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Cell Cycle Development

Meeting Review

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The Keystone Symposium on the Cell Cycle and Development brought together biologists with an interest in how cell cycle control is integrated into the ontogenetic program of multicellular organisms, and showcased research using a wide variety of systems from both animals and plants. A clear indication from the meeting is that this research is changing the conventional wisdom on both cell cycle control and development.

A defining feature of life is a cell's ability to reproduce, which it does through a complex regulatory program commonly referred to as the cell cycle. The cell cycle coordinates replication of the genome and subsequent segregation of each genomic replicate into a new daughter cell, a process whose reiteration leads to the exponential proliferation of cells. Over the last 25 years, much has been learned about the molecular genetics of eukaryotic cell cycle control in single-cell organisms such as yeast, as well as in cultured mammalian cells, which have provided a model system for the study of cell cycle disregulation in cancer. The latter often involves mutations in genes required in multicellular organisms for the subjugation of cell cycle control to the higher level ontogenetic program, which generally entails developmental transformation and ultimate cessation of the cell cycle. Given this connection, it is surprising that in recent years the field of developmental biology has paid relatively little attention to cell cycle control (compared to other aspects of development such as patterning and differentiation), and conversely cell cycle biologists have to a large extent failed to address the subject of development. However, a reunion is currently underway that is sparking paradigm shifts in both fields. The Keystone Symposium on the Cell Cycle and Development, organized by Dirk Inzé, Bruce Edgar, and Jacqueline Lees and held in Snowbird, Utah in January 2004, was a landmark event in this reunion, bringing together biologists studying cell cycle control in the context of a large number of developmental systems from both animals and plants.

The presentations varied in their emphasis on either cell cycle control or development, as might be expected at a meeting that aims to reintegrate fields that have followed divergent trajectories. Because of this fact and the large variety of subjects that were covered, only a subset of the presentations will be reviewed here, and these will invariably reflect my own interests. Talks dealing with cell cycle development in animals and plants are reviewed in separate sections, a device that was not used at the meeting but that I find to be natural given fundamental differences in animal and plant development.

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In his keynote address, Marc Kirschner set the tone by presenting recent unpublished work aimed at defining the role of the anaphase promoting complex (APC) and UbcH10 in cyclin A metabolism and the G1 phase of the cell cycle. The take-home lesson was that rather than being driven by extrinsic signals, G1 may actually be regarded as an autonomous extension of mitosis. In other words, the oscillatory forces that carry cells through all four phases of the cycle are likely to be entirely intrinsic to the cellular machinery, which is modulated by (and not strictly dependent on) extrinsic signals. This point may eventually appear obvious in retrospect, but it has not been the conventional wisdom of cell cycle research concerned with the G1 to S phase transition in mammalian cells, which has been viewed as requiring the mitogenic signal-driven activities of G1-specific regulators such as cyclin D/ckd4 and cyclin E/cdk2. Thus, the recent findings that mice lacking the cyclin E gene are largely viable (Geng et al., 2003), as are mice lacking cdk2 (Berthet et al., 2003; presented in a talk by Philipp Kaldis) were probably disturbing to anyone who had assumed that cyclin E/cdk2 was an integral component of the G1→S phase transition. In this new perspective, G1 regulators such as cyclin D/cdk4, cyclin E/cdk2, and their various inhibitors might be viewed not as devices that are intrinsically required for cell cycle control, but rather as inventions for integrating cell cycle control into the higher level developmental program of multicellular organisms. In the opinion of this reviewer, findings consonant with such a view provided perhaps the single strongest underlying current running through the meeting.

The Coordination of Cell Proliferation, Differentiation, and Morphogenesis

In animal development, cell proliferation and cell differentiation are tightly linked and coordinated through signaling that regulates the expression of cell cycle control genes (Figure 1). Among these are the cdk inhibitors, which are not only required for timely exit from the cell cycle during differentiation, but also for the maintenance of cell cycle arrest in differentiated cells. As an example of the latter, Martine Roussel presented recent work showing that the cyclin D/cdk4 inhibitor $p19^{Ink4d}$ is required to maintain sensory hair cells of the inner ear in a postmitotic state (Chen et al., 2003). In mice lacking *Ink4d*, the sensory hair cells differentiate normally during embryogenesis but are progressively lost in the adult after reentering the cell cycle and undergoing apoptosis (Chen et al., 2003). Thus, the terminally differentiated state is not necessarily incompatible with the resumption of cell division, which must at least in some cases be actively inhibited. The converse, that is, a requirement for factors that actively maintain the proliferative state of progenitor cells, can also be true. Ed Levine described work from his lab showing that in the developing retina, expression of the *Chx10* homeodomain gene

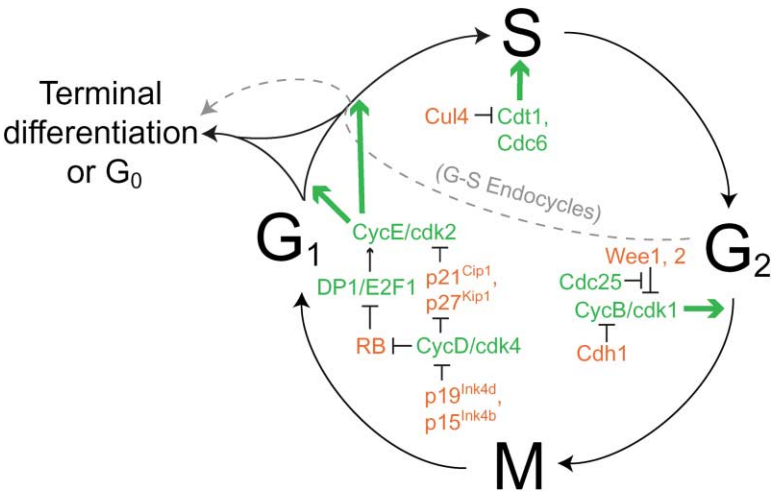

Figure 1. A Simplified Schematic of the Somatic Cell Cycle and Some of the Control Proteins Mentioned in This Review

Proteins that stimulate the indicated phase or phase transition are shown in green, while inhibitory proteins are shown in red. The developmental coordination of cell proliferation, differentiation, and morphogenesis involves the regulated expression of these proteins, typically at the transcriptional level, and often in response to intercellular signaling. Terminal differentiation involves downregulation of stimulatory activities and upregulation of inhibitory activities, leading to cell cycle exit (usually during G1). Differentiated cells can also reenter the cell cycle and, at least in some cases, are actively prevented from doing so by the activities of cell cycle inhibitors. Regulated expression of cell cycle control proteins is also used to developmentally modify the cell cycle, for example, into an endoreduplication cycle (endocycle) that bypasses mitosis in preparation for some types of cell differentiation.

promotes proliferation, by modulating the activity of the $p27^{Kip1}$ cdk2 inhibitor (Green et al., 2003). This is likely to occur in part through the sequestration of p27 by cyclin D1, which may be a direct target of Chx10 (Green et al., 2003). Thus, active maintenance of the proper balance between proliferation and differentiation is an integral function of the developmental program.

Paolo Dotto further elaborated on this theme in his presentation of research on the proliferation and differentiation of skin cells, suggesting that the transitions from one compartment to another (self-renewing↔transit amplifying↔growth arrested↔terminally differentiated) can in principle go both ways and thus represent a dynamic equilibrium determined by the levels of cell cycle regulators that are responsive to extracellular signals (Okuyama et al., 2004). As an example, the $p21^{Cip1}$ cdk inhibitor, which is upregulated in growth-arrested keratinocytes, is transcriptionally activated by signaling via Notch1, and this is required for the irreversible commitment to differentiation (Rangarajan et al., 2001; Topley et al., 1999). A similar situation is obtained with TGFβ-mediated control of cell proliferation in epithelial cells, which was the subject of a presentation by Joan Seoane from the laboratory of Joan Massagué. TGFβ signaling downregulates c-*myc* while upregulating expression of both $p15^{Ink4b}$ (Seoane et al., 2001) and $p21^{Cip1}$. The transcriptional effector of TGFβ signaling is the phosphorylated Smad3/4 transcription factor, which typically requires DNA binding cofactors for effective binding and activity. In the *p21Cip1* promoter, the cofactor required for Smad3/4 activity is the FoxO transcription factor, and this activity is inhibited by PI3K/AKT signaling and by the transcription factor FoxG1 (Seoane et al., 2004). Thus, $p21^{Cip1}$-dependent cell cycle arrest in a variety of developmental contexts reflects the integration of inputs from different signaling pathways on the *p21Cip1* promoter.

The developmental decision to continue cycling is typically made prior to the restriction point in G1, and work in mammalian cell culture has shown that cells are driven past the restriction point by the activities of cyclin D/cdk4/6 and cyclin E/cdk2 (Sherr, 2000). Peter Sicinski reviewed work from his lab in which D-type cyclins have been knocked out, either singly or in combination (Ciemerych et al., 2002; Sicinska et al., 2003), while Hiroaki Kiyokawa reviewed work from his lab on the knockout of cdk4 (Zou et al., 2002). While much of the presented work from the Sicinski lab is unpublished and cannot be described here (stay tuned!), a take-home lesson is that like the cyclinE/cdk2 knockouts, these studies have led to some unexpected conclusions about the role of cyclinD/cdk4 in mammalian development. The emerging concept is that the major role of the cyclin D/cdk4 system is modulating the cell cycle in response to mitogenic developmental signals. In vertebrates, this is likely to be facilitated by the presence of three cyclin D genes, giving greater developmental flexibility. A poster presented by Valerie Lobjois from the laboratory of Fabienne Pituello showed that cyclin D1 and cyclin D2 have complementary expression patterns in the developing chick spinal cord and respond differently to fgf8 and shh, two of the major signaling systems that pattern the neural tube. Cyclin D2 is activated by fgf8 signaling in the caudal region of the neural plate, whereas cyclin D1 expression is repressed by fgf8 signaling in that region, but activated by shh signaling in the closing neural tube. As with many other families of regulatory proteins in vertebrates, what is not clear is to what extent the different cyclin D proteins are functionally distinct, or whether they merely represent expression variants required to fulfill a generic cyclin D function in response to different developmental signals.

Downstream of cyclin D/ckd4 activity and at the core of the G1/S machinery is the E2F/RB transcriptional regulatory system. The E2F genes encode both activators and repressors that function at different phases of the cell cycle, and in different developmental contexts. The conventional view is that, along with their heterodimeric partner DP1, E2F proteins bind to the promoters of cell cycle genes activated at the G1/S transition, an

activity that is modulated by physical interactions with RB pocket proteins; the interaction between E2Fs and RB family members is in turn regulated by phosphorylation of RB by cdks. As is the case with the D and E cyclins and their associated kinases, the conventional wisdom on E2Fs requires some revision. Nick Dyson described recent work on the two *Drosophila* E2Fs, which are functionally antagonistic in cell cycle control: the activator dE2F1 promotes proliferation, while the repressor dE2F2 does the opposite (Frolov et al., 2001). However, combined loss of function shows that the dE2Fs are not essential for cell cycle progression, although they are required for cell cycle-regulated gene expression. Microarray screens using probes prepared from cultured SL2 cells treated with RNAi for each dE2F have revealed distinct but overlapping sets of genes regulated by each transcription factor (Dimova et al., 2003). In general, dE2F1 is required to activate genes associated with cell cycle progression, while dE2F2 represses genes associated with a variety of developmentally regulated, differentiated cellular functions not associated with the cell cycle (Dimova et al., 2003). Unlike the case with the cell cycle genes, the latter repressive functions are not antagonized by E2F1, but rather are likely to be alleviated by a variety of other transcription factors in promoter context-specific mechanisms that respond to developmental signals (Dimova et al., 2003). Thus, the E2F/RB gene regulatory network is not simply dedicated to driving the cell cycle but is actually integral to the developmental coordination of cell proliferation and differentiation.

Jacqueline Lees presented unpublished work that addresses the role of the E2F proteins in the regulation of the tumor suppressor $p19^{ARF}$. Her group has shown that E2F contributes to transcriptional repression of *Arf* in normal, unstressed cells and to the activation that occurs in response to oncogenic challenge. This analysis demonstrates that *Arf* is a genuine E2F-responsive gene that is regulated in a different manner from classic E2F targets.

The retinoblastoma (RB) proteins oppose cell cycle progression via their association with E2Fs, but they also interact with other transcription factors. Philip Hinds presented work on the role of RB in osteogenesis and osteosarcoma, which is commonly associated with loss of the RB gene. In bone development, RB physically interacts via its pocket domain with the Runx2 transcription factor to activate transcription of osteoblast differentiation genes (Thomas et al., 2001), and loss of RB function is associated with a defect in osteoblast differentiation and a retention of proliferative capacity. *Runx2* participates in each step along the developmental trajectory from pluripotent mesenchymal cell to mature osteoblast, reflecting an involvement of Runx genes in both cell proliferation and differentiation. A fundamental question regarding the biology of Runx proteins is how their activity is modulated during the transition from a proliferative to a differentiated state (Coffman, 2003). The answer undoubtedly lies in large part in the specific protein-protein interactions engaged by Runx proteins, for example the Runx2-RB interaction during osteoblast differentiation, and the Runx2-Twist interaction that was recently shown by Bialek et al. ([2004], this issue of *Developmental Cell*) to prevent premature differentiation of osteoblast progenitor cells.

The *Drosophila* eye presents a particularly good genetic model for research on the mechanisms that coordinate proliferation and differentiation, and several presentations described work using this system. The laboratories of Georg Halder and Iswar Hariharan have each used eye phenotype in mutagenesis screens to look for genes required for the control of growth and/or cell proliferation. These screens have revealed two new genes—*hippo* and *salvador*—the products of which limit cell number in the eye both by blocking proliferation (correlated with downregulation of cyclin E) and promoting apoptosis (correlated with phosphorylation of DIAP1; Harvey et al., 2003; Tapon et al., 2002; Udan et al., 2003). *Hippo* encodes a kinase that interacts with the scaffold protein Salvador (Harvey et al., 2003; Udan et al., 2003) in a complex with the warts protein kinase, mutations in which were previously shown to give a overgrowth phenotype similar to that found in *hippo* and *salvador* mutants (Justice et al., 1995; Xu et al., 1995). In their screen, the Hariharan lab also discovered archipelago, the F-box protein that targets cyclin E for degradation (Moberg et al., 2001), and *Tsc1* and *Tsc2*, genes that are required to maintain cells in G0 arrest by antagonizing genes that promote cell growth and entry into S phase (Tapon et al., 2001).

Helena Richardson described genes discovered in a screen for modifiers of a cyclin E mutant eye phenotype (rough eye caused by loss of cell proliferation). Two suppressors recovered in the screen, *scribble* and *lgl*, encode cytoarchitectural proteins that function in the maintenance of apico-basal polarity and are required both for cell shape and to control proliferation within the epithelium. *Scrib* mutant clones within the eye disc display ectopic cyclin E expression and cell proliferation (that can be at least partially accounted for by ectopic hedgehog signaling), which is counteracted by JNK signal-mediated compensatory cell death. The latter can be overcome by oncogenetically activated ras or notch signaling, resulting in an aggressive tumorogenesis phenotype in the eye disc that provides a good model for multistep carcinogenesis (Brumby and Richardson, 2003). As shown by Wei Du in his talk, hedgehog signaling is important for the synchronous entry into S phase during the second mitotic wave in the eye disc, and this is in part mediated by direct binding of the Ci transcription factor to the cyclin E promoter (Duman-Scheel et al., 2002). A theme that emerged from this talk as well as several others is that developmental patterning signals such as hedgehog coordinate cell proliferation and differentiation by regulating the expression of both cell-type-specific genes and cell cycle control genes. Of course, a deep understanding of how this is actually achieved will ultimately require detailed knowledge of the architecture of the relevant *cis*-regulatory systems and gene-regulatory networks within which they are linked.

During animal development, cell cycle control is not only important for cell differentiation but also critical for morphogenesis. Paul Mueller presented data from *Xenopus* showing that the Wee2 kinase, which inactivates cdk1, is expressed in the paraxial mesoderm, wherein it is required to maintain the low mitotic index

that is observed in that tissue. Morpholino antisense-mediated knockdown of Wee2 leads to defective convergent extension, as do other methods of artificially advancing the cell cycle (Leise and Mueller, 2004). In general, the process of cell division is likely to be incompatible with morphogenetic movements: it has recently been shown that Wee1-mediated cell cycle arrest is required for *Xenopus* gastrulation (Murakami et al., 2004), and in flies the *tribbles* gene is required to downregulate the products of *string* and *twine* and thereby block cell division during formation of the ventral furrow (Grosshans and Wieschaus, 2000; Mata et al., 2000; Seher and Leptin, 2000).

Growth and the Cell Cycle

Several presentations dealt with the topic of the cell cycle in relation to growth. George Thomas discussed work from his lab on the role of ribosomal biogenesis in regulating cell proliferation. Using liver regeneration as a model system, Thomas and colleagues have shown that conditional deletion of the ribosomal protein S6 gene does not cause defective liver growth in a fasting/refeeding regime, but it does cause defective regeneration after hepatectomy, as a result of a failure in cell proliferation associated with a loss of cyclin E expression (Volarevic et al., 2000). However, recent unpublished work shows that rescue of cyclin E expression does not restore the S6-deficient hepatocytes ability to enter S phase. Microarray experiments indicate that the S6-deficient hepatocytes fail to upregulate several cell cycle control genes including E2F1, while overexpressing several cell cycle inhibitors. Based on the results obtained from $S6^{+/-}$ heterozygous hepatocytes, which do continue to proliferate (albeit more slowly), it was hypothesized that entry into S phase is modulated by a ribosome-counting mechanism, whereas complete loss of ribosomal biogenesis activates a cell cycle checkpoint.

Bruce Edgar presented work using *Drosophila* to define the roles of insulin signaling and the dMyc transcription factor in cell growth. Activation of the insulin receptor, which normally occurs in response to nutrients, promotes growth by alleviating the TSC1/2-mediated inhibition of Rheb, a GTP binding protein that stimulates glucose uptake and activates the TOR protein kinase (Saucedo et al., 2003). Insulin signaling is therefore a mechanism for tuning growth rate to nutrient availability (Britton et al., 2002). dMyc is also involved in promoting cell growth (Edgar et al., 2001; Johnston et al., 1999), but it does so independent of insulin signaling and nutrient availability, and instead is likely to be modulated by various developmental signaling pathways. Among the dMyc-responsive genes are those involved in ribosome biogenesis (Orian et al., 2003), including rRNA synthesis, a RNA polymerase I-dependent function that is not directly modulated by insulin signaling. Consistent with this role, dMyc overexpression causes an increase in the size of nucleoli, whereas underexpression of dMyc does the opposite. It has been suggested that the role of myc in ribosomal biogenesis may contribute significantly to its oncogenic potential (Ruggero and Pandolfi, 2003). Interestingly, neither dMyc nor the insulin signaling pathway affects the expression of cell cycle control genes that have been tested, suggesting that their roles in promoting cell growth may be linked indirectly to a role in cell division.

Martin Raff presented recently published work showing that unlike yeast cells, mammalian cells do not have a cell size checkpoint that couples cell cycle progression to cell growth (Conlon and Raff, 2003). Rather, cell growth and the cell division cycle are independently controlled by extracellular signals (growth factors and mitogens, respectively). The reason that mammalian cells do not need a cell size checkpoint is that unlike yeast cells, they maintain a constant growth rate, independent of size (Conlon and Raff, 2003). Since the overall rate of protein synthesis increases with cell size, a constant growth rate would require that the rate of protein degradation also increase as cells get larger, indicating that protein anabolism is somehow coupled to catabolism. For sympathetic neurons, NGF is required to shut down protein degradation when protein synthesis is blocked by cycloheximide, suggesting that in these cells such coupling is itself dependent on extracellular signals. Thus, by coupling the rates of protein synthesis and degradation to extracellular signaling, mammalian cells have dispensed with the need for a cell size checkpoint, and this may allow for more flexibility in the developmental modulation of growth and cell cycle control in animals.

DNA Replication and Developmental Modification of the Cell Cycle

A primary function of the cell cycle is replication of the genome, and in the prototypical cell cycle redundant mechanisms exist to ensure that this only happens once per cycle. A failure in these mechanisms often leads to inappropriate rereplication of DNA, which in turn can contribute to genomic instability leading to cancer. Edward Kipreos discussed findings from his lab showing that in *C. elegans*, CUL-4 is required to prevent rereplication of the genome in the larval somatic cells by participating in the destruction of the licensing factor CDT-1 following S phase initiation (Zhong et al., 2003). In a related vein, Brian Calvi presented unpublished work showing that in the *Drosophila* eye disc, phosphorylation by cyclin E/cdk2 is required to target CDT1/DUP for destruction following entry into S phase.

On the other hand, not all cells divide after replicating their DNA: certain cell types have modified cell cycles in which the entire genome is endoreduplicated, or specific genes amplified by multiple rounds of local replication. This is often a developmental strategy that facilitates high levels of specific gene expression in support of specific differentiated cell functions, for example amplification of genes encoding chorion proteins (structural proteins of the eggshell) in *Drosophila* follicle cells. Addressing the question of how generally this developmental strategy is used in follicle cells, Terry Orr-Weaver presented results of a differential microarray screen to detect amplified genes (Claycomb et al., 2004). Differentially amplified loci were found to be clustered into four genomic intervals, two of which were not previously known to be amplified. While the level of amplification was found to be modest (4- to 6-fold), it was developmentally significant and required for normal levels of

expression of genes within the amplicons. In the case of the *yellow-g* gene (which encodes a crosslinking enzyme within one of the amplicons), a failure of amplification leads to a loss of the structural integrity of the chorion. Thus, in some instances of cell differentiation, differential gene amplification appears to be used as an alternative strategy to differential transcriptional activation to support high levels of gene expression.

What regulates the developmental transition from a normal mitotic cycle to an endocycle? Hannele Ruohala-Baker presented work from her lab showing that the Notch pathway regulates this transition in the follicle cell lineage, in part by controlling expression of $cdc25^{string}$, $p21^{dacapo}$, and Cdh1. Delta expression in the germline signals via the notch receptor in follicle cells, and this is required to downregulate both $cdc25^{string}$ and $p21^{dacapo}$ while upregulating Cdh1 (Deng et al., 2001; Schaeffer et al., 2004) (H.R. Shcherbata et al., submitted). This leads to inhibition of both the G2→M transition and G1 arrest while promoting entry into S phase, thereby modifying the cell cycle into an endocycle that bypasses mitosis.

Plant Cell Cycle Development

All of the themes discussed above are also relevant to plant development, but with numerous twists that reflect fundamental differences between the ways that plants and animals develop. Dirk Inzé set the stage by noting that cell cycle control is somewhat more complex in plants than in animals. While plants have most of the core cell cycle components found in animals (e.g., A-type cdks), they also have plant-specific components (e.g., B-type cdks). Jim Murray noted that organogenesis in plants occurs throughout the life of the plant concomitant with growth within organ primordial (meristems), which is very different from animals, in which organogenesis occurs during embryogenesis and is followed by growth in postembryonic development. Two alternate theories have been proposed for the integration of cell division into plant development. The "organismal" theory holds that cell division occurs as a consequence rather than a cause of plant growth, which accounts (for example) for the ability of leaves to compensate for cell proliferation defects by increased cell growth. The "cell" theory holds that cell division itself drives growth of the plant. While some experimental results are consistent with the organismal view, others support the cell theory, and Dirk Inzé has recently proposed that the two views are too polarized (Beemster et al., 2003). Instead, cell division, differentiation, and morphogenesis can be seen as being integrated into a higher level ontogenetic program by signaling systems within growth zones (Beemster et al., 2003). As in animal development, the problem then becomes elucidating how the relevant signaling systems interact with the cell cycle machinery.

Keiko Torii showed that ERECTA receptor serine/threonine kinases play key roles in promoting cell proliferation in *Arabidopsis* (Shpak et al., 2003), by activating the expression of some core cell cycle regulators. Jim Murray discussed the role of D-type cyclins, which, as in animals, respond to extracellular cues (sucrose or hormones) to promote cell proliferation in the leaf shoot apical meristem, a function that mirrors that of cyclin D in animal development. As in animals, differentiation requires timely cell cycle exit during G1, and this is counteracted by overexpression of CYCD3 (Dewitte et al., 2003). Plant development makes extensive use of endoreduplication during growth and differentiation, with different cell types having different ploidy. Yukiko Mizukami described the effect on *Arabidopsis* and tobacco leaf epidermal cell differentiation and patterning of enforcing mitotic cell cycles in cells that normally endoreduplicate (using constitutive CYCD1/3 expression), and vice versa (using constitutive AtFZR/Cdh-1 expression). Despite extreme alterations in cell cycle patterns, leaf epidermal cell differentiation and cell-type-specific marker expression occur independently of ploidy levels. This suggests that the cell cycle and cell differentiation are separable yet normally coupled processes during leaf epidermal development. In a similar vein, Eva Kondorosi described the effects of modulation of APC activity by two distinct types of cdh-1 activators that display distinct expression domains and functions; for example, the *ccs52A* gene is expressed throughout the cell cycle and is specifically required for endoreduplication and consequent differentiation of root nodules in the legume *Medicago truncatula* (Vinardell et al., 2003), whereas *ccs52B* is cell cycle regulated and plays a role in mitotic exit (Tarayre et al., 2004). Finally, Crisanto Gutierrez described work showing how regulation of the G1→S transition by the modulation of the E2F/RB system as well as components of the DNA replication machinery (e.g., CDC6, CDT1) coordinates the transition from mitotic cell cycle to endocycle and terminal differentiation in a cell type-specific manner (Castellano et al., 2001; Ramirez-Parra et al., 2003).

Future Prospects

The field of developmental biology has traditionally been subdivided into areas of interest delimited by different aspects of the developmental process—cell fate specification, differentiation, pattern formation, morphogenesis, etc. Cell cycle development has not received as much attention as these other areas. A clear indication from this meeting was that this neglect is now being remedied, and it is to be hoped that this reflects a larger revolution in thinking about development, a process whose different aspects are integrated by gene regulatory networks that determine when, where, and at what levels the relevant molecular activities for any given aspect are deployed. Much is now known about the relevant molecular activities that modulate the cell cycle during organismal development. How they are linked into the gene regulatory networks that coordinate cell cycle control with other aspects of development such as differentiation and morphogenesis will be a major area for future research.

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