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Divide and conquer: development and cell cycle genes in plant transformation

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Genetic transformation and regeneration of transgenic plants remains unfeasible for the majority of plant species. We propose that inducible expression and/or suppression of the genes that control the cell cycle and development, by altering chromatin structure and exerting epigenetic control of gene expression, might substantially improve competence for transformation and/or regeneration. Transformation efficiency was higher in cells with nuclei at the S and G2 phases, and manipulating the genes whose activation or silencing promote the G1–S transition has increased both transient and stable transformation. Controlling the cell cycle directly, using *RBR* and *VIP1*, or indirectly, through hormone regulation using *IPT* and *ESR1*, has improved rates of stable transformation. Other target genes that might promote incorporation of DNA and/or pluripotency of cells include *HP1, CycD3* and *CycD1*. The availability of large EST databanks, complete plant-genome sequences and/or inducible gene expression systems create opportunities for testing homologous genes to increase competence of transformation and regeneration.

Introduction

Twenty years have passed since the first regeneration of genetically transformed plants [1]. For most plant species, however, the process is still unfeasible or highly inefficient [2], making it unavailable, for practical purposes, as a routine plant-breeding tool. Furthermore, the rate of publications on transgenic technology development in plants has lagged in recent years, threatening to undermine progress in both the science and the application [3]. Transformation requires the delivery of DNA into the nucleus of cells and its insertion into the genome, and regeneration requires the multiplication and differentiation of transformed cells into organs or embryos. Several options exist to deliver DNA into plant cells [4] and although the methods available could be improved, insertion of DNA into the cell might not be considered the major obstacle to transformation.

Phytohormones, such as auxins, are powerful tools to stimulate cell division and modify the competence of cells [5] but they are often inadequate for promoting efficient transformation. Zuo *et al.* [6] summarized the potential uses of cyclin D3, histone H2A (H2A), cytokinin synthesis and receptor genes to improve regeneration and as a means to generate marker-free transgenic plants: they identified several opportunities as to how these genes might be harnessed to improve transformation systems. In this review, we discuss developmental genes, with a focus on those regulating the G1–S transition in the cell cycle, as a means to overcome the limitations in genetic transformation and regeneration.

The processes of plant transformation and regeneration from a transgenic cell can stall at several key points, including: (1) the acquisition of a stem-cell-like state in cells as they re-enter the cell cycle [7]; (2) progression between the G1 and S phases of the cycle [8,9] (Box 1); and (3) the differentiation of the transformed cell into a new embryo or meristem [10–13]. In this manuscript, we consider transformation to be the recovery of cells expressing a foreign gene for use as a selectable marker, and regeneration to be the organogenesis or embryogenesis from the genetically transformed cells.

We will first discuss the basic concepts of de- and/or re-differentiation and the genes that control it. Next, we will explore genes that either suppress or promote G1–S transition and, later, those that facilitate gene uptake or directly influence the regeneration process (Table 1). We will conclude by discussing strategies to use these genes, in combination with inducible expression systems, to improve transformation and regeneration efficiency.

The concept of dedifferentiation: heterochromatin remodeling

The differentiated cell

A characteristic of multicellular organisms is that most of their cells are non-proliferating differentiated cells in which DNA replication factors are absent or functionally inactive [14]. Differentiated cells at the quiescent phase (G0) are characterized by DNA compaction: methylation of cytosine residues in the DNA attracts methyl-CpG-binding proteins, which associate with histone deacetylases, and compact the DNA, thereby facilitating histone methylation [15]. In addition, methylated histone tails attract heterochromatin protein 1 (HP1), which causes further compaction of the DNA or the formation of heterochromatin [16]. Transcriptionally active genes are associated with euchromatic areas, whereas heterochromatin contains mostly transcriptionally inactive genes [17,18]. However, even within euchromatic regions, condensed areas of heterochromatin are common. The *HP1*-like genes in plants (*LHP1* and *TFL2*) promote DNA

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Box 1. Summary of phases in the plant cell cycle and their terminology

Interphase
G0 (Gap0): resting period, non dividing
G1 (Gap1): growth, preparation for DNA synthesis
S DNA synthesis
G2 (Gap2): growth

Mitosis
M Cell division

For an animated summary of the cell cycle see http://www.cellsalive.com/cell_cycle.htm

condensation and repression of transcription [16] – a characteristic of differentiated cells.

Acquiring pluripotentiality

For a transgenic, differentiated cell to attain competence for regeneration it must adopt a 'stem-cell-like' state, which confers pluripotentiality, and then re-enter the S phase of the cell cycle. Both of these steps involve chromatin remodeling [7], although the G0–G1 transition involves chromatin relaxation rather than DNA synthesis [19]. In general, the competence of a cell to switch fate or acquire pluripotentiality [7] is accompanied by post-translational modifications of histone H3, redistribution of heterochromatin protein 1 (HP1), changes in DNA-methylation patterns and activation of silent genes such as *VIP1* and *no apical meristem* (*NAM*)-like genes [17].

Cell phases – genes that regulate the G1–S transition

The control of the G1–S transition is crucial to the commitment to further cell division or differentiation in eukaryotic cells [20]. G1–S transition is driven by the E2F–RBR pathway, where *E2F* is a family of transcription activators that are normally repressed in differentiated cells by retinoblastoma-related protein (RBR) [20,21] and are up-regulated before entering S phase [22]. Upon phosphorylation by D- and A-type cyclins, in association with their cyclin kinase partners, RBR releases the E2F factors, enabling the G1–S transition to occur [20,21,23]. The transcription activation factor E2Fa acts in combination with its dimerization partner DPa, and among the hundreds of genes targeted by E2Fa–DPa [24] are D-type cyclins (*CycD*), which provide feedback to the release of E2F factors in the E2F–RBR pathway [20,21]. It has been shown that *Cyclin D3* (*CycD3*) expression is modulated in plant cells by the hormone cytokinin [25]. Furthermore, overexpression of *CycD3* can eliminate the requirement for exogenous cytokinin in the induction and proliferation of callus cells, and shows increased transcription of the S-phase-associated histone *H4* (*H4*) [25]. *Cyclin D1* (*CycD1*) can also accelerate the cell cycle in tobacco cells by enhancing both G1–S entry and progression through S and G2 phases [26].

E2Fa activates and E2Fc represses progression through G1–S phase

Transgenic plants co-overexpressing *E2Fa* and its dimerization partner *DPa* showed increased cell proliferation; their cotyledons contained approximately twice the number of cells as wild type. This phenotype was accompanied by a significantly higher proportion of 2C and 4C nuclei and two additional endocycles compared with the control [27]. Transcriptome analysis of E2Fa–DPa-overexpressing plants showed the up-regulation of 14 genes that are involved in DNA replication (and/or modification) and S phase onset, including *histone acetylase B (HAT B)*, *topoisomerase 6*, *DNA gyrase*, *CDKB1*, and histones *H1*, *H2A*, *H2B* and *H4* [24]. However, not all E2F factors positively regulate the G1–S transition. *E2Fc*, another member of the *E2F* family, is a negative regulator of the E2F–RBR pathway. E2Fc represses the entry of cells into S phase by competitive inhibition for the DPa subunit of the E2Fa–DPa transcription factor complex [23,28]. In summary, either overexpression of E2Fa or silencing of E2Fc could be potentially used to induce pluripotency.

Table 1. Examples of genes known to facilitate T-DNA integration or that control developmental processes required for plant regeneration

Gene	Name	Function	Refs
Acquisition of pluripotency and G1–S transition			
RBR1	Retinoblastoma-related protein	Negatively regulates the family of transcription factors E2F. Inactivation or RBR1 results in expression of E2F regulated genes and G1–S transition.	[61]
CycD3	Cyclin D3	Up-regulated by nutrient availability and plant hormones, involved in acquisition of competence for organogenesis and control of progression of the cell cycle.	[25]
E2Fa	Transcription factor	In association with DPa (dimer partner a), strongly up-regulates genes involved in DNA replication and regulates G1–S phase progression in the cell cycle.	[27]
H2A	Histone 2A	Facilitates the integration of the T-DNA into the host genome and participates in chromatin remodelling.	[32]
LHP1	Like-heterochromatin protein 1	Promotes DNA condensation and transcription repression.	[60]
Organogenesis and somatic embryogenesis (hormone receptors and response)			
PID	Pinoid	A serine–threonine kinase that functions as a positive regulator of polar auxin transport.	[62]
CUC	Cup-shaped cotyledon	*CUC1* and *CUC2* are required for the separation of cotyledon primordia and for the formation of a functional shoot meristem.	[49]
ESR1	Enhancer of shoot regeneration	Increased efficiency of shoot regeneration from root explants in the presence of cytokinin.	[63]
IPT	Isopentenyl–transferase	Bacterial gene that increases cytokinin levels leading to generation of shoots from transformed plant cells.	[64]

Links between G1–S transition and regeneration of transgenic plants

Differentiated plant cells are normally at the quiescent G0 phase, and to insert foreign DNA into their genome they need to re-enter the cell cycle. Several studies have shown that the phase of the plant cell cycle at the time of transformation is a major determinant of transformation and regeneration efficiency. Transformation frequency is positively associated with cell division or dedifferentiation, with higher transformation observed in cells with nuclei at the S and G2 phases of the cycle [8,9,29]. Using flow cytometry, Villemont *et al.* [29] found that the highest stable transgene expression in *Petunia hybrida* takes place when cells are at S phase during transformation with *Agrobacterium*. No transformation occurred if cells were at G0–G1 (quiescent phase), and only transient transgene expression occurred when cells were at M phase [29]. Flow cytometric studies in citrus showed that a high frequency of actively dividing cells in S phase resulted in high rates of stable integration of DNA when co-cultivated with *Agrobacterium* in a medium rich in auxins [9].

The regeneration frequency of transgenic explants has also been linked to the cell cycle phase at the time of transformation. There was a strong positive correlation observed between the frequency of S and G2 nuclei and regeneration ability in *Petunia hybrida*, and a strong negative correlation between frequency of G0 and G1 nuclei and percent of regeneration after using *Agrobacterium* transformation [8]. A similar phenomenon was observed in *Brassica oleracea*, with the highest transformation efficiencies and regeneration rates observed in explants with higher proportions of 4C nuclei [30].

Histone H2A and VIP1 facilitate the insertion of foreign DNA into the plant genome

Histone H2A is part of the ATP-dependent machinery of histone variant exchange, which occurs during chromatin remodeling and epigenetic control of transcription [31]. A *H2A*-like protein was also one of the genes up-regulated by the co-expression of *E2Fa–DPa* and S phase onset [24]. A mutation in *H2A-1* rendered plants resistant to genetic transformation with *Agrobacterium*, whereas its overexpression in wild-type plants increased transformation efficiency by twofold [32]. Transient expression of *H2A* also improved the rate of stable transformation [32].

VIP1 (*VirE2 interacting protein 1*) is a gene present in *Arabidopsis*; it is normally silent in differentiated cells but was up-regulated during the acquisition of pluripotentiality [17]. Further research determined that VIP1 interacts with VirE2 from *Agrobacterium* and with several histones, facilitating the integration of the foreign DNA into the plant genome [33,34]. Although the regeneration capacity of explants overexpressing *VIP1* is not increased [34], its overexpression increased the rate of transient and stable plant transformation [35] (Figure 1). VIP1 has a nuclear localization signal, and interacts not only with VirE2 but also with the plant H2A [34], H2B, H3 and H4 [33]. It has been proposed that the VIP1–histone association might trigger the proteolytic uncoating of the T-DNA, enabling its integration [33].

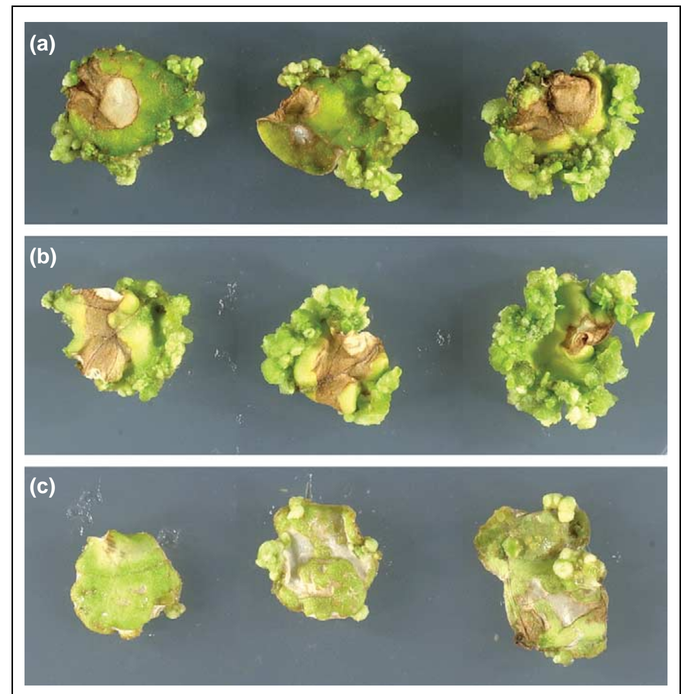

Figure 1. Enhanced transformation using *VIP1*. **(a)** and **(b)** Leaf disks of two independent tobacco lines previously transformed with *VIP1*, and **(c)** non-transgenic wild-type submitted to a second transformation with the *HPTII* antibiotic resistance gene, followed by regeneration in the presence of hygromycin. Left to right, disks treated with *Agrobacterium* cultures at 0.1, 0.5 and 1.0 OD (optical density), respectively. Lines harbouring *VIP1* showed higher transformation efficiency. Reprinted from Tzfira *et al.* reference [34].

Relieving the blockage on regeneration

Silencing of retinoblastoma-related protein (RBR) leads to increased transformation and regeneration

The activation of the mitotic cell cycle and entry into S phase is controlled by a pathway that involves the activation of E2F through the hyperphosphorylation and inactivation of RBR [23]: the abundance of RBR protein is highest in differentiated tissues (23). Plant geminiviruses normally invade differentiated cells with no active DNA replication; therefore, they have developed strategies to reactivate DNA synthesis. At least two of their proteins, Rep and RepA, repress the plant cell RBR and, thus, activate the replication machinery required to amplify the viral genome [14]. In tobacco cells, constitutive expression of the viral RepA protein induced cell division and conferred a high-competence transformation phenotype, whereas *RepA* expression in maize resulted in calli that remained embryogenic and were readily regenerable, suggesting that RepA was able to overcome the G0–G1 blockage [36].

The phosphorylation of RBR by complexes of D-type cyclins and cyclin kinases (CycD–CDKs) resulted in its dissociation from E2F transcription factors, and enabled activation of the *E2F* target genes needed for entry into S phase [37]. Experimentally, virus-induced gene silencing (VIGS) of *RBR* in tobacco resulted in the up-regulated transcription of *E2F* and S phase active genes, such as *ribonucleotide reductase* (*RNR*), *proliferating cell nuclear antigen* (*PCNA*), *minichromosome maintenance* (*MCM*), *histone H1* and *replication origin protein* (*CDC6*), in addition to an increased proportion of 4C nuclei [38].

Calreticulins and calpains can regulate regeneration

Fluctuations of Ca^{2+} concentration in subcellular compartments can act as a signal of cell stress, in communication between the plasma membrane and organelles, and in the control of the cell cycle [39]. Phytocalpains and calreticulins are examples of calcium-binding proteins that have shown association with the plant cell cycle and regeneration and, therefore, might facilitate transformation. Calpains are cytoplasmic, calcium-dependent cysteine proteases that differ in their Ca^{2+} requirements, whereas calreticulins reside in the lumen of the endoplasmic reticulum, where they bind Ca^{2+} with high capacity [40].

Rice suspension cultures loose their regenerability during time, a phenomenon that has been associated with an increased expression of calreticulin phosphoprotein pp56 (pp56). Rice suspension cultures tested for the abundance of phosphoprotein pp56 showed increased expression from between one and twenty-four-months, an increase that was associated with the loss of the ability of the cells to regenerate [41]. By contrast, constitutive expression of another calreticulin-encoding gene, *BrCRT1*, was shown to be a positive regulator of plant regeneration in *Brassica rapa* [42].

Calpains appear to have an important role in the cell cycle: *defective kernel 1* (*DEK1*) encodes a calcium-activated protease in maize; silencing of the *Nicotiana DEK1* homolog *NbDEK* resulted in high transcript levels of the S-phase genes (*PCNA*, *RNR*, *CDC6*), *CycD2*, *CycD3*, *RBR*, *E2F*, *E2F*-regulated genes and *KNOX* (*knotted 1-like homeobox*); and silencing of *NbDEK* caused hyperproliferation of epidermal cells and produced stems with ectopic division of epidermal cells [43].

Auxin receptors and/or transporters affect the G1–S transition

Plant tissues have the potential to regenerate through organogenesis or somatic embryogenesis, depending on the type and proportion of auxins and cytokinins present in culture media; these same two hormones control the cell cycle [5]. In tobacco cells, the ubiquitin proteolytic system (SCF) is required for chromatin decondensation and reactivation of S phase [19] – in these cells auxin appears to trigger E2Fc degradation through the AXR1–SCF pathway [5], where *AXR1* encodes a protein involved in RUB (Ub-related) modification of *CUL1* and, therefore, is a regulator of the E3 ligase–SCF activity. As discussed above, E2Fc prevents the progression through G1–S; therefore, its degradation facilitates G1–S transition [5]. SCF complex also catalyzes the destruction of Aux–IAA transcription factors, triggered by the interaction of auxin with the transport-inhibitor response protein 1 (TIR1) [44]. Aux–IAA proteins actually suppress auxin-induced gene expression, and high auxin levels accelerate their destruction [44].

Cytokinin synthesis and receptors contribute to regeneration

Overexpression of cytokinin synthesis and signaling genes (*IPT*, *ESR1* and *CKI1*) have been shown to improve the efficiency of regeneration [6]. Using microarray analysis, *Rap2.6L*, a gene related to shoot regeneration from root explants, was identified in *Arabidopsis*. *Rap2.6L* is a member of the *AP2–ERBP* transcription-factor gene family and is up-regulated in the presence of cytokinin [45].

The root or shoot decision: PIN polarization precludes organ formation

Although usually not reported, for some plant species, it is common to obtain genetically transformed calli that never regenerate into new plants, for example, *Phaseolus coccineus* [46]. We propose the use of genes involved in cell polarization and early meristem formation to overcome this type of limitation. Polar auxin transport generates gradients that underlie the tropic growth response, leading to apical–basal patterning [11]. PINOID (PID) is a serine–threonine kinase that regulates auxin transport and is the major determinant of PINFORMED (PIN) protein localization (i.e. PIN1, PIN2 and PIN4) [11]. In shoot cells, where PID is normally expressed, PIN1 is located on the apical membranes, whereas in *pid* mutants, PIN1 is mistargetted to basal membranes [11]: constitutive expression of *PID* causes loss of meristem identity in the primary root. PIN proteins focus and stabilize auxin effects in both proximo–distal and lateral dimensions. Hence, PIN proteins direct the expression of the auxin-inducible *PLETHORA* (*PLT*) genes, which specify the positions of the quiescent centre and stem cells in the root [10]. PIN proteins were proposed to restrict *PLT* expression in the basal embryo region to initiate root primordium formation, whereas *PLT* genes maintain *PIN* transcription to stabilize the position of the distal-stem cell niche [10]. In laser-ablated *Arabidopsis* roots, a concerted change in expression domains of *PLT*, *SHORTROOT* (*SHR*) and *SCARECROW* (*SCR*) signal the new PIN polarity, which re-establishes auxin transport and facilitates the regeneration process [47].

Making the aerial part starting from meristems

Most of the aerial parts of the plant are derived from the shoot apical meristem (SAM). Cup-shaped cotyledon (CUC) proteins are transcription factors that regulate the expression of target genes involved in meristem formation, through specific recognition by NAC domains (*Petunia* NAM and *Arabidopsis* ATAF1, ATAF2, and CUC2) [48]. In *Arabidopsis*, the *CUC* genes *CUC1* and *CUC2* act upstream of the *STM* (*SHOOTMERISTEMLESS*) gene, whereas the double mutant *cuc1 cuc2* results in an absence of meristem [49]. CUC proteins transactivate genes involved in SAM formation and organ separation, where *CUC3* encodes a major determinant of boundary specification, and *CUC2* is essential for shoot apical meristem initiation [12]. Both *CUC1* and *CUC2* are post-translationally regulated by micro RNA miR164 [50,51]. Phylogenetic analysis and expression patterns of members of the *NAC* family showed that the paralogs *ZmNAM1* and *ZmNAM2* in maize, *NAM* in *Petunia hybrida*, *CUC2* in *Arabidopsis* and *CUP* in *Antirrhinum majus* contribute to SAM establishment [13]. The C-terminal domain (CTD) of *CUC* genes has

Table 2. Examples of plant genes that should be useful for overcoming blockages on transformation or regeneration

Acquisition of pluripotentiality and heterochromatin remodeling	Drive G1 to S transition to make cells transformable	Regeneration
HP1 ↓ [16–19]	*E2Fa–DPa* ↑ [20,21,23,24,27]	*PID* ↑ (shoot formation) [10,11]
RBR ↓ [7,14,20,21,36]	*CycD3* ↑ [25]	*PID* ↓ (root formation) [10,11]
VIP1 ↑ [17,34,35]	*BrCRT1* ↑ [42]	*CUC1* and *CUC2* ↑ [12,48,49]
H2A ↑ [24,31,32]	*CycD1* ↑ [26]	*ZmNAM1* or *ZmNAM2* ↑ [13]
Histone deacetylases ↓ [16,17]	*CycD* and *CycB Kinases* ↑ [20,21,23,24,37]	*IPT* ↑ [6]
Histone acetylase HAT B ↑ [24]	*DEK1* ↓ [43]	*ESR1* ↑ [6]
	E2Fc ↓ [5,23,28]	*RBR* ↑ [20,21]
	RBR ↓ [22]	

↑: induced or transient up-regulation required; ↓: induced or transient down-regulation required. See text for abbreviations.

transactivation activity but the functional specificity of *CUC* in promoting adventitious shoot formation resides in their NAC domain. Ectopic expression of *CUC1* and *CUC2* increases the frequency of adventitious shoot formation [48].

Inducible Promoters

The evidence presented above suggests that genes that affect chromatin remodeling or the cell cycle might be important tools for improving transformation or regeneration efficiency. However, it is also abundantly clear that their constitutive activation or silencing would prevent normal development. Published examples include suppression of the RBR protein by VIGS (leading to ectopic cell division and a lack of differentiation) [38], suppression of phytocalpain *NbDEK1* (inhibiting cell differentiation) [43] and overexpression of the calreticulin BrCRT1 (retarding plant growth) [42]. There are several inducible systems available for the spatial and temporal control of gene expression. These can minimize the detrimental effects of developmental genes when used to promote transformation: they can directly induce or suppress gene expression or cause DNA rearrangements that cause permanent gain or loss of expression. Inducible systems include those regulated by tetracycline, steroids, insecticides, ethanol and copper [52]. Precise control of transcription is achievable using inducible promoters in combination with microinduction, where the chemical inducer is embedded in a paste and applied under the microscope [53]. Microinduction has been used in combination with a tetracycline-inducible promoter driving expression of expansin and phragmoplastin, enabling control of gene expression in small areas within the meristem [53,54]. Among the inducible systems, the alcohol-inducible switch – based on components of the *alc* regulon, including AlcR transcriptional activator and alcA promoter of *Aspergillus nidulans* [55] – has been considered as one of the most promising in a wide range of model and crop plants. It is particularly suitable for functional genomics studies because of its high specificity and efficiency [56,57]. The *alc* switch enables temporal and spatial regulation of gene expression, and it has been successfully used for efficient induced expression and reliable RNA silencing of developmental genes such as *STM*, *CycD1* and *CycD3* [26,57].

Conclusion

The process of making a transgenic plant can stall at various points, including acquisition of pluripotentiality, progress into the S phase of the cycle – which makes cells competent for transformation – and differentiation of transformed cells into new embryos or meristems. Many of the genes that have major roles in these processes have been identified, and the manipulation of several of them has been shown to affect the rate of transformation and/or regeneration. Depending on the point at which a blockage appears to occur, different strategies for the temporal and spatial induction of gene expression or repression are needed. Several options for selectively up- or down-regulating key genes are briefly outlined in Table 2.

The effectiveness of induced cell cycle-related genes in causing cell nuclei to enter S phase can be assessed by several methods, including the study of simple phenotypes such as rate of shoot meristem production. To aid recognition of meristemoids at early stages in development, the use of transgenic marker genotypes, such as those containing a *WUSCHEL*–reporter or *STM*–reporter construct [58,59], would be useful. In addition, monitoring of S-phase-specific genes, such as *RNR*, *MCM*, *CDC6*, *PCNA*, *H2A*, *H4* and *E2Fa* [23–25,38], might give detailed insights into the mechanisms of cell cycle modulation: using microarray analysis, >100 genes related to S phase have been identified in *Arabidopsis* after overexpression of *E2Fa–DPa* [24]. In addition, changes in heterochromatin remodeling, DNA or histone methylation and histone acetylation and deacetylation can be directly monitored by a variety of methods [17–19,60]. With the diversity of genomic databases, regeneration systems and developmental obstacles in various species, we anticipate a rich variety of approaches and successes in upcoming years.

References

1 Horsch, R.B. *et al*. (1985) A simple and general method for transferring genes into plants. *Science* 227, 1229–1231

2 Gelvin, S.B. (2003) *Agrobacterium*-mediated plant transformation: the biology behind the 'gene-jockeying' tool. *Microbiol. Mol. Biol. Rev.* 67, 16–37

3 Vain, P. (2005) Plant transgenic science knowledge. *Nat. Biotechnol.* 23, 1348–1349

4 Sharma, K.K. *et al*. (2005) Genetic transformation technology: status and problems. *In Vitro* Cell. Dev. Biol. Plant 41, 102–112

5 del Pozo, J.C. *et al*. (2005) Hormonal control of the plant cell cycle. *Physiol. Plant.* 123, 173–183

6 Zuo, J. *et al*. (2002) Marker-free transformation: increasing transformation frequency by the use of regeneration-promoting genes. *Curr. Opin. Biotechnol.* 13, 173–180

7 Grafi, G. (2004) How cells dedifferentiate: a lesson from plants. *Dev. Biol.* 268, 1–6

8 Lai, Y.C. and Chen, L.F.O. (2002) Flow cytometric analysis of nuclear cell cycle phases in relation to plant regeneration in *Petunia hybrida*. *J. Genet. Mol. Biol.* 13, 13–20

9 Peña, L. *et al*. (2004) Early events in *Agrobacterium*-mediated genetic transformation of citrus explants. *Ann. Bot. (Lond.)* 94, 67–74

10 Blilou, I. *et al*. (2005) The PIN auxin efflux facilitator network controls growth and patterning in *Arabidopsis* roots. *Nature* 433, 39–44

11 Friml, J. *et al*. (2004) A PINOID-dependent binary switch in apical–basal PIN polar targeting directs auxin efflux. *Science* 306, 862–865

12 Vroemen, C.W. *et al*. (2003) The *CUP-SHAPED COTYLEDON3* gene is required for boundary and shoot meristem formation in *Arabidopsis*. *Plant Cell* 15, 1563–1577

13 Zimmermann, R. and Werr, W. (2005) Pattern formation in the monocot embryo as revealed by *NAM* and *CUC3* orthologues from *Zea mays* L. *Plant Mol. Biol.* 58, 669–685

14 Gutierrez, C. (2000) DNA replication and cell cycle in plants: learning from geminiviruses. *EMBO J.* 19, 792–799

15 Ng, H.H. and Bird, A. (1999) DNA methylation and chromatin modification. *Curr. Opin. Genet. Dev.* 9, 158–163

16 Wagner, D. (2003) Chromatin regulation of plant development. *Curr. Opin. Plant Biol.* 6, 20–28

17 Avivi, Y. *et al*. (2004) Reorganization of specific chromosomal domains and activation of silent genes in plant cells acquiring pluripotentiality. *Dev. Dyn.* 230, 12–22

18 Hsieh, T.F. and Fischer, R.L. (2005) Biology of chromatin dynamics. *Annu. Rev. Plant Biol.* 56, 327–351

19 Zhao, J. *et al*. (2001) 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, 22772–22778

20 Shen, W.H. (2001) The plant cell cycle: G1/S regulation. *Euphytica* 118, 223–232

21 Dewitte, W. and Murray, J.A.H. (2003) The plant cell cycle. *Annu. Rev. Plant Biol.* 54, 235–264

22 Williams, L. *et al*. (2003) Chromatin reorganization accompanying cellular dedifferentiation is associated with modifications of histone H3, redistribution of HP1, and activation of E2F-target genes. *Dev. Dyn.* 228, 113–120

23 de Jager, S.M. *et al*. (2005) The developmental context of cell-cycle control in plants. *Semin. Cell Dev. Biol.* 16, 385–396

24 Vlieghe, K. *et al*. (2003) Microarray analysis of *E2Fa–DPa*-overexpressing plants uncovers a cross-talking genetic network between DNA replication and nitrogen assimilation. *J. Cell Sci.* 116, 4249–4259

25 Riou-Khamlichi, C. *et al*. (1999) Cytokinin activation of *Arabidopsis* cell division through a D-type cyclin. *Science* 283, 1541–1544

26 Koroleva, O.A. *et al*. (2004) *CycD1*, a putative G1 cyclin from *Antirrhinum majus*, accelerates the cell cycle in cultured tobacco BY-2 cells by enhancing both G1/S entry and progression through S and G2 phases. *Plant Cell* 16, 2364–2379

27 de Veylder, L. *et al*. (2002) Control of proliferation, endoreduplication and differentiation by the *Arabidopsis* E2Fa–DPa transcription factor. *EMBO J.* 21, 1360–1368

28 Vanneste, S. *et al*. (2005) Auxin regulation of cell cycle and its role during lateral root initiation. *Physiol. Plant.* 123, 139–146

29 Villemont, E. *et al*. (1997) Role of the host cell cycle in the *Agrobacterium*-mediated genetic transformation of *Petunia*: evidence of an S-phase control mechanism for T-DNA transfer. *Planta* 201, 160–172

30 Chen, L.F.O. *et al*. (2001) Transformation of broccoli (*Brassica oleracea* var. *italica*) with isopentenyltransferase gene via *Agrobacterium tumefaciens* for post-harvest yellowing retardation. *Mol. Breed.* 7, 243–257

31 Mizuguchi, G. *et al*. (2004) ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. *Science* 303, 343–348

32 Mysore, K.S. *et al*. (2000) An *Arabidopsis* histone *H2A* mutant is deficient in *Agrobacterium* T-DNA integration. *Proc. Natl. Acad. Sci. U. S. A.* 97, 948–953

33 Loyter, A. *et al*. (2005) The plant VirE2 interacting protein 1. A molecular link between the *Agrobacterium* T-complex and the host cell chromatin? *Plant Physiol.* 138, 1318–1321

34 Li, J. *et al*. (2005) Uncoupling of the functions of the *Arabidopsis* VIP1 protein in transient and stable plant genetic transformation by *Agrobacterium*. *Proc. Natl. Acad. Sci. U. S. A.* 102, 5733–5738

35 Tzfira, T. *et al*. (2002) Increasing plant susceptibility to *Agrobacterium* infection by overexpression of the *Arabidopsis* nuclear protein VIP1. *Proc. Natl. Acad. Sci. U. S. A.* 99, 10435–10440

36 Gordon-Kamm, W. *et al*. (2002) Stimulation of the cell cycle and maize transformation by disruption of the plant retinoblastoma pathway. *Proc. Natl. Acad. Sci. U. S. A.* 99, 11975–11980

37 den Boer, B.G.W. and Murray, J.A.H. (2000) Control of plant growth and development through manipulation of cell-cycle genes. *Curr. Opin. Biotechnol.* 11, 138–145

38 Park, J.A. *et al*. (2005) Retinoblastoma protein regulates cell proliferation, differentiation, and endoreduplication in plants. *Plant J.* 42, 153–163

39 Kadota, Y. *et al*. (2005) Cell-cycle-dependent regulation of oxidative stress responses and Ca^{2+} permeable channels NtTPC1A/B in tobacco BY-2 cells. *Biochem. Biophys. Res. Commun.* 336, 1259–1267

40 Nagata, T. *et al*. (2004) Comparative analysis of plant and animal calcium signal transduction element using plant full-length cDNA data. *Mol. Biol. Evol.* 21, 1855–1870

41 Li, Z. and Komatsu, S. (2000) Molecular cloning and characterization of calreticulin, a calcium-binding protein involved in the regeneration of rice cultures suspension cells. *Eur. J. Biochem.* 267, 737–745

42 Jin, Z.L. *et al*. (2005) Over-expression of Chinese cabbage calreticulin 1, *BrCRT1*, enhances shoot and root regeneration, but retards plant growth in transgenic tobacco. *Transgenic Res.* 14, 619–626

43 Ahn, J.W. *et al*. (2004) Phytocalpain controls the proliferation and differentiation fates of cells in plant organ development. *Plant J.* 38, 969–981

44 Dharmasiri, N. *et al*. (2005) The F-box protein TIR1 is an auxin receptor. *Nature* 435, 441–445

45 Che, P. *et al.* (2005) *Rap2.6L* is involved in a transcription factor network during shoot development in *Arabidopsis*. 16th International Conference on *Arabidopsis* Research, TAIR accession number 501716888 (http://www.arabidopsis.org/servlets/TairObject?type=publication&id=501716888)

46 Zambre, M. *et al*. (2001) Regeneration of fertile plants from callus in *Phaseolus polyanthus* Greenman (year bean). *Ann Bot (Lond)* 88, 371–377

47 Xu, J. *et al*. (2006) A molecular framework for plant regeneration. *Science* 311, 385–388

48 Taoka, K. *et al*. (2004) The NAC domain mediates functional specificity of *CUP-SHAPED COTYLEDON* proteins. *Plant J.* 40, 462–473

49 Aida, M. *et al*. (1997) Genes involved in organ separation in *Arabidopsis*: an analysis of the *CUP-SHAPED COTYLEDON* mutant. *Plant Cell* 9, 841–857

50 Laufs, P. *et al*. (2004) MicroRNA regulation of the *CUC* genes is required for boundary size control in *Arabidopsis* meristems. *Development* 131, 4311–4322

51 Mallory, A.C. *et al*. (2004) MicroRNA regulation of NAC-domain targets is required for proper formation and separation of adjacent embryonic, vegetative, and floral organs. *Curr. Biol.* 14, 1035–1046

52 Padidam, M. (2003) Chemically regulated gene expression in plants. *Curr. Opin. Plant Biol.* 6, 169–177

53 Pien, S. *et al*. (2001) Local expression of expansin induces the entire process of leaf development and modifies leaf shape. *Proc. Natl. Acad. Sci. U. S. A.* 98, 11812–11817

54 Wyrzykowska, J. and Fleming, A. (2003) Cell division pattern influences gene expression in the shoot apical meristem. *Proc. Natl. Acad. Sci. U. S. A.* 100, 5561–5566

55 Caddick, M.X. *et al*. (1998) An ethanol-inducible gene switch for plants used to manipulate carbon metabolism. *Nat. Biotechnol.* 16, 177–180

56 Filichkin, S. *et al.* Alcohol-inducible gene expression in transgenic *Populus*. *Plant Cell Rep.* DOI: 10.1007/S00299-005-0112-3 (http://

www.springerlink.com/(d1kwim45shxuix55eanpj245)/app/home/contribution.asp?referrer=parent&backto=issue,35,73;journal,1,251;linkingpublicationresults,1:100383,1)

57 Li, R. *et al*. (2005) Ethanol-inducible gene expression system and its applications in plant functional genomics. *Plant Sci.* 169, 463–469

58 Laux, T. *et al*. (1996) The *WUSCHEL* gene is required for shoot and floral meristem integrity in *Arabidopsis*. *Development* 122, 87–96

59 Lenhard, M. *et al*. (2002) The *WUSCHEL* and *SHOOTMERISTEMLESS* genes fulfill complementary roles in *Arabidopsis* shoot meristem regulation. *Development* 129, 3195–3206

60 Gaudin, V. *et al*. (2001) Mutations in *Like-Heterochromatin Protein 1* affect flowering time and plant architecture in *Arabidopsis*. *Development* 128, 4847–4858

61 Grafi, G. *et al*. (1996) A maize cDNA encoding a member of the retinoblastoma protein family: involvement in endoreduplication. *Proc. Natl. Acad. Sci. U. S. A.* 93, 8962–8967

62 Bennett, S.R.M. *et al*. (1995) Morphogenesis in *pinoid* mutants of *Arabidopsis thaliana*. *Plant J.* 8, 505–520

63 Banno, H. *et al*. (2001) Overexpression of *Arabidopsis ESR1* induces initiation of shoot regeneration. *Plant Cell* 13, 2609–2618

64 Kunkel, T. *et al*. (1999) Inducible isopentenyl transferase as a high-efficiency marker for plant transformation. *Nat. Biotechnol.* 17, 916–919

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