, Tanaka T, et al. 2007. Novel receptor-like kinase ALE2 controls shoot development by specifying epidermis in *Arabidopsis*. *Development* 134:1643–52
146. Tang G, Reinhart BJ, Bartel DP, Zamore PD. 2003. A biochemical framework for RNA silencing in plants. *Genes Dev.* 17:49–63
147. Torres-Ruiz RA, Jürgens G. 1994. Mutations in the *FASS* gene uncouple pattern formation and morphogenesis in *Arabidopsis* development. *Development* 120:2967–78
148. Treml BS, Winderl S, Radykewicz R, Herz M, Schweizer G, et al. 2005. The gene *ENHANCER OF PINOID* controls cotyledon development in the *Arabidopsis* embryo. *Development* 132:4063–74
149. Tucker MR, Hinze A, Tucker EJ, Takada S, Jürgens G, Laux T. 2008. Vascular signalling mediated by ZWILLE potentiates WUSCHEL function during shoot meristem stem cell development in the *Arabidopsis* embryo. *Development* 135:2839–43
150. Tzafrir I, Pena-Muralla R, Dickerman A, Berg M, Rogers R, et al. 2004. Identification of genes required for embryo development in Arabidopsis. *Plant Physiol.* 135:1206–20
151. Ueda M, Zhang Z, Laux T. 2011. Transcriptional activation of *Arabidopsis* axis patterning genes *WOX8/9* links zygote polarity to embryo development. *Dev. Cell* 20:264–70
152. Vroemen CW, Mordhorst AP, Albrecht C, Kwaaitaal MA, de Vries SC. 2003. The *CUP-SHAPED COTYLEDON3* gene is required for boundary and shoot meristem formation in Arabidopsis. *Plant Cell* 15:1563–77
153. Waites R, Selvadurai HR, Oliver IR, Hudson A. 1998. The *PHANTASTICA* gene encodes a MYB transcription factor involved in growth and dorsoventrality of lateral organs in *Antirrhinum*. *Cell* 93:779–89
154. Waki T, Hiki T, Watanabe R, Hashimoto T, Nakajima K. 2011. The *Arabidopsis* RWP-RK protein RKD4 triggers gene expression and pattern formation in early embryogenesis. *Curr. Biol.* 21:1277–81
155. Wang H, Ngwenyama N, Liu Y, Walker JC, Zhang S. 2007. Stomatal development and patterning are regulated by environmentally responsive mitogen-activated protein kinases in *Arabidopsis*. *Plant Cell* 19:63–73
156. Weijers D, Geldner N, Offringa R, Jürgens G. 2001. Seed development: early paternal gene activity in *Arabidopsis*. *Nature* 414:709–10
157. Weijers D, Schlereth A, Ehrismann JS, Schwank G, Kientz M, Jürgens G. 2006. Auxin triggers transient local signaling for cell specification in *Arabidopsis* embryogenesis. *Dev. Cell* 10:265–70
158. Weijers D, Van Hamburg J-P, Van Rijn E, Hooykaas PJJ, Offringa R. 2003. Diphtheria toxin-mediated cell ablation reveals interregional communication during Arabidopsis seed development. *Plant Physiol.* 133:1882–92

159. Weir I, Lu J, Cook H, Causier B, Schwarz-Sommer Z, Davies B. 2004. *CUPULIFORMIS* establishes lateral organ boundaries in *Antirrhinum*. *Development* 131:915–22
160. Williams L, Grigg SP, Xie M, Christensen S, Fletcher JC. 2005. Regulation of *Arabidopsis* shoot apical meristem and lateral organ formation by microRNA *miR166g* and its *AtHD-ZIP* target genes. *Development* 132:3657–68
161. Wu G, Lin WC, Huang T, Poethig RS, Springer PS, Kerstetter RA. 2008. KANADI1 regulates adaxial-abaxial polarity in *Arabidopsis* by directly repressing the transcription of *ASYMMETRIC LEAVES2*. *Proc. Natl. Acad. Sci. USA* 105:16392–97
162. Wu KL, Guo ZJ, Wang HH, Li J. 2005. The WRKY family of transcription factors in rice and *Arabidopsis* and their origins. *DNA Res.* 12:9–26
163. Wu X, Chory J, Weigel D. 2007. Combinations of *WOX* activities regulate tissue proliferation during *Arabidopsis* embryonic development. *Dev. Biol.* 309:306–16
164. Wysocka-Diller JW, Helariutta Y, Fukaki H, Malamy JE, Benfey PN. 2000. Molecular analysis of SCARECROW function reveals a radial patterning mechanism common to root and shoot. *Development* 127:595–603
165. Xu J, Zhang H-Y, Xie C-H, Xue H-W, Dijkhuis P, Liu C-M. 2005. *EMBRYONIC FACTOR 1* encodes an AMP deaminase and is essential for the zygote to embryo transition in Arabidopsis. *Plant J.* 42:743–56
166. Yanai O, Shani E, Dolezal K, Tarkowski P, Sablowski R, et al. 2005. *Arabidopsis* KNOXI proteins activate cytokinin biosynthesis. *Curr. Biol.* 15:1566–71
167. Yang JY, Chung MC, Tu CY, Leu WM. 2002. *OSTF1*: a HD-GL2 family homeobox gene is developmentally regulated during early embryogenesis in rice. *Plant Cell Physiol.* 43:628–38
168. Yang X, Zhang X. 2010. Regulation of somatic embryogenesis in higher plants. *Crit. Rev. Plant Sci.* 29:36–57
169. Yin Y, Vafeados D, Tao Y, Yoshida S, Asami T, Chory J. 2005. A new class of transcription factors mediates brassinosteroid-regulated gene expression in *Arabidopsis*. *Cell* 120:249–59
170. Zhao J, Xin H, Qu L, Ning J, Peng X, et al. 2011. Dynamic changes of transcript profiles after fertilization are associated with de novo transcription and maternal elimination in tobacco zygote, and mark the onset of the maternal-to-zygotic transition. *Plant J.* 65:131–45
171. Zhou G-K, Kubo M, Zhong R, Demura T, Ye Z-H. 2007. Overexpression of miR165 affects apical meristem formation, organ polarity establishment and vascular development in *Arabidopsis*. *Plant Cell Physiol.* 48:391–404
172. Zhu H, Hu F, Wang R, Zhou X, Sze SH, et al. 2011. *Arabidopsis* Argonaute10 specifically sequesters miR166/165 to regulate shoot apical meristem development. *Cell* 145:242–56
173. Zimmermann R, Werr W. 2005. Pattern formation in the monocot embryo as revealed by *NAM* and *CUC3* orthologues from *Zea mays* L. *Plant Mol. Biol.* 58:669–85

7.3. Eigenanteil an Publikationen

Daniel Slane[*], Jixiang Kong[*], Kenneth W. Berendzen, Joachim Kilian, Agnes Henschen, Martina Kolb, Markus Schmid, Klaus Harter, Ulrike Mayer, Ive De Smet, Martin Bayer[1] and Gerd Jürgens. Cell type-specific transcriptome analysis in the early *Arabidopsis thaliana* embryo. Manuskript angenommen zur Publikation bei *Development*. * Gleichwertiger Beitrag.

Bei der Ausarbeitung des Grundkonzeptes sowie der ursprünglichen experimentellen Planung war ich hauptsächlich beteiligt unter Anleitung von Ive de Smet und Gerd Jürgens. Bei der weiteren experimentellen Planung sowie Deutung der Ergebnisse, der Etablierung der Methodik, Generierung der Expressionsdaten und deren *in vivo* Bestätigung teilte ich die Arbeit mit Jixiang Kong. Die *in silico* Auswertung der Expressionsdaten geht zum Grossteil auf mich zurück. Zur Erstellung des Manuskripts leistete ich einen größeren Beitrag.

Lau, S., Slane, D., Herud, O., Kong, J. and Jurgens, G. (2012). Early embryogenesis in flowering plants: setting up the basic body pattern. *Annu Rev Plant Biol* **63**, 483-506.

In diesem Übersichtsartikel wurde der Teil “SHOOT MERISTEM SPECIFICATION AND COTYLEDON INITIATION“ von mir verfasst, andere Teile wurden teilweise von mir mitgestaltet.

8. Weitere Publikationen

Reichardt, I., Slane, D., El Kasmi, F., Knöll, C., Fuchs, R., Mayer, U., Lipka, V. and Jürgens, G. (2011). Mechanisms of functional specificity among plasma-membrane syntaxins in Arabidopsis. *Traffic* **12**, 1269-1280.

9. Referenzliste

Abe, M., Takahashi, T. and Komeda, Y. (2001). Identification of a *cis*-regulatory element for L1 layer-specific gene expression, which is targeted by an L1-specific homeodomain protein. *Plant J.* **26**, 487-494.

Abe, M., Katsumata, H., Komeda, Y. and Takahashi, T. (2003). Regulation of shoot epidermal cell differentiation by a pair of homeodomain proteins in *Arabidopsis*. *Development* **130**, 635-643.

Aida, M., Ishida, T. and Tasaka, M. (1999). Shoot apical meristem and cotyledon formation during *Arabidopsis* embryogenesis: interaction among the *CUP-SHAPED COTYLEDON* and *SHOOT MERISTEMLESS* genes. *Development* **126**, 1563-1570.

Aida, M., Ishida, T., Fukaki, H., Fujisawa, H. and Tasaka, M. (1997). Genes involved in organ separation in Arabidopsis: an analysis of the cup-shaped cotyledon mutant. *Plant Cell* **9**, 841-857.

Autran, D., Baroux, C., Raissig, M. T., Lenormand, T., Wittig, M., Grob, S., Steimer, A., Barann, M., Klostermeier, U. C., Leblanc, O. et al. (2011). Maternal epigenetic pathways control parental contributions to *Arabidopsis* early embryogenesis. *Cell* **145**, 707-719.

Barthelson, R. A., Lambert, G. M., Vanier, C., Lynch, R. M. and Galbraith, D. W. (2007). Comparison of the contributions of the nuclear and cytoplasmic compartments to global gene expression in human cells. *BMC Genomics* **8**, 340.

Barton, M. K. and Poethig, R. S. (1993). Formation of the shoot apical meristem in *Arabidopsis thaliana*: an analysis of development in the wild type and in the *shoot meristemless* mutant. *Development* **119**, 823-831.

Bayer, M., Nawy, T., Giglione, C., Galli, M., Meinnel, T. and Lukowitz, W. (2009). Paternal control of embryonic patterning in *Arabidopsis thaliana*. *Science* **323**, 1485-1488.

Belmonte, M. F., Kirkbride, R. C., Stone, S. L., Pelletier, J. M., Bui, A. Q., Yeung, E. C., Hashimoto, M., Fei, J., Harada, C. M., Munoz, M. D. et al. (2013). Comprehensive developmental profiles of gene activity in regions and subregions of the Arabidopsis seed. *Proc Natl Acad Sci U S A* **110**, E435-444.

Benkova, E., Michniewicz, M., Sauer, M., Teichmann, T., Seifertova, D., Jürgens, G. and Friml, J. (2003). Local, efflux-dependent auxin gradients as a common module for plant organ formation. *Cell* **115**, 591-602.

Berger, F. and Twell, D. (2011). Germline specification and function in plants. *Annu Rev Plant Biol* **62**, 461-484.

Birnbaum, K., Shasha, D. E., Wang, J. Y., Jung, J. W., Lambert, G. M., Galbraith, D. W. and Benfey, P. N. (2003). A gene expression map of the Arabidopsis root. *Science* **302**, 1956-1960.

Birnbaum, K., Jung, J. W., Wang, J. Y., Lambert, G. M., Hirst, J. A., Galbraith, D. W. and Benfey, P. N. (2005). Cell type-specific expression profiling in plants via cell sorting of protoplasts from fluorescent reporter lines. *Nat Methods* **2**, 615-619.

Bonner, W. A., Hulett, H. R., Sweet, R. G. and Herzenberg, L. A. (1972). Fluorescence Activated Cell Sorting. *Rev Sci Instrum* **43**, 404-409.

Boyes, D. C., Zayed, A. M., Ascenzi, R., McCaskill, A. J., Hoffman, N. E., Davis, K. R. and Gorlach, J. (2001). Growth stage-based phenotypic analysis of Arabidopsis: a model for high throughput functional genomics in plants. *Plant Cell* **13**, 1499-1510.

Brady, S. M., Orlando, D. A., Lee, J. Y., Wang, J. Y., Koch, J., Dinneny, J. R., Mace, D., Ohler, U. and Benfey, P. N. (2007). A high-resolution root spatiotemporal map reveals dominant expression patterns. *Science* **318**, 801-806.

Breuninger, H., Rikirsch, E., Hermann, M., Ueda, M. and Laux, T. (2008). Differential expression of *WOX* genes mediates apical-basal axis formation in the *Arabidopsis* embryo. *Dev. Cell* **14**, 867-876.

Byrne, M. E., Simorowski, J. and Martienssen, R. A. (2002). *ASYMMETRIC LEAVES1* reveals *knox* gene redundancy in *Arabidopsis*. *Development* **129**, 1957-1965.

Byrne, M. E., Barley, R., Curtis, M., Arroyo, J. M., Dunham, M., Hudson, A. and Martienssen, R. A. (2000). *Asymmetric leaves1* mediates leaf patterning and stem cell function in *Arabidopsis*. *Nature* **408**, 967-971.

Casson, S., Spencer, M., Walker, K. and Lindsey, K. (2005). Laser capture microdissection for the analysis of gene expression during embryogenesis of Arabidopsis. *Plant J* **42**, 111-123.

Chandler, J. W., Cole, M. and Werr, W. (2008). The role of DORNROESCHEN (DRN) and DRN-LIKE (DRNL) in Arabidopsis embryonic patterning. *Plant Signal Behav* **3**, 49-51.

Cheng, Y., Qin, G., Dai, X. and Zhao, Y. (2008). *NPY* genes and AGC kinases define two key steps in auxin-mediated organogenesis in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* **105**, 21017-21022.

Cole, M., Chandler, J., Weijers, D., Jacobs, B., Comelli, P. and Werr, W. (2009). DORNROSCHEN is a direct target of the auxin response factor MONOPTEROS in the Arabidopsis embryo. *Development* **136**, 1643-1651.

Cong, L., Ran, F. A., Cox, D., Lin, S., Barretto, R., Habib, N., Hsu, P. D., Wu, X., Jiang, W., Marraffini, L. A. et al. (2013). Multiplex genome engineering using CRISPR/Cas systems. *Science* **339**, 819-823.

De Smet, I., Lau, S., Mayer, U. and Jürgens, G. (2010). Embryogenesis - the humble beginnings of plant life. *Plant J.* **61**, 959-970.

De Smet, I., Vassileva, V., De Rybel, B., Levesque, M. P., Grunewald, W., Van Damme, D., Van Noorden, G., Naudts, M., Van Isterdael, G., De Clercq, R. et al. (2008). Receptor-like kinase ACR4 restricts formative cell divisions in the Arabidopsis root. *Science* **322**, 594-597.

Deal, R. B. and Henikoff, S. (2010). A simple method for gene expression and chromatin profiling of individual cell types within a tissue. *Dev Cell* **18**, 1030-1040.

Deal, R. B. and Henikoff, S. (2011). The INTACT method for cell type-specific gene expression and chromatin profiling in Arabidopsis thaliana. *Nat Protoc* **6**, 56-68.

Dinneny, J. R., Long, T. A., Wang, J. Y., Jung, J. W., Mace, D., Pointer, S., Barron, C., Brady, S. M., Schiefelbein, J. and Benfey, P. N. (2008). Cell identity mediates the response of Arabidopsis roots to abiotic stress. *Science* **320**, 942-945.

Emmert-Buck, M. R., Bonner, R. F., Smith, P. D., Chuaqui, R. F., Zhuang, Z., Goldstein, S. R., Weiss, R. A. and Liotta, L. A. (1996). Laser capture microdissection. *Science* **274**, 998-1001.

Friml, J., Vieten, A., Sauer, M., Weijers, D., Schwarz, H., Hamann, T., Offringa, R. and Jürgens, G. (2003). Efflux-dependent auxin gradients establish the apical-basal axis of *Arabidopsis*. *Nature* **426**, 147-153.

Friml, J., Yang, X., Michniewicz, M., Weijers, D., Quint, A., Tietz, O., Benjamins, R., Ouwerkerk, P. B., Ljung, K., Sandberg, G. et al. (2004). A PINOID-dependent binary switch in apical-basal PIN polar targeting directs auxin efflux. *Science* **306**, 862-865.

Friml, J., Yang, X., Michniewicz, M., Weijers, D., Quint, A., Tietz, O., Benjamins, R., Ouwerkerk, P. B. F., Ljung, K., Sandberg, G. r. et al. (2004). A PINOID-dependent binary switch in apical-basal PIN polar targeting directs auxin efflux. *Science* **306**, 862 -865.

Furutani, M., Vernoux, T., Traas, J., Kato, T., Tasaka, M. and Aida, M. (2004). *PIN-FORMED1* and *PINOID* regulate boundary formation and cotyledon development in *Arabidopsis* embryogenesis. *Development* **131**, 5021-5030.

Girke, T., Todd, J., Ruuska, S., White, J., Benning, C. and Ohlrogge, J. (2000). Microarray analysis of developing Arabidopsis seeds. *Plant Physiol* **124**, 1570-1581.

Grigg, S. P., Galinha, C., Kornet, N., Canales, C., Scheres, B. and Tsiantis, M. (2009). Repression of apical homeobox genes is required for embryonic root development in *Arabidopsis*. *Curr. Biol.* **19**, 1485-1490.

Haecker, A., Gross-Hardt, R., Geiges, B., Sarkar, A., Breuninger, H., Herrmann, M. and Laux, T. (2004). Expression dynamics of WOX genes mark cell fate decisions during early embryonic patterning in Arabidopsis thaliana. *Development* **131**, 657-668.

Hamamura, Y., Nagahara, S. and Higashiyama, T. (2012). Double fertilization on the move. *Curr Opin Plant Biol* **15**, 70-77.

Hamann, T., Mayer, U. and Jürgens, G. (1999). The auxin-insensitive *bodenlos* mutation affects primary root formation and apical-basal patterning in the *Arabidopsis* embryo. *Development* **126**, 1387-1395.

Huang, F., Zago, M. K., Abas, L., van Marion, A., Galvan-Ampudia, C. S. and Offringa, R. (2010). Phosphorylation of conserved PIN motifs directs *Arabidopsis* PIN1 polarity and auxin transport. *Plant Cell* **22**, 1129-1142.

Jacob, Y., Mongkolsiriwatana, C., Veley, K. M., Kim, S. Y. and Michaels, S. D. (2007). The nuclear pore protein AtTPR is required for RNA homeostasis, flowering time, and auxin signaling. *Plant Physiol* **144**, 1383-1390.

Jeong, S., Palmer, T. M. and Lukowitz, W. (2011a). The RWP-RK Factor *GROUNDED* Promotes Embryonic Polarity by Facilitating YODA MAP Kinase Signaling. *Curr. Biol.* **21**, 1268-1276.

Jeong, S., Bayer, M. and Lukowitz, W. (2011b). Taking the very first steps: from polarity to axial domains in the early Arabidopsis embryo. *J. Exp. Bot.* **62**, 1687-1697.

Johri, B. M., Ambegaokar, K. B. and Srivastava, P. S. (1992). Comparative Embryology of Angiosperms. Berlin: Springer-Verlag.

Jürgens, G. (2001). Apical-basal pattern formation in Arabidopsis embryogenesis. *EMBO J.* **20**, 3609-3616.

Kerk, N. M., Ceserani, T., Tausta, S. L., Sussex, I. M. and Nelson, T. M. (2003). Laser capture microdissection of cells from plant tissues. *Plant Physiol* **132**, 27-35.

Lau, S., Slane, D., Herud, O., Kong, J. and Jurgens, G. (2012). Early embryogenesis in flowering plants: setting up the basic body pattern. *Annu Rev Plant Biol* **63**, 483-506.

Lau, S., Ehrismann, J. S., Schlereth, A., Takada, S., Mayer, U. and Jürgens, G. (2010). Cell-cell communication in *Arabidopsis* early embryogenesis. *Eur. J. Cell Biol.* **89**, 225-230.

Laux, T., Mayer, K. F. X., Berger, J. and Jürgens, G. (1996). The *WUSCHEL* gene is required for shoot and floral meristem integrity in *Arabidopsis*. *Development* **122**, 87-96.

Le, B. H., Cheng, C., Bui, A. Q., Wagmaister, J. A., Henry, K. F., Pelletier, J., Kwong, L., Belmonte, M., Kirkbride, R., Horvath, S. et al. (2010). Global analysis of gene activity during Arabidopsis seed development and identification of seed-specific transcription factors. *Proc Natl Acad Sci U S A* **107**, 8063-8070.

Lenhard, M., Jürgens, G. and Laux, T. (2002). The *WUSCHEL* and *SHOOTMERISTEMLESS* genes fulfil complementary roles in *Arabidopsis* shoot meristem regulation. *Development* **129**, 3195-3206.

Lie, C., Kelsom, C. and Wu, X. (2012). WOX2 and STIMPY-LIKE/WOX8 promote cotyledon boundary formation in Arabidopsis. *Plant J* **72**, 674-682.

Lohmann, J. U., Hong, R. L., Hobe, M., Busch, M. A., Parcy, F., Simon, R. and Weigel, D. (2001). A molecular link between stem cell regulation and floral patterning in Arabidopsis. *Cell* **105**, 793-803.

Lu, P., Porat, R., Nadeau, J. A. and O'Neill, S. D. (1996). Identification of a meristem L1 layer-specific gene in Arabidopsis that is expressed during embryonic pattern formation and defines a new class of homeobox genes. *Plant Cell* **8**, 2155-2168.

Lukowitz, W., Roeder, A., Parmenter, D. and Somerville, C. (2004). A MAPKK kinase gene regulates extra-embryonic cell fate in *Arabidopsis*. *Cell* **116**, 109-119.

Lynn, K., Fernandez, A., Aida, M., Sedbrook, J., Tasaka, M., Masson, P. and Barton, M. K. (1999). The *PINHEAD/ZWILLE* gene acts pleiotropically in *Arabidopsis* development and has overlapping functions with the *ARGONAUTE1* gene. *Development* **126**, 469-481.

Mansfield, S. G. and Briarty, L. G. (1991). Early embryogenesis in *Arabidopsis thaliana*. II. The developing embryo. *Can. J. Bot.* **69**, 461-476.

Mao, G., Meng, X., Liu, Y., Zheng, Z., Chen, Z. and Zhang, S. (2011). Phosphorylation of a WRKY transcription factor by two pathogen-responsive MAPKs drives phytoalexin biosynthesis in *Arabidopsis*. *Plant Cell* **23**, 1639-1653.

Mayer, U., Büttner, G. and Jürgens, G. (1993). Apical-basal pattern formation in the *Arabidopsis* embryo: studies on the role of the *gnom* gene. *Development* **117**, 149-162.

Mosher, R. A. and Melnyk, C. W. (2010). siRNAs and DNA methylation: seedy epigenetics. *Trends Plant Sci* **15**, 204-210.

Mustroph, A., Zanetti, M. E., Jang, C. J. H., Holtan, H. E., Repetti, P. P., Galbraith, D. W., Girke, T. and Bailey-Serres, J. (2009). Profiling translatomes of discrete cell populations resolves altered cellular priorities during hypoxia in Arabidopsis. **106**, 18843-18848.

Nawy, T., Bayer, M., Mravec, J., Friml, J., Birnbaum, K. D. and Lukowitz, W. (2010). The GATA factor *HANABA TARANU* is required to position the proembryo boundary in the early *Arabidopsis* embryo. *Dev. Cell* **19**, 103-113.

Nodine, M. D. and Bartel, D. P. (2012). Maternal and paternal genomes contribute equally to the transcriptome of early plant embryos. *Nature* **482**, 94-97.

Nodine, M. D., Yadegari, R. and Tax, F. E. (2007). *RPK1* and *TOAD2* Are Two Receptor-like Kinases Redundantly Required for *Arabidopsis* Embryonic Pattern Formation *Dev. Cell* **12**, 943-956.

Palovaara, J., Saiga, S. and Weijers, D. (2013). Transcriptomics approaches in the early Arabidopsis embryo. *Trends Plant Sci* **18**, 514-521.

Rademacher, E. H., Lokerse, A. S., Schlereth, A., Llavata-Peris, C. I., Bayer, M., Kientz, M., Freire Rios, A., Borst, J. W., Lukowitz, W., Jurgens, G. et al. (2012). Different auxin response machineries control distinct cell fates in the early plant embryo. *Dev Cell* **22**, 211-222.

Schmid, M., Davison, T. S., Henz, S. R., Pape, U. J., Demar, M., Vingron, M., Scholkopf, B., Weigel, D. and Lohmann, J. U. (2005). A gene expression map of Arabidopsis thaliana development. *Nat Genet* **37**, 501-506.

Sivaramakrishna, D. (1978). Size relationships of apical cell and basal cell in two-celled embryos in angiosperms. *Can. J. Bot.* **56**, 1434-1438.

Slotkin, R. K., Vaughn, M., Borges, F., Tanurdzic, M., Becker, J. D., Feijo, J. A. and Martienssen, R. A. (2009). Epigenetic reprogramming and small RNA silencing of transposable elements in pollen. *Cell* **136**, 461-472.

Spencer, M. W., Casson, S. A. and Lindsey, K. (2007). Transcriptional profiling of the Arabidopsis embryo. *Plant Physiol* **143**, 924-940.

Spinelli, S. V., Martin, A. P., Viola, I. L., Gonzalez, D. H. and Palatnik, J. F. (2011). A mechanistic link between *STM* and *CUC1* during *Arabidopsis* development. *Plant Physiol.* **156**, 1894-1904.

Steiner, F. A., Talbert, P. B., Kasinathan, S., Deal, R. B. and Henikoff, S. (2012). Cell-type-specific nuclei purification from whole animals for genome-wide expression and chromatin profiling. *Genome Res* **22**, 766-777.

Takada, S. and Jürgens, G. (2007). Transcriptional regulation of epidermal cell fate in the *Arabidopsis* embryo. *Development* **134**, 1141-1150.

Takada, S., Hibara, K., Ishida, T. and Tasaka, M. (2001). The *CUP-SHAPED COTYLEDON1* gene of *Arabidopsis* regulates shoot apical meristem formation. *Development* **128**, 1127-1135.

Tanaka, H., Watanabe, M., Sasabe, M., Hiroe, T., Tanaka, T., Tsukaya, H., Ikezaki, M., Machida, C. and Machida, Y. (2007). Novel receptor-like kinase ALE2 controls shoot development by specifying epidermis in *Arabidopsis*. *Development* **134**, 1643-1652.

Treml, B. S., Winderl, S., Radykewicz, R., Herz, M., Schweizer, G., Hutzler, P., Glawischnig, E. and Ruiz, R. A. (2005). The gene *ENHANCER OF PINOID* controls cotyledon development in the *Arabidopsis* embryo. *Development* **132**, 4063-4074.

Ueda, M., Zhang, Z. and Laux, T. (2011). Transcriptional activation of *Arabidopsis* axis patterning genes *WOX8/9* links zygote polarity to embryo development. *Dev. Cell* **20**, 264-270.

Wang, H., Ngwenyama, N., Liu, Y., Walker, J. C. and Zhang, S. (2007). Stomatal development and patterning are regulated by environmentally responsive mitogen-activated protein kinases in *Arabidopsis*. *Plant Cell* **19**, 63-73.

Webb, M. C. and Gunning, B. E. (1991). The microtubular cytoskeleton during development of the zygote, proemhryo and free-nuclear endosperm in Arabidopsis thaliana (L.) Heynh. *Planta* **184**, 187-195.

Wendrich, J. R. and Weijers, D. (2013). The Arabidopsis embryo as a miniature morphogenesis model. *New Phytol* **199**, 14-25.

Wu, X., Chory, J. and Weigel, D. (2007). Combinations of *WOX* activities regulate tissue proliferation during *Arabidopsis* embryonic development. *Dev. Biol.* **309**, 306-316.

Xiang, D., Venglat, P., Tibiche, C., Yang, H., Risseeuw, E., Cao, Y., Babic, V., Cloutier, M., Keller, W., Wang, E. et al. (2011). Genome-wide analysis reveals gene expression and metabolic network dynamics during embryo development in Arabidopsis. *Plant Physiol* **156**, 346-356.

Yadav, R. K., Girke, T., Pasala, S., Xie, M. and Reddy, G. V. (2009). Gene expression map of the Arabidopsis shoot apical meristem stem cell niche. *Proc Natl Acad Sci U S A* **106**, 4941-4946.

Zanetti, M. E., Chang, I. F., Gong, F., Galbraith, D. W. and Bailey-Serres, J. (2005). Immunopurification of polyribosomal complexes of Arabidopsis for global analysis of gene expression. *Plant Physiol* **138**, 624-635.

Zhang, C., Barthelson, R. A., Lambert, G. M. and Galbraith, D. W. (2008). Global characterization of cell-specific gene expression through fluorescence-activated sorting of nuclei. *Plant Physiol* **147**, 30-40.

Zhu, H., Hu, F., Wang, R., Zhou, X., Sze, S. H., Liou, L. W., Barefoot, A., Dickman, M. and Zhang, X. (2011). *Arabidopsis* Argonaute10 specifically sequesters miR166/165 to regulate shoot apical meristem development. *Cell* **145**, 242-256.

Zong, Q., Schummer, M., Hood, L. and Morris, D. R. (1999). Messenger RNA translation state: the second dimension of high-throughput expression screening. *Proc Natl Acad Sci U S A* **96**, 10632-10636.