Genomics 100 (2012) 116–124

Contents lists available at SciVerse ScienceDirect

Genomics

journal homepage: www.elsevier.com/locate/ygeno

A genome-wide transcriptome profiling reveals the early molecular events during callus initiation in *Arabidopsis* multiple organs☆

Ke Xu [a,b], Jing Liu [a,b], Mingzhu Fan [a,b], Wei Xin [a], Yuxin Hu [a,c], Chongyi Xu [a,*]

[a] Key Laboratory of Plant Molecular Physiology, Institute of Botany, Chinese Academy of Sciences, Beijing, 100093, China
[b] Graduate School of Chinese Academy of Sciences, Beijing, 100093, China
[c] National Center for Plant Gene Research, Beijing, 100093, China

ARTICLE INFO

Article history:
Received 26 January 2012
Accepted 24 May 2012
Available online 1 June 2012

Keywords:
Arabidopsis thaliana
Callus initiation
Microarray
Transcriptome

ABSTRACT

Induction of a pluripotent cell mass termed callus is the first step in an in vitro plant regeneration system, which is required for subsequent regeneration of new organs or whole plants. However, the early molecular mechanism underlying callus initiation is largely elusive. Here, we analyzed the dynamic transcriptome profiling of callus initiation in *Arabidopsis* aerial and root explants and identified 1342 differentially expressed genes in both explants after incubation on callus-inducing medium. Detailed categorization revealed that the differentially expressed genes were mainly related to hormone homeostasis and signaling, transcriptional and post transcriptional regulations, protein phosphorelay cascades and DNA- or chromatin-modification. Further characterization showed that overexpression of two transcription factors, *HB52* or *CRF3*, resulted in the callus formation in transgenic plants without exogenous auxin. Therefore, our comprehensive analyses provide some insight into the early molecular regulations during callus initiation and are useful for further identification of the regulators governing callus formation.

Crown Copyright © 2012 Published by Elsevier Inc. All rights reserved.

1. Introduction

Plant cells have been thought to have pluripotency because they have the ability to regenerate the fully arrayed plant organs from already differentiated tissues under appropriate culture conditions. More than half a century ago, Skoog and Miller showed that the developmental fates of tobacco (*Nicotiana tabacum*) pith tissue could be directed by plant hormones auxin and cytokinin to regenerate a whole plant in an in vitro system [1]. Since then, similar manipulations have been widely practiced in a variety of plant species and the concept for in vitro plant regeneration has been well established. Generally, a mass of pluripotent cells called callus could be induced from explants on the medium with an optimal concentration of auxin and cytokinin, and subsequent culture of the callus with high cytokinin/auxin ratio leads to shoot regeneration while the low ratio favors root formation. Although such in vitro regeneration system has become a platform to improve the agronomic traits of plants through micropropagation and transgenic modification, the molecular mechanisms underlying in vitro plant regeneration and how two simple plant hormones direct these developmental fates are largely unknown.

Callus is a high proliferating cell mass that has long been inferred to be a dedifferentiated tissue, and the genome has undergone reprogramming to restore stem cell state and pluripotency is acquired during the callus formation [2,3]. Some evidences from the cell culture appear to support this notion. For examples, tobacco protoplasts require two rounds of chromatin decondensation for cells to re-enter cell division and form callus [4] and the acquisition of competence for switching cell fate correlates with chromatin reorganization, redistribution of HP1 protein and an increase in acetylated histone H3 at K9 and K14 [5]. In *Arabidopsis* callus cells, pericentric heterochromatin is dissociated on a large scale and sequentially reassembled [6], and the changes in telomere length, telomerase activity and heterochromatin redistribution have been observed during removal of the somatic cell walls [6–8]. However, some recent works suggest that callus induction in multiple organs may originate predominantly from a pre-existing population of pericycle or pericycle-like cells within some explants and callus forms via differentiation of the root meristem-like tissue [9,10]. A recent study also shows that cambial meristematic cells derived from the cambium of *Taxus cuspidata* are likely stem-like cells and can differentiate at high frequency [11]. Although some previous works attempted to dissect the callus formation of *Arabidopsis* root or cotyledon explants at transcriptome and proteome levels [2,3,12],

☆ Microarray data from this article have been deposited with GenBank Data Libraries under Accession No. GSE29543.

* Corresponding author. Fax: +86 10 62836950.
E-mail addresses: xuke@ibcas.ac.cn (K. Xu), liujing@ibcas.ac.cn (J. Liu), fmingzhu@ibcas.ac.cn (M. Fan), xwforever48@ibcas.ac.cn (W. Xin), huyuxin@ibcas.ac.cn (Y. Hu), xuchongyi@ibcas.ac.cn (C. Xu).

0888-7543/$ – see front matter. Crown Copyright © 2012 Published by Elsevier Inc. All rights reserved.
doi:10.1016/j.ygeno.2012.05.013

less is known about the common molecular events at callus initiation stages among different explants and how plant hormone signals are involved.

To gain insight into the common molecular events at the early stage of callus formation, we investigated the dynamic gene expression profiles in *Arabidopsis* aerial and root explants on CIM within 96 h and analyzed the differentially expressed genes in both explants. The outcome of this work summarizes the very early molecular events generally occurring in multiple organs during callus induction and yields a reliable source of genes for comprehensive understanding of the regulatory networks governing callus initiation.

2. Materials and methods

2.1. Plant materials and culture conditions

All *Arabidopsis thaliana* (Columbia-0) seeds were germinated on the MS (Murashige and Skoog) medium at 22 ± 1 °C with a 16 h light/8 h dark photoperiod. Aerial and root fragments from 10-day-old seedlings were transferred to the callus-inducing medium (CIM: B5 solid medium with 2.2 μM 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.2 μM kinetin, supplemented with 0.5 g l^{-1} MES, 20 g l^{-1} glucose and 2.5 g l^{-1} phytagel) and harvested at 0, 12, 24, 48 and 96 h for microarray analysis and morphological observation. The aerial explants contained the whole aerial parts above the root–hypocotyl junction of 10-day-old seedlings.

2.2. RNA extraction and microarray analyses

Total RNA was isolated using a guanidine thiocyanate extraction buffer described in the previous report [13]. The integrity of RNA was monitored by denaturing agarose gel electrophoresis. Microarray experiments were performed according to the standard Affymetrix protocol. Expression levels were estimated from Affymetrix hybridization intensity data using MicroArray Suite 5.0 (Affymetrix 2001). The data were normalized using the robust multiarray averaging (RMA) method and the $\log_2$ values were used for comparison of the three independent replicates. The relative ratios were the expression level of genes both *Arabidopsis* explants incubated on CIM for different lengths of time versus that of original explants. The genes showing significant changes in their expression were selected by applying a *t*-test (one-way ANOVA Welch *t*-test, and an FDR (false discovery rate) corrected $P<0.05$) and a cutoff value of two-fold change during callus induction. To test the hybridization quality, "*Arabidopsis* control genes" coding for GAPDH, actin, tubulin, ubiquitin, and several ribosomal RNAs (25S, 5S), spotted by the manufacturer, were verified. The expression ratios of the control genes were consistently in the range of 0.81–1.29. Microarray data reported in this article have been submitted to GEO (NCBI) [GenBank: GSE29543].

2.3. Real-time quantitative RT-PCR

Total RNA was treated with RNase-free Dnase I, according to the manufacturer's instructions (Qiagen, http://www.qiagen.com), and was quantified by 260/280-nm UV light absorption. About 1 μg total RNA was reverse transcribed using the Supertranscript III RT kit (Invitrogen). cDNA diluted ten times was used for real-time quantitative RT-PCR (qPCR). Eight genes that demonstrated differential expression in the microarray data were selected for verifying the reliability of the microarray data. In addition, the expression patterns of the reported root meristem genes, *WOX5* and *PLT1* [10] were detected by qPCR in the transgenic plants. Primers were designed to amplify products less than 200 bases and are listed in Supplemental Table 1. qPCR was performed with a Rotor-Gene 3000 thermocycler (Corbett Research, Sydney, Australia) with the SYBR® Premix Ex Taq™ II kit (Takara, Dalian, China). The efficiency of amplification of various RNAs was assessed relative to the amplification of transcripts for *ACTIN2* gene (*At3g18780*). RNA samples were assayed in triplicate. The relative expression values were calculated using a modified $2^{-\Delta\Delta CT}$ method [14].

2.4. Plasmid construction and Arabidopsis transformation

The coding regions (CDS) of *HB52* and *CRF3* were cloned into pVIP96 [15] for generation of the *Pro35S:HB52* and *Pro35S:CRF3* constructs, respectively, and the plasmids were introduced into *Arabidopsis* by *Agrobacterium tumefaciens* with the floral dipping method [16]. Primers are shown in Supplemental Table 1.

3. Results

3.1. Callus initiation occurs within 96 h in Arabidopsis multiple organs

To examine the callus formation in different explants, we first incubated the *Arabidopsis* aerial and root fragments of 10-day-old seedling on CIM for 0, 12, 24, 48 and 96 h, and observed the callus initiation process in cotyledon, hypocotyl and root explants at each time point (Fig. 1). There was no microscopical change in different organs within 24 h of the incubation (Figs. 1A–C, F–H, K–M). However, the periclinal and anticlinal divisions were successively observed in the xylem pericycle cells of hypocotyls at 48 h, resulting in some protuberances positioned along longitudinal direction (Fig. 1I). At 96 h, cell divisions took place in the pericycle cells of roots and some small protuberances formed (Fig. 1E). Although no visible protuberance formed in the cotyledons until 96 h, cell division resumed exclusively in the vascular tissues of cotyledon petioles (Fig. 1O and Supplemental Fig. 1D). As protuberances mark the resuming of cell division for subsequent callus formation, our observations clearly indicate that the callus initiation in *Arabidopsis* multiple organs occurs within 96 h on CIM.

3.2. General features of gene expression profiles in callus initiation

Previous studies have shown that incubation of *Arabidopsis* root explants on CIM for 48 h was sufficient to confer the pluripotency of shoot regeneration on explants [17] and our observation also demonstrated that callus initiation happened within 96 h in multiple organs on CIM (Fig. 1). To explore the early molecular events during callus induction, we used *Arabidopsis* Affymetrix ATH1 GeneChips with a total number of 22746 probe sets corresponding to about 80% of known genes [18], and carried out a comprehensive analysis of gene expression of both root explants and aerial explants, which included hypocotyls and other aboveground parts of seedlings, when incubated on CIM for 0, 12, 24, 48 and 96 h. The resulting data were subjected to the robust multiarray averaging and $\log_2$ transformation before analysis (Supplemental Table 2), and very strong correlations were found for all time points within three biological replicates (referred to as A0, A12, A24, A48, A96, R0, R12, R24, R48 and R96), demonstrating the reliable reproducibility of gene expression profiles (Supplemental Table 3).

With a significance level of an FDR adjusted $P<0.05$ and a cut-off of a two-fold ratio of expression, 589 genes were up- or down-regulated in the aerial explants, 2053 were identified in root explants, and 106 genes were overlapped in both explants incubated on CIM for 12 h. Similarly, the distinct and overlapped genes responsive to CIM at 24, 48 and 96 h were identified in two explants (Fig. 2). Overall, a total of 3618 genes in the aerial explants, and 6929 genes in the root explants were identified to be up- or down-regulated during at least one time point after explants were cultured on CIM, among which 1342 genes were overlapped in both explants (Supplemental Table 4,

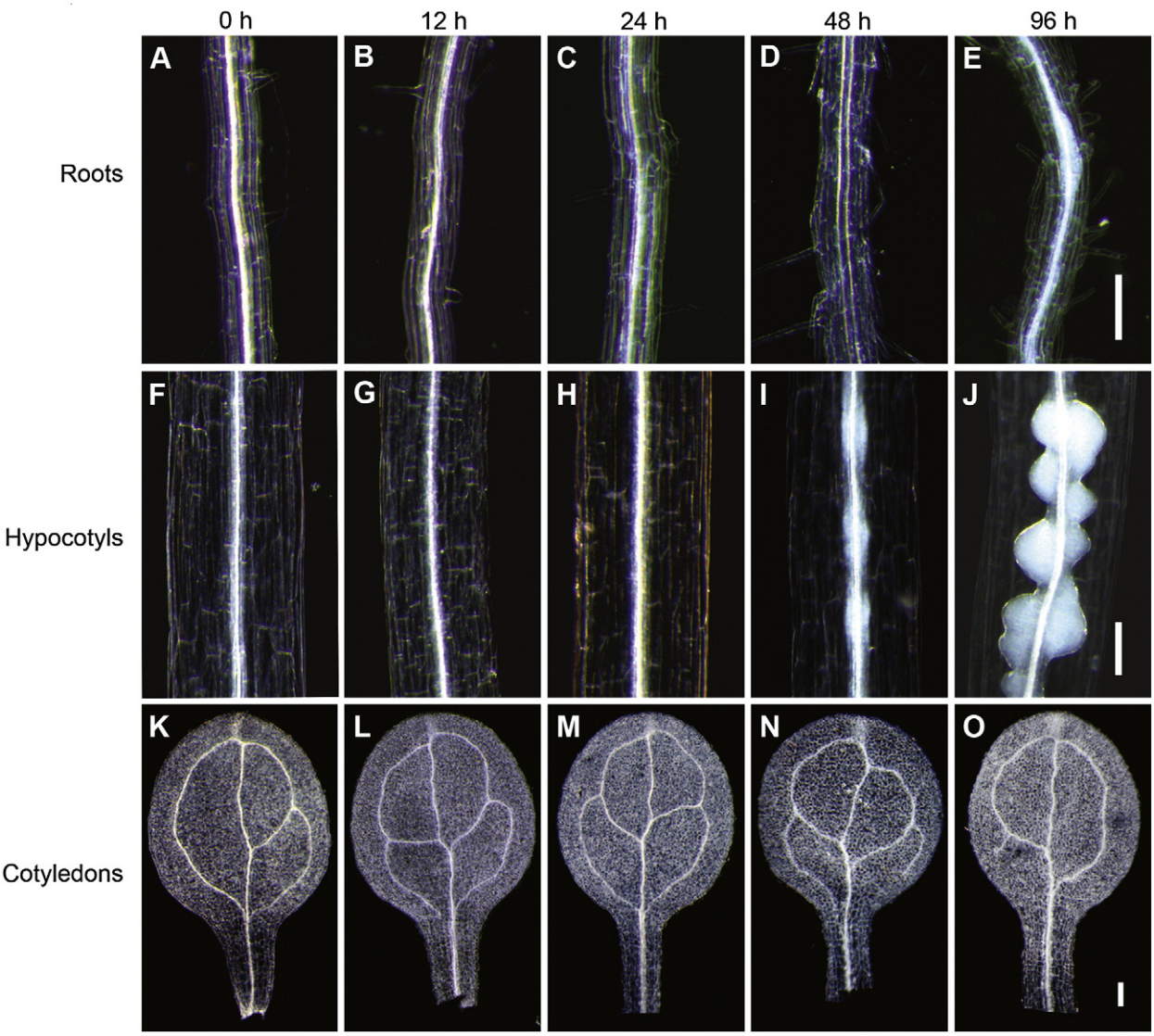

Fig. 1. Callus initiation in *Arabidopsis* multiple organs. Morphology of root (A–E), hypocotyl (F–J), and cotyledon (K–O) on CIM for times indicated. Bars = 100 μm. At least 50 samples of each organ were incubated on CIM and visualized under a microscope.

5 and 6). Because previous work showed that callus formation from *Arabidopsis* multiple organs including root, cotyledon and petal explants follows the same developmental pathway [10], we focused mainly on the overlapped genes that were up- or down-regulated in both aerial and root explants on CIM within 96 h.

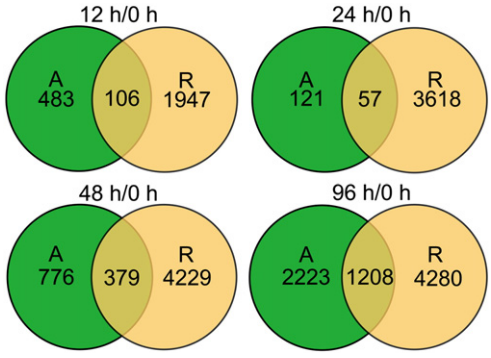

Fig. 2. Venn diagram showing numbers of genes differentially expressed in both explants during the callus induction. Numbers in circles indicate genes that exhibited ≥two-fold change in expression. The space of each group is not proportional to the gene numbers represented. A and R represent aerial and root explants, and time points are shown on top of each Venn diagram, respectively. Each Venn diagram represents relationship of differentially regulated genes identified in the two explants within the 96 h induction period.

3.3. Verification of microarray results by qPCR

To validate the gene expression profiles revealed by microarray, we performed real-time quantitative RT-PCR (qPCR) analyses of the dynamic expression of eight genes chosen from microarray data, among which seven (*At1g80370*, *At1g76520*, *At1g65920*, *At5g02760*, *At5g46280*, *HB52* and *CRF3*) were up-regulated and one (*At2g01520*) was down-regulated. As shown in Supplemental Fig. 2, despite varied quantitative levels of some genes, qPCR data indeed recapitulated the expression trends observed in microarray experiments. Thus, we conclude that the gene expression profiles revealed by the microarray data reliably reflect the genome-wide transcriptome changes during callus initiation process.

3.4. The expression of genes involved in hormone homeostasis and signaling during callus initiation

Because CIM contains a high concentration of auxin that has been considered to be required for callus induction, we first paid our attention

to the genes involved in auxin homeostasis, signaling and transport, including *TIR1*, *ABP1*, *Aux/IAAs*, *SAURs*, *GH3s*, *ARFs* and those encoding auxin influx and efflux carriers. Among 29 *Aux/IAAs*, six *IAA* genes (*IAA5*, *IAA19*, *IAA20*, *IAA16*, *IAA30* and *IAA13*) were induced by CIM and their expression profiles were similar between both explants during the incubation times. Of 20 putative *GH3s* in *Arabidopsis*, the expressions of six genes (*At1g59500*, *At2g23170*, *At4g27260*, *WES1*, *JAR1* and *At2g14960*) were changed at the different stages. Only *ARF5* was up-regulated at 96 h among 23 *ARFs*. For the genes encoding auxin influx and efflux carriers, *At2g17500* was induced at 12 h, while up-regulation of *PIN1* was detected at 48 and 96 h. However, genes related to auxin biosynthesis and auxin reception, such as *TIR1* and its homologues, as well as *ABP1*, did not show obvious alterations in their expressions during this process (Supplemental Table 7).

CIM also contains a low concentration of cytokinin, we further examined the expression of genes involved in cytokinin signaling through a multistep His-to-Asp phosphorelay [19,20], and found that the genes encoding key components of cytokinin signaling including the receptor histidine kinases (HKs), histidine phosphotransfer proteins (HPts), and A- and B-type response regulators (ARRs), did not show any significant change during incubation. Only two genes involved in cytokinin degradation, *CKX5* and *CKX3*, were detected to be up-regulated in the both explants (Supplemental Table 7).

Interestingly, although CIM does not contain ethylene and gibberellin, the expression profiles revealed that expressions of several genes involved in ethylene and gibberellin signaling were apparently changed in both explants during callus induction (Supplemental Table 7), suggesting that these hormone signals might be indirectly involved in callus initiation.

3.5. A large number of transcription factors are differentially expressed during callus induction

We surprisingly noticed that a large number of transcription factor (TF) genes, including those encoding APETALA2 (AP2), NAM/ATAF1/CUC2 (NAC), LATERAL ORGAN BOUNDARIES DOMAIN (LBD), WRKY, MYB, Zinc-finger, HOMEOBOX-LEUCINE ZIPPER (HB) and BASIC/HELIX–LOOP–HELIX (bHLH) proteins, were differentially expressed before and after the explants were transferred on CIM, among which AP2, NAC, LBD and WRKY belong to the plant-specific TF families (Table 1 and Supplemental Table 7).

AP2 family has been shown to be involved in the determination of organ identity [21]. Among 146 putative *Arabidopsis AP2* genes, *At5g61890* and *At5g13330* showed early response to CIM from 12 to 48 h, *CRF3* was up-regulated throughout the incubation excluding 24 h, up-regulation of *PUCHI* was detected from 24 to 96 h, while *PLT1* and *EDF3* were only induced at 96 h in both explants (Table 1). Similarly, the expressions of six NAC domain family genes (*NAC2*, *NAC047*, *NAC058*, *NAM*, *NAC084* and *NAC094*) were found to be up-regulated throughout callus induction (Table 1). This finding is consistent with the previous observation that callus induction is associated with the hypomethylation-dependent up-regulation of a few members of the NAC family [8,22].

The *Arabidopsis* genome has 43 members of LBD transcription factors. Some *LBD* genes were directly regulated by AUXIN RESPONSE FACTOR (ARF) proteins and involved in organ cell specification or lateral root formation [23–25]. Interestingly, five *LBD* genes were found to be significantly induced during early stages of callus induction. *LBD16*, *LBD17*, *LBD18* and *LBD29* were induced in all the time points examined,

Table 1
Differentially expressed transcription factors in callus induction.

Locus ID	Annotation[a]	$Log_2$12h/0 h[b]		$Log_2$24h/0 h		$Log_2$48h/0 h		$Log_2$96h/0 h	
		R[c]	A[d]	R	A	R	A	R	A
AP2-EREBP									
At5g61890	AP2 domain transcription factor	3.15[e]	1.98	2.33	1.60	1.77	1.97	–[f]	–
At5g53290	AP2 domain transcription factor	2.98	3.33	–	–	2.94	3.82	3.01	3.95
At5g13330	AP2 domain transcription factor	1.54	2.09	1.47	2.55	1.33	2.65	–	–
At5g18560	AP2 domain transcription factor (PUCHI)	–	–	3.83	2.99	4.38	4.19	4.24	3.91
At3g20840	AP2 domain transcription factor (PLT1)	–	–	–	–	–	–	3.45	3.30
At3g25730	AP2 domain transcription factor (EDF3)	–	–	–	–	–	–	1.32	3.30
NAC									
At5g39610	NAM family protein (NAC2)	1.93	2.92	1.56	2.88	–	–	–	–
At3g04070	NAM family protein (NAC047)	1.62	2.79	–	–	–	–	–	–
At3g18400	NAM family protein (NAC058)	–	–	2.43	1.65	2.32	3.23	–	–
At1g52880	NAM family protein (NAM)	–	–	–	–	3.43	1.41	2.01	1.35
At5g14000	NAM family protein (NAC084)	–	–	–	–	1.70	2.99	3.18	4.54
At5g39820	NAM family protein (NAC094)	–	–	–	–	–	–	4.28	5.78
LBD									
At3g58190	LOB domain protein (LBD29)	5.50	5.19	6.44	6.35	5.73	7.73	2.94	8.66
At2g42440	LOB domain protein (LBD17)	4.70	5.06	4.73	6.21	3.95	6.03	1.79	5.78
At2g42430	LOB domain protein (LBD16)	2.81	2.84	2.88	3.20	3.18	3.58	3.00	3.47
At2g45420	LOB domain protein (LBD18)	4.46	3.59	6.40	5.41	6.16	5.71	5.96	6.55
At1g31320	LOB domain protein (LBD4)	–	–	–	–	–	–	4.45	1.57
WRKY									
At3g01970	WRKY family transcription factor (WRKY45)	–	–	2.52	2.75	2.34	3.29	1.62	4.23
At2g47260	WRKY family transcription factor (WRKY23)	–	–	1.47	1.79	1.77	3.40	1.85	4.26
At5g52830	WRKY family transcription factor (WRKY27)	–	–	1.28	1.61	–	–	–	–
At2g46130	WRKY family transcription factor (WRKY43)	–	–	–	–	1.32	3.02	–	–
At3g56400	WRKY family transcription factor (WRKY70)	−2.12	−1.13	–	–	–	–	–	–
At2g21900	WRKY family transcription factor (WRKY59)	–	–	–	–	−3.21	−3.00	–	–

[a] Annotation is provided by Harvest website (http://harvest.ucr.edu/) and is based on best BLASTX match against *Arabidopsis* TAIR database or UniRef 90 protein database.
[b] Log_2 ratio: the normalized intensity values of gene expression of *Arabidopsis* aerial and root explants incubated on CIM for 12, 24, 48 and 96 h versus 0 h.
[c] R: root explants.
[d] A: aerial explants.
[e] Positive or negative number: up-regulated or down-regulated fold change of gene expression.
[f] –: absolute value of fold change of gene regulation<2. The note is same as below.

while *LBD4* was only up-regulated at 96 h (Table 1). Coincidentally, overexpression of *PtaLBD1* was reported to enhance callus formation in low auxin concentration medium [26]. Furthermore, some *WRKY* genes have been reported to be constitutively expressed in habituated calluses [27] or induced by cytokinin [28], and we also observed that four *WRKY* genes (*WRKY45*, *WRKY23*, *WRKY27* and *WRKY43*) were up-regulated and two genes (*WRKY70* and *WRKY59*) were down-regulated in at least one time point (Table 1).

MYB is a large TF family in both animal and plant genomes, and this family has been categorized to A-MYB, B-MYB and C-MYB subfamilies. In animals, appropriate B-MYB expression is critical for the maintenance of chromosome stability and pluripotent embryonic stem (ES) cells [29] and for the cell cycle progression through G2/M [30]. In plants, MYB has been implicated in controlling cell development, hormone and environmental responses [31]. Our close look at the expression of *MYB* genes during the callus induction revealed that five genes (*MYB112*, *MYB14*, *MYB63*, *MYB94* and *At4g39160*) were up-regulated and four (*MYB28*, *LCL1*, *MYB48* and *At1g19000*) were down-regulated (Supplemental Table 7), suggesting that the MYB family may also be important for callus initiation. In addition, there were three other TF family genes that were differentially expressed in this process, i.e., Zinc-finger, HB and bHLH. In particular, 15 Zinc-finger TF genes were up-regulated and ten genes were down-regulated. Expressions of ten *HB* genes were changed after explants were incubated on CIM, and six *bHLH* genes were up-regulated while six were down-regulated by CIM respectively in this process (Supplemental Table 7). Our findings strongly suggest that these TFs play important roles in callus initiation process.

3.6. Protein phosphorelay cascades are involved in regulation of callus initiation

Overexpression of a receptor kinase interacting with the sulfated peptide phytosulfokine (PSK) in carrot cells caused an enhanced callus growth in response to PSK [32]. In this study, we found that among the genes annotated as leucine-rich transmembrane protein kinases, eight genes (*At1g25320*, *At5g53320*, *At3g24240*, *At1g34110*, *HAE*, *RUL1*, *TMKL1* and *At5g48940*) were up-regulated and four genes (*At5g37450*, *At5g59680*, *RLK* and *At3g03770*) were down-regulated. Serine/threonine protein kinases function in auxin and brassinosteroid signaling [33,34]. *CTR1*, which encodes a serine/threonine protein kinase, was moderately up-regulated throughout the process. In addition, the other kinase families also obviously changed. Moreover, our microarray data showed that three genes (*At5g02760*, *ABI1* and *At1g34750*) encoding PP2C-type phosphatases were significantly induced on CIM (Supplemental Table 7). Therefore, differential expression of kinase and phosphatase genes implies that reversible protein phosphorylation is involved in control of callus induction.

3.7. Epigenetic modifications are active during callus initiation

Previous works in plants suggest that the loss of the differentiated state in protoplasts is accompanied by global changes in DNA methylation and histone modification [35], and histone modifications are involved in the regulation of chromatin structure and gene silencing [36]. Notably, we found that the transcript abundances of genes related the epigenetic modifications were altered in the callus induction process in both explants. These genes included those encoding chromatin remodeling factors, heterochromatin formation factors, histone deacetylase, methyltransferase and acetyltransferase. 11 WD-40 repeat family proteins, which belong to chromatin remodeling factors, were up-regulated by CIM. Three genes encoding SET domain-containing proteins, which fall into heterochromatin formation factors (histone methyltransferases), were moderately up-regulated at 48 and/or 96 h. Of the 23 putative histone deacetylases genes in *Arabidopsis* genome, three (*HDA13*, *HD2A* and *HDA9*) were up-regulated (Table 2). By contrast, the genes involved in other histone modifications including those encoding histone acetyltransferases and demethylases were not differentially expressed. In addition, one methyltransferase gene (*At1g66690*) was up-regulated only at 12 h, ten were up-regulated at 48 and/or 96 h, and five were down-regulated at the different stages and two acetyltransferases (*HLS1* and *GNAT*) were up-regulated at 48 and/or 96 h (Supplemental Table 7).

3.8. Genes related to cell division are induced at late stages

As expected, a high proportion of the genes associated with cell division were up-regulated during callus induction (Table 3), including those encoding cell cycle regulatory proteins, chromatin structural proteins and proteins related to DNA synthesis machinery. We took a

Table 2
Transcript profiles of epigenetic genes in callus initiation.

Locus ID	Annotation	$Log_2$12h/0 h		$Log_2$24h/0 h		$Log_2$48h/0 h		$Log_2$96h/0 h	
		R	A	R	A	R	A	R	A
Chromatin remodeling factors									
At3g10530	WD-40 repeat family protein	2.46	1.42	–	–	–	–	–	–
At2g18900	WD-40 repeat family protein	2.35	1.70	–	–	2.03	1.03	2.09	1.04
At4g04940	WD-40 repeat family protein	2.10	1.50	–	–	–	–	1.55	1.17
At1g15440	WD-40 repeat family protein (PWP2)	1.53	1.31	–	–	–	–	–	–
At5g11240	WD-40 repeat family protein	1.68	1.10	–	–	–	–	–	–
At5g15550	WD-40 repeat family protein	–	–	–	–	–	–	1.35	1.17
At5g63010	WD-40 repeat family protein	–	–	–	–	–	–	1.29	1.60
At1g49910	WD-40 repeat family protein	–	–	–	–	–	–	1.77	1.11
At4g28450	WD-40 repeat family protein	–	–	–	–	–	–	1.41	1.14
At3g19590	WD-40 repeat family protein	–	–	–	–	–	–	2.27	1.02
At3g10530	WD-40 repeat family protein	–	–	–	–	–	–	1.58	1.21
Heterochromatin formation factors									
At2g19640	SET domain-containing protein (ASHR2)	–	–	–	–	1.38	2.69	1.42	2.91
At4g30860	SET domain-containing protein (ASHR3)	–	–	–	–	–	–	2.38	1.00
At1g01920	SET domain-containing protein	–	–	–	–	–	–	2.14	1.02
Histone modification									
At2g27840	histone deacetylase-related (HDA13)	2.47	1.32	2.97	1.04	2.41	1.30	1.00	1.06
At3g44750	histone deacetylase (HD2A)	–	–	–	–	1.41	1.57	1.62	1.88
At3g44680	histone deacetylase, putative (HDA9)	–	–	–	–	–	–	1.33	1.21

Table 3
Expression profiles of genes related to cell division.

Locus ID	Annotation	$Log_2$12h/0 h		$Log_2$24h/0 h		$Log_2$48h/0 h		$Log_2$96h/0 h	
		R	A	R	A	R	A	R	A
Cell cycle-related genes									
At1g80370	Cyclin, putative (CYCA2;4)	–	–	–	–	–	–	3.40	1.47
At4g37490	G2/mitotic-specific cyclin (CYC1)	–	–	–	–	–	–	3.08	1.40
At1g44110	Cyclin, putative (CYCA1;1)	–	–	–	–	–	–	2.99	1.07
At1g76310	Cyclin, putative (CYCB2;4)	–	–	–	–	–	–	2.54	1.05
At1g20590	Cyclin, putative	–	–	–	–	–	–	2.51	1.34
At3g11520	Cyclin, putative (CYC2)	–	–	–	–	–	–	2.11	1.82
At3g50070	Cyclin family protein (CYCD3;3)	–	–	–	–	–	–	1.89	1.62
At2g27970	Cyclin-dependent kinase, putative/CDK (CKS2)	–	–	–	–	–	–	1.73	1.89
At1g78770	Cell division cycle family protein (APC6)	–	–	–	–	–	–	1.99	1.31
Histone genes									
At2g38810	Histone H2A, putative (HTA8)	–	–	–	–	–	–	2.61	2.10
At1g54690	Histone H2A, putative (HTA3)	–	–	–	–	–	–	1.21	1.50
At5g54640	Histone H2A (HTA1)	–	–	–	–	–	–	1.07	1.02
At5g22880	Histone H2B, putative (HTB2)	–	–	–	–	–	–	1.70	1.10
At5g59910	Histone H2B (HTB4)	–	–	–	–	–	–	1.38	1.87
At3g53650	Histone H2B, putative	–	–	–	–	–	–	1.23	2.13
At3g46030	Histone H2B, putative (HTB11)	–	–	–	–	–	–	1.00	1.06
At1g07820	Histone H4	–	–	–	–	–	–	1.78	1.01
Chromosome structure and DNA replication									
At1g65920	Regulator of chromosome condensation (RCC1)	–	–	–	–	2.79	1.69	4.48	1.86
At5g33300	Chromosome-associated kinesin-related	–	–	–	–	1.66	1.29	1.86	1.79
At2g07690	Minichromosome maintenance protein (MCM5)	–	–	–	–	–	–	2.89	1.21
At5g60870	Regulator of chromosome condensation (RCC1)	–	–	–	–	–	–	1.06	1.29
At5g46280	DNA replication licensing factor (MCM3)	–	–	–	–	–	–	2.57	1.05
At2g16440	DNA replication licensing factor (MCM4)	–	–	–	–	–	–	2.35	1.28
At5g49010	DNA replication protein-related (EMB2812)	–	–	–	–	–	–	1.32	1.18

closer look at the expression level of cyclins, cyclin-dependent kinases, and histone subunit genes in the two explants. Seven (*CYCA2;4*, *CYC1*, *CYCA1;1*, *CYCB2;4*, *At1g20590*, *CYC2* and *CYCD3;3*) of 31 cyclins or cyclin homologues were specifically up-regulated at 96 h. Only one (*CKS2*) of 14 cyclin-dependent kinases (CDKs) and CDK homologues was identified to be up-regulated at 96 h. Among the nucleosome components, three (*HTA8*, *HTA3* and *HTA1*) of 13 *Histone H2A*, four (*HTB2*, *HTB4*, *At3g53650* and *HTB11*) of 11 *Histone H2B* and one (*At1g07820*) of eight *Histone H4* genes were up-regulated specifically at 96 h. Interestingly, the expressions of all the cell division-related genes were up-regulated after 48 h, suggesting that the resuming cell division may be a result of callus forming process. Consistent with this, several chromosome structural genes (*RCC1*, *At5g33300*, *MCM5* and *RCC1*) and DNA replication genes (*MCM3*, *MCM4* and *EMB2812*) appeared to be up-regulated slightly earlier or at the same time as did the cell division genes (Table 3).

3.9. Protein turnover in the callus induction

Activation of genes whose products are involved in the ubiquitin proteolytic pathways during callus induction has been reported previously [4,8,37]. Consistent with this, our expression profiling also revealed that some transcripts up-regulated in the callus induction were predicted to encode proteins involved in the ubiquitin–proteasome pathways, including two E3 genes (*ASK3* and *ASK18*), one E1 gene (*UBA2*) and one E2 gene (*UBC20*) (Supplemental Table 7). In our studies, six genes encoding the F-box protein family were up-regulated with different expression patterns and only one gene (*At3g61060*) was down-regulated during the incubation time (Supplemental Table 7). Consistently, the previous work showed the F-box protein Skp2 regulates G1/S transition by controlling the degradation of p27/Kip.1, which plays a critical role in the pathogenesis of many human tumors [38].

3.10. Changes of other family genes

Interestingly, we also found that other six family genes were up-regulated during the early stage of callus induction, including those encoding the GDSL-motif lipase/hydrolase family protein, ABC transporter family protein, pentatricopeptide (PPR) repeat-containing protein, VQ motif-containing protein, short-chain dehydrogenase/reductase (SDR) and polygalacturonase (Supplemental Table 7). The gene expressions of the proton-dependent oligopeptide transport proteins and major latex protein-related/MLP-related proteins were uniquely down-regulated in this process (Supplemental Table 7).

3.11. Overexpression of two transcription factors rapidly responsive to CIM initiates callus without exogenous phytohormone

To further explore the key regulators involved in callus initiation, ten genes (*At5g61890*, *CRF3*, *NAC2*, *MYB112*, *ATL8*, *HB52*, *At2g42280*, *At2g26290*, *At3g10530* and *At2g27840*) were preferentially selected for further functional confirmation because their expressions were highly and rapidly induced within 12 h when both two explants were incubated on CIM. We generated transgenic *Arabidopsis* plants overexpressing each of these genes and carefully examined their phenotypes. Fortunately, the whole or parts of roots of the recovered T1 *Pro35S:HB52* seedling or the root tips of T1 *Pro35S:CRF3* seedlings formed callus-like structures when grown on the medium without exogenous phytohormone (Figs. 3A and B), and about 16% and 6% of seedlings displayed varying degrees of phenotypic characteristics in 548 *HB52* and 304 *CRF3* T1 transformants, respectively. We also excised root explants of transgenic *HB52* or *CRF3* plants on hormone free medium, and observed that enhanced callus-like structure forming in these root explants (Supplemental Fig. 3). To investigate the property of the callus-like structures, we detected the expressions of the root meristem genes in the transgenic plants and found that the expressions of *WOX5* and *PLT1* were dramatically increased in the

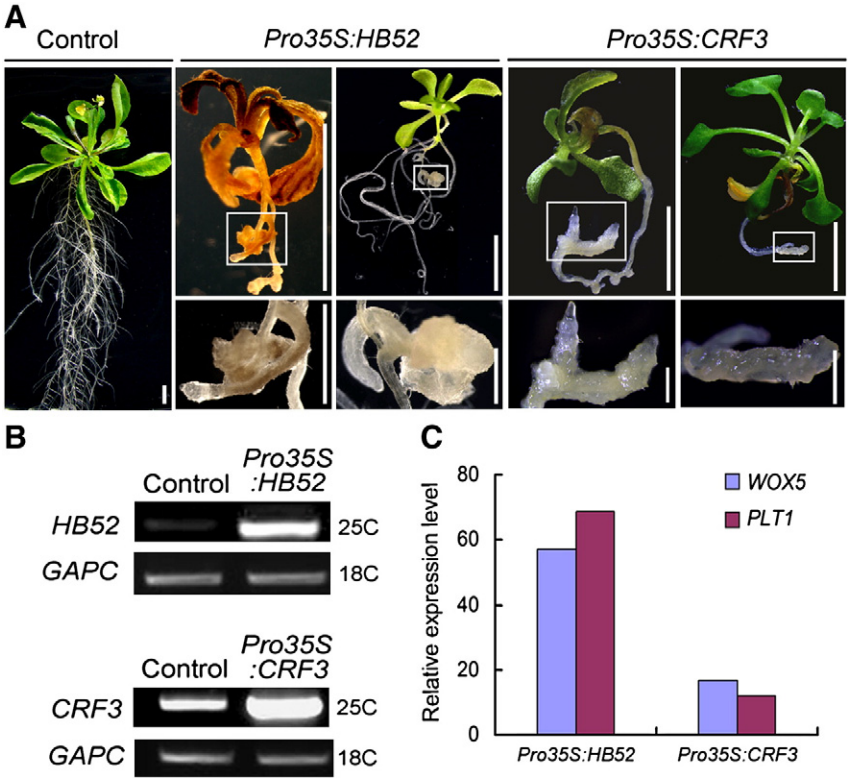

Fig. 3. Overexpression of *HB52* or *CRF3* forms callus. (A) Phenotype of the recovered *Pro35S:HB52* and *Pro35S:CRF3* T1 seedlings. The control and transgenic *Pro35S:HB52* and *Pro35S:CRF3* seedlings (upper panel) were germinated and grown on B5 medium without exogenous hormone for 40 days. Bars = 5 mm. The enlarged images of the regions circled by squares were shown in the lower panel. Bars = 1 mm. (B) RT-PCR analysis of *HB52* and *CRF3* expression in *Pro35S:HB52* and *Pro35S:CRF3* T1 mixed seedlings. The expression of *GAPC* was used as an internal control. Note that the control plant harbored an empty vector. (C) Quantitative real-time RT-PCR (qRT-PCR) analyses of *WOX5* and *PLT1* in the roots of the 40-day-old strong phenotypic *Pro35S:HB52* and *Pro35S:CRF3* T1 mixed seedlings. The expression level of the two genes in *Pro35S:HB52* and *Pro35S:CRF3* T1 mixed seedlings after normalized to *ACTIN2* was compared to that in control plants.

root of 40-day-old T1 *Pro35S:HB52* or *Pro35S:CRF3* plants (Fig. 3C), indicating that the callus induced by the two genes resembles that induced by CIM.

4. Discussion

Identification of genes expressed during callus induction at genome level is a vital approach towards understanding the regulatory circuits that control the callus induction process. In the study, we focused on the 1342 overlapped genes that differentially expressed in both aerial and root explants on CIM within 96 h and summarized the early common molecular events of callus formation. Our analyses not only identified a number of differently expressed genes related to hormones during callus initiation, but also revealed that a large number of genes that encode several TFs were highly induced in the process. Furthermore, we also observed that the expressions of some epigenetic modification genes were active and protein phosphorelay cascades were involved in callus induction. In addition, several genes involved in protein turnover, particularly members of the F-box protein family, were regulated during the callus induction.

With *Arabidopsis* root explants incubated on CIM for 48 and 96 h, Che et al. have examined 4939 differentially expressed genes at 96 h of incubation [12]. In our study, 5488 genes were identified to be up- or down-regulated after root explants were cultured on CIM for 96 h. A total of 2656 genes were overlapped in both results (Supplemental Fig. 4 and Supplemental Table 8). Furthermore, Che et al. have summarized 16 up-regulated genes with stage-specific patterns at 48 and 96 h of incubation by principal component analysis [12]. Consistent with this, all the 16 genes were all up-regulated in our results. Most of them were almost the same at the level of quantitative analysis in both results (Supplemental Table 9). Therefore, our gene expression profiles provide reliable data for characterizing the molecular events of callus initiation.

Recent studies showed calluses from aerial and root explants incubated on CIM were characterized by the ectopic activation of root marker genes, including *SCR*, *SHR* and *WOX5* [10]. In our results, the expressions of *SCR* and *SHR* were gradually up-regulated in root explants during callus initiation, and a notable change was more than an eight-fold increase at 96 h of incubation (Supplemental Table 10), which was consistent with the previous report [10]. Similarly, the up-regulated expression patterns of *SCR* and *SHR* also emerged in aerial explants, but their changes were less than two fold within 96 h on CIM (Supplemental Table 10), and consistent with this, the significant signals of the two marker genes were detected in aerial explants incubated on CIM containing a higher level of 2,4-D until 10 days [10]. The decline of the *GL2* non-hair epidermal marker gene in aerial explants was obviously lower than that in root explants throughout the incubation (Supplemental Table 10), which is identical to the disappearance of *GL2* signals in two explants in the previous report [10]. More differentiated cells have more difficultly returning to the cell cycle [39]. This may result in lower numbers of up- or down-regulated genes in the more differentiated aerial explants versus higher numbers in root ones during the early stage of callus induction. Besides the expressions of these root marker genes, we also found that some genes, including *IAA19*, *PIN1*, *LBD16*, *LBD17*, *LBD18*, *LBD29*, *PUCHI*, *CKX3* and *CKX5*, were highly and steadily induced in *Arabidopsis* aerial and root explants incubated on CIM during the callus induction (Table 1 and Supplemental Table 7), and these genes have been reported to modulate the initiation and emergence of lateral roots [23,24,40–43]. Our data further strengthen that callus formation and lateral root formation are under the same genetic control at the initiation step [10]. It is an interesting molecular event that some genes regulating a lateral root

development program are also involved in the control of callus initiation program. A future work is to dissect the relationship of callus initiation and lateral root initiation via the same development program.

Among *Aux/IAA* genes, *IAA5* and *IAA19* were rapidly and highly induced during callus induction (Supplemental Table 7), which is consistent with previous findings that the two genes were up-regulated by CIM [12] and highly expressed in the habituated calluses [27]. These observations suggest that IAA5 and IAA19 are likely to be important factors for triggering callus induction. Although in a typical plant tissue culture, the ratios of auxin to cytokinin are implicated to be critical in cell fate determination in in vitro systems [1], our expression profiles showed that only two cytokinin degradation genes, *CKX5* and *CKX3*, were up-regulated in the two explants (Supplemental Table 7), suggesting that cytokinin may play a coordinating role for callus induction. Consistent with this, a previous work also found that in a culture of root explants on modified CIM containing only auxin 2,4-D induced callus [44]. Furthermore, our studies imply that both ethylene and gibberellin homeostasis and signaling are involved in the callus induction process though these two exogenous hormones are not preset and supplemented in the CIM.

Plant callus formation is considered to be analogous to the induction of animal pluripotent stem (iPS) cells mediated by enforced expression of a few TFs, including Oct4, Sox2 and Nanog, because plant callus cells and animal iPS cells share a common characteristic known as pluripotency [45]. Similarly, a large number of TF families were identified to be differentially expressed during callus induction, including AP2, WRKY, NAC, LBD, MYB, Zinc finger, HB and bHLH (Table 1 and Supplemental Table 7). Among the families of TFs examined, all the genes of AP2, NAC and LBD families, which are plant-specific TF families, displayed an up-regulated pattern throughout the process. Particularly, overexpression of two TFs genes, *HB52* and *CRF3*, exhibited spontaneous callus formation phenotype without exogenous phytohormone in some organs of transgenic plants (Fig. 3). These observations strongly suggest that transcriptional regulation may be an important regulatory mechanism underlying the control of formation of pluripotent cells in plants and animals. On the other hand, recent works suggest that callus originates from pericycle or pericycle-like cells [9,10], arguing that the cell reprogramming occurs during callus induction. Here, we identified some epigenetic genes of chromatin remodeling factors, histone modification factors, methyltransferase and acetyltransferase that were differentially expressed during the early stage of callus induction (Table 2 and Supplemental Table 7). The dynamic expressions of these genes strongly suggest that epigenetic modification may be important for callus formation. Finally, a very interesting observation was that cell division-related genes, including cell cycle-related genes, histone genes, genes encoding chromosome structure and DNA replication, were up-regulated only after 48 h (Table 3). Thus, it is likely that cell division may be a consequence of callus induction for the formation of a mass of callus cells.

Taken together, our results provided extensive clues to elucidate the regulatory mechanisms associated with the early events of callus induction. The CIM activates auxin signaling pathway, resulting in transcription regulation of a large number of TF genes, which in turn triggers dynamic changes of cell states via other molecular events, including protein phosphorelay and turnover, and epigenetic modification of chromatin and DNA, thus rendering cells with competency and resuming cell division to form the callus. More importantly, our approach yields a reliable source of novel genes for further functional studies to comprehensively understand active regulatory networks and indentify key regulators governing the callus induction, which might be difficult by classical forward-genetics approaches. Additional studies will be required to further address the function of *HB52* and *CRF3* in plant regeneration system. This will include their temporal and spatial expressions during the callus induction. Also, because it is difficult to test the pluripotency of the callus-like structures in the constitutively over-expressing *HB52* and *CRF3* plants, the connection of inducible transgene expression to the plant regeneration ability will have to be analyzed. In addition, their signaling pathway involved in callus induction will have to be characterized.

Supplementary materials related to this article can be found online at http://dx.doi.org/10.1016/j.ygeno.2012.05.013.

Acknowledgments

This work was supported by the Ministry of Science and Technology of China (2007CB948200) and the National Natural Science Foundation of China (30900108).

References

[1] F. Skoog, C.O. Miller, Chemical regulation of growth and organ formation in plant tissues cultured in vitro, Symp. Soc. Exp. Biol. 54 (1957) 118–130.
[2] B.R. Chitteti, Z. Peng, Proteome and phosphoproteome dynamic change during cell dedifferentiation in *Arabidopsis*, Proteomics 7 (2007) 1473–1500.
[3] B.R. Chitteti, F. Tan, H. Mujahid, B.G. Magee, S.M. Bridges, Z. Peng, Comparative analysis of proteome differential regulation during cell dedifferentiation in *Arabidopsis*, Proteomics 8 (2008) 4303–4316.
[4] J. Zhao, N. Morozova, L. Williams, L. Libs, Y. Avivi, G. Grafi, Two phases of chromatin decondensation during dedifferentiation of plant cells: distinction between competence for cell fate switch and a commitment for S phase, J. Biol. Chem. 276 (2001) 22772–22778.
[5] L. Williams, J. Zhao, N. Morozova, Y. Li, Y. Avivi, G. Grafi, Chromatin reorganization accompanying cellular dedifferentiation is associated with modifications of histone H3, redistribution of HP1, and activation of E2F-target genes, Dev. Dyn. 228 (2003) 113–120.
[6] F. Tessadori, et al., Large-scale dissociation and sequential reassembly of pericentric heterochromatin in dedifferentiated *Arabidopsis* cells, J. Cell Sci. 120 (2007) 1200–1208.
[7] J. Fajkus, J. Fulnečková, M. Hulánová, K. Berková, K. Říha, R. Matyásek, Plant cells express telomerase activity upon transfer to callus culture, without extensively changing telomere lengths, Mol. Gen. Genet. 260 (1998) 470–474.
[8] G. Grafi, H. Ben-Meir, Y. Avivi, M. Moshe, Y. Dahan, A. Zemach, Histone methylation controls telomerase-independent telomere lengthening in cells undergoing dedifferentiation, Dev. Biol. 306 (2007) 838–846.
[9] R. Atta, et al., Pluripotency of *Arabidopsis* xylem pericycle underlies shoot regeneration from root and hypocotyl explants grown in vitro, Plant J. 57 (2009) 626–644.
[10] K. Sugimoto, Y. Jiao, E.M. Meyerowitz, *Arabidopsis* regeneration from multiple tissues occurs via a root development pathway, Dev. Cell 18 (2010) 463–471.
[11] E.K. Lee, et al., Cultured cambial meristematic cells as a source of plant natural products, Nat. Biotechnol. 28 (2010) 1213–1217.
[12] P. Che, D.J. Gingerich, S. Lall, S.H. Howell, Global and hormone-induced gene expression changes during shoot development in *Arabidopsis*, Plant Cell 14 (2002) 2771–2785.
[13] Y. Hu, F. Bao, J. Li, Promotive effect of brassinosteroids on cell division involves a distinct *CycD3*-induction pathway in *Arabidopsis*, Plant J. 24 (2000) 693–701.
[14] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ Method, Methods 25 (2001) 402–408.
[15] Y. Hu, Q. Xie, N.H. Chua, The *Arabidopsis* auxin-inducible gene *ARGOS* controls lateral organ size, Plant Cell 15 (2003) 1951–1961.
[16] S.J. Clough, A.F. Bent, Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*, Plant J. 16 (1998) 735–743.
[17] P. Che, S. Lall, S.H. Howell, Developmental steps in acquiring competence for shoot development in *Arabidopsis* tissue culture, Planta 226 (2007) 1183–1194.
[18] K. Yamada, et al., Empirical analysis of transcriptional activity in the *Arabidopsis* genome, Science 302 (2003) 842–846.
[19] C.E. Hutchison, J.J. Kieber, Cytokinin signaling in *Arabidopsis*, Plant Cell 14 (2002) S47–S59 (Suppl.).
[20] T. Kakimoto, Perception and signal transduction of cytokinins, Annu. Rev. Plant Biol. 54 (2003) 605–627.
[21] J.L. Riechmann, E.M. Meyerowitz, The AP2/EREBP family of plant transcription factors, Biol. Chem. 379 (1998) 633–646.
[22] Y. Avivi, et al., Reorganization of specific chromosomal domains and activation of silent genes in plant cells acquiring pluripotentiality, Dev. Dyn. 230 (2004) 12–22.
[23] H.W. Lee, N.Y. Kim, D.J. Lee, J. Kim, *LBD18/ASL20* regulates lateral root formation in combination with *LBD16/ASL18* downstream of *ARF7* and *ARF19* in *Arabidopsis*, Plant Physiol. 151 (2009) 1377–1389.
[24] Y. Okushima, H. Fukaki, M. Onoda, A. Theologis, M. Tasaka, ARF7 and ARF19 regulate lateral root formation via direct activation of *LBD/ASL* genes in *Arabidopsis*, Plant Cell 19 (2007) 118–130.
[25] H. Iwakawa, et al., 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 (2002) 467–478.
[26] Y.S. Yordanov, S. Regan, V. Busov, Members of the LATERAL ORGAN BOUNDARIES DOMAIN transcription factor family are involved in the regulation of secondary growth in *Populus*, Plant Cell 22 (2010) 3662–3677.

[27] M.S. Pischke, E.L. Huttlin, A.D. Hegeman, M.R. Sussman, A transcriptome-based characterization of habituation in plant tissue culture, Plant Physiol. 140 (2006) 1255–1278.
[28] S. Hoth, et al., Monitoring genome-wide changes in gene expression in response to endogenous cytokinin reveals targets in *Arabidopsis thaliana*, FEBS Lett. 554 (2003) 373–380.
[29] K.V. Tarasov, et al., B-MYB is essential for normal cell cycle progression and chromosomal stability of embryonic stem cells, PLoS One 3 (2008) e2478.
[30] J.L. Shepard, et al., A zebrafish *bmyb* mutation causes genome instability and increased cancer susceptibility, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 13194–13199.
[31] A. Feller, K. Machemer, E.L. Braun, E. Grotewold, Evolutionary and comparative analysis of MYB and bHLH plant transcription factors, Plant J. 66 (2011) 94–116.
[32] Y. Matsubayashi, M. Ogawa, A. Morita, Y. Sakagami, An LRR receptor kinase involved in perception of a peptide plant hormone, phytosulfokine, Science 296 (2002) 1470–1472.
[33] J.M. Pérez-Pérez, M.R. Ponce, J.L. Micol, The *ULTRACURVATA2* gene of *Arabidopsis* encodes an FK506-binding protein involved in auxin and brassinosteroid signaling, Plant Physiol. 134 (2004) 101–117.
[34] L.Z. Tao, A.Y. Cheung, H.M. Wu, Plant Rac-like GTPases are activated by auxin and mediate auxin-responsive gene expression, Plant Cell 14 (2002) 2745–2760.
[35] M. Berdasco, et al., Promoter DNA hypermethylation and gene repression in undifferentiated *Arabidopsis* cells, PLoS One 3 (2008) e3306.
[36] P. Loidl, A plant dialect of the histone language, Trends Plant Sci. 9 (2004) 84–90.
[37] E. Jamet, A. Durr, Y. Parmentier, M.C. Criqui, J. Fleck, Is ubiquitin involved in the dedifferentiation of higher plant cells? Cell Differ. Dev. 29 (1990) 37–46.
[38] D. Schiffer, P. Cavalla, V. Fiano, C. Ghimenti, R. Piva, Inverse relationship between p27/Kip. 1 and the F-box protein Skp2 in human astrocytic gliomas by immunohistochemistry and Western blot, Neurosci. Lett. 328 (2002) 125–128.
[39] Y. Yu, Z. Feng, G. Wang, F. Li, X. Du, J. Zhu, Initiation of dedifferentiation and structural changes in in vitro cultured petiole of *Arabidopsis thaliana*, Protoplasma 241 (2010) 75–81.
[40] K. Tatematsu, et al., *MASSUGU2* encodes Aux/IAA19, an auxin-regulated protein that functions together with the transcriptional activator NPH4/ARF7 to regulate differential growth responses of hypocotyl and formation of lateral roots in *Arabidopsis thaliana*, Plant Cell 16 (2004) 379–393.
[41] E. Benková, et al., Local, efflux-dependent auxin gradients as a common module for plant organ formation, Cell 115 (2003) 591–602.
[42] A. Hirota, T. Kato, H. Fukaki, M. Aida, M. Tasaka, The auxin-regulated AP2/EREBP gene *PUCHI* is required for morphogenesis in the early lateral root primordium of *Arabidopsis*, Plant Cell 19 (2007) 2156–2168.
[43] T. Werner, V. Motyka, V. Laucou, R. Smets, H. Van Onckelen, T. Schmulling, Cytokinin-deficient transgenic *Arabidopsis* plants show multiple developmental alterations indicating opposite functions of cytokinins in the regulation of shoot and root meristem activity, Plant Cell 15 (2003) 2532–2550.
[44] S.P. Gordon, M.G. Heisler, G.V. Reddy, C. Ohno, P. Das, E.M. Meyerowitz, Pattern formation during de novo assembly of the *Arabidopsis* shoot meristem, Development 134 (2007) 3539–3548.
[45] K. Takahashi, S. Yamanaka, Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors, Cell 126 (2006) 663–676.