Cell cycle: **To differentiate or not to differentiate?**

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Developmental regulation of the cell cycle is an important determinant of tissue size and shape. Equally important is regulated withdrawal from the cell cycle to allow cells to differentiate. Recent evidence supports a direct link between transcriptional regulation of the cell cycle machinery and cell differentiation.

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We often think of cell-cycle progression and differentiation as two distinct and mutually exclusive processes during development. When cells continue to cycle they do not differentiate, and when cells terminally differentiate they no longer cycle. Thus, there is a temporal coupling between withdrawal from the cell cycle and differentiation. But what are the mechanisms that achieve such coupling? Several recent studies have provided new insights into this issue. In one instance, from *Drosophila*, the same transcription factor appears to simultaneously stimulate both cell-cycle arrest and neural differentiation [1].

Our current knowledge of cell-cycle regulation provides a foundation upon which we can build mechanistic connections between cell division and terminal differentiation. Progression through the cell cycle is governed mainly by the activation and deactivation of a collection of cyclin-dependent kinases (Cdks), which control specific regulatory steps in the cell cycle such as the G1–S and G2–M transitions. In order for cell cycle arrest to accompany differentiation, it is necessary either to downregulate positive regulators of Cdks, such as the cyclins, or to activate negative regulators of Cdks, such as Cdk inhibitors. As we discuss below, cells have been found to use each of these general mechanisms.

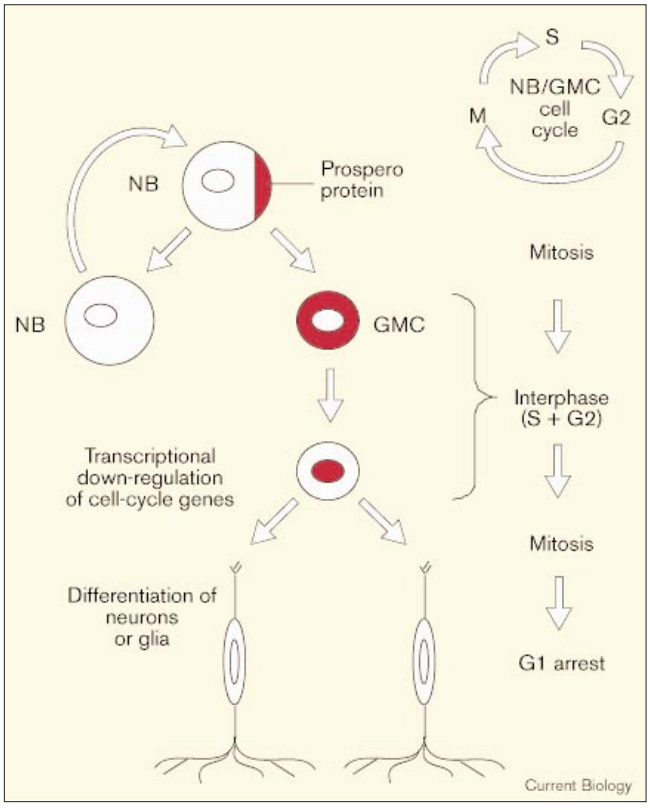
Experimental manipulation of cell-cycle regulators allows one to directly address causality when examining connections between the cell cycle and differentiation. Although a temporal coupling of cell-cycle arrest and terminal differentiation is common during development, and therefore may seem obligatory, let us first explore whether there are instances when the two events can be uncoupled. It is easy to imagine that arresting the cell cycle would permit a cell to begin the differentiation process. Accordingly, it seems unlikely that a typical cell could terminally differentiate and subsequently re-enter the cell cycle. But differentiated myocytes overexpressing the G1–S activator E2F-1 incorporate bromodeoxyuridine (BrdU) into nuclei, indicating entry into the S phase of the cell cycle can still occur in these cells [2]. So differentiation does not necessarily preclude progress through the cell cycle, suggesting that active maintenance of cell-cycle arrest is an important aspect of the differentiated state. Some species have evolved to use this plasticity to their advantage. Certain amphibians, such as the newt, have the ability to regenerate lost tissue. In cell culture, differentiated newt myotubes will re-enter the cell cycle in response to a ligand produced by activation of the thrombin protease, which is required for blood clotting [3]. This cellular response is likely to be a key aspect of the limb regenerative capability of these animals.

Conversely, does cell-cycle arrest always result in differentiation? A number of recent experiments indicate that the answer to this question is no — cell-cycle arrest may be necessary for differentiation, but it is not always sufficient. Oligodendrocyte differentiation involves both changes in gene expression and concurrent cell cycle arrest. In order to determine whether cell-cycle arrest is sufficient to cause differentiation, Tang *et al.* [4] inhibited proliferation of oligodendrocyte precursors *in vitro* by overexpressing p27, a well-known Cdk inhibitor. They discovered that the precursors stopped dividing, but did not express proteins associated with differentiation. This suggests that cell-cycle arrest *per se* is not sufficient for differentiation, and that other signaling pathways are also needed.

Interestingly, these other signaling pathways can also be regulated by molecules that are better known to control the cell cycle. For instance, the *Xenopus* Cdk inhibitor $p27^{Xic1}$ was found to induce Müller glial cells in the developing retina [5]. In this case, one might expect that this cell-cycle regulator was acting simply to terminate the cell cycle, thereby allowing the determination of glial cells from retinoblasts. But cell-cycle arrest caused by treatment of *Xenopus* embryos with the DNA synthesis inhibitors hydroxyurea or aphidicolin was not sufficient to promote differentiation of retinoblasts into glia. Similarly, dominant-negative forms of Cdk2 and Cdc2 did not induce glia. It seems, therefore, that $p27^{Xic1}$ induces Müller glial cell formation not solely by preventing cell-cycle progression, but by inducing other targets that affect cell differentiation.

In order to understand how the coordination of cell-cycle arrest and differentiation is achieved, it would be useful to

Figure 1



Prospero regulates cell cycle exit in the *Drosophila* CNS. *Drosophila* neuroblast stem cells (NBs) and ganglion mother cells (GMCs) divide via a rapid, G2-regulated cell cycle that lacks a recognizable G1 phase. Prospero protein, shown in red, is synthesized by neuroblasts and localized to the cell cortex. Cytokinesis of the neuroblast occurs in such a way that Prospero is deposited exclusively into the daughter ganglion mother cell, and not the regenerated daughter neuroblast. During interphase of a ganglion mother cell, Prospero translocates to the nucleus, where it enacts a transcription program that initiates neural differentiation. Prospero also induces G1 cell-cycle arrest in the two daughter cells generated by ganglion mother cell division, and this occurs at least in part from transcriptional downregulation of cell-cycle activators.

identify known developmental regulators or pathways that directly link these two events. An excellent example is provided by the transcription factor Prospero, which plays an important role in the developing *Drosophila* central nervous system (CNS) [1]. The *Drosophila* CNS develops from a group of stem cells called neuroblasts, which produce specific neurons or glia depending on the neuroblast's position within the embryo. Neuroblasts divide asymmetrically, giving rise to another neuroblast and a smaller cell called a ganglion mother cell. Each ganglion mother cell divides once, producing two daughters that then differentiate into neurons or glia (Figure 1). Prospero is produced by neuroblasts, but is asymmetrically located within the neuroblast mother such that it is specifically inherited by the daughter ganglion mother cell following mitosis and cytokinesis of the neuroblast (Figure 1).

In the ensuing ganglion mother cell interphase, Prospero is translocated to the nucleus, where it is thought to direct gene expression that results in differentiation of the two daughters generated by ganglion mother cell division. Prospero is found only transiently in the nucleus of newborn neurons, and is not present in mature neurons. Prospero's role in specifying neuronal cell fates in the *Drosophila* CNS is well-documented. As the movement of Prospero into the nucleus of the ganglion mother cell coincides with the cell's decision to exit the cell cycle and terminally differentiate following mitosis (Figure 1), Li and Vaessin [1] analyzed Prospero's role in regulating mitotic activity in the CNS. If cell-cycle arrest and differentiation are truly coupled, one would expect that cells prevented from differentiating would continue to cycle. Indeed, *prospero* mutant embryos contain ectopic mitotic activity throughout the CNS. The converse is also true: ectopic mis-expression of *prospero* precociously arrests cell division. One of Prospero's roles in CNS development is thus to control cell-cycle progression.

But how does Prospero achieve such control? Prospero is a transcription factor, and regulated gene expression is known to play an important role in cell-cycle control. Vassein and Li [1] therefore examined the expression of various genes encoding cell-cycle regulators after genetic manipulation of Prospero function. They found that *prospero* mutants display inappropriately increased levels of *cycA*, *cycE*, and *cdc25^{string}* RNAs, all of which encode stimulators of cell division. Conversely, when *prospero* is overexpressed, the same cell-cycle regulatory genes are now transcriptionally suppressed. Thus, there is a striking correlation between Prospero's ability to modulate the expression of cell-cycle regulators and its ability to affect cell-cycle progression.

These data suggest a model in which Prospero functions both to terminate cell proliferation by transcriptional suppression of cell-cycle activators and simultaneously to induce a differentiation program, effectively coupling the two events. Despite the appeal of this model, cause and effect still remain to be firmly established. This is because most positive regulators of the cell cycle are usually downregulated during differentiation. Consequently, the correlation between transcriptional shut off and cell-cycle exit could be just that — a correlation — rather than a mechanism for terminating proliferation. For instance, a curious result that seems at odds with the model is that the *Drosophila* homolog of the retinoblastoma protein (pRb), a well known negative regulator of the cell cycle, is also transcriptionally downregulated in response to Prospero activity. Studies aimed at determining how directly Prospero regulates the transcription of these cell-cycle regulators — for example, does it bind to upstream control elements of target genes — should be informative.

A different mechanism through which developmentally important transcription factors can stimulate cell-cycle arrest is through the induction of Cdk inhibitors. There is some evidence for this, both from *in vitro* cell culture studies and from genetic analyses in animals. For example, in double mutant mice lacking the function of both the p21 and $p27^{kip1}$ Cdk inhibitors, numerous cell types fail to differentiate during embryonic development [6,7]. Muscle formation is dramatically affected; myoblasts cannot properly withdraw from the cell cycle in response to differentiation signals and consequently overproliferate [7]. As with the *Xenopus* Müller glia described above, Cdk inhibitors may also play a direct role in stimulating differentiation. In myoblasts, $p27^{kip1}$ activates pRb, which is inhibited by Cdk-mediated phosphorylation. The pRb protein is thought to act as a transcriptional cofactor with myogenic transcription factors, such as MyoD and myogenin. Although the signals that activate p21 and $p27^{kip1}$ during muscle differentiation remain unknown, these Cdk inhibitors clearly provide a crucial link between cell-cycle arrest and muscle differentiation.

As in *Drosophila*, cell-type specific transcription factors that act in the mammalian nervous system may provide a way of linking cell-cycle arrest and differentiation. Neural basic helix-loop-helix (bHLH) transcription factors are capable of driving neural differentiation when transiently expressed in a pluripotent cell line [8]. These same bHLH proteins also initiate cell-cycle withdrawal, as evidenced by decreased BrdU incorporation, and induce elevated levels of the Cdk inhibitor $p27^{Kip1}$. It thus appears that, within the nervous system, there is direct communication between factors affecting differentiation and key components of the cell cycle machinery.

Additional evidence for transcriptional regulation as a mechanism for controlling the cell cycle during differentiation has also come from work on the nematode *Caenorhabditis elegans*. The *C. elegans cki-1* gene encodes a member of the CIP/KIP family of Cdk inhibitors and links postembryonic developmental decisions to cell-cycle decisions [9]. The *cki-1* gene is expressed in both differentiating cells and postembryonic cells arrested in G1. Studies using constructs encoding fusion proteins between Cki-1 and the green fluorescent protein (GFP) showed that the 5′ upstream sequences of the *cki-1* gene governed these patterns of expression, indicative of transcriptional control. *In vivo* inhibition of *cki-1* function by RNA-mediated interference (RNAi) in some cases caused a failure both to withdraw from the cell cycle and to differentiate. Genetic analysis has implicated several well known signaling pathways in the regulation of *cki-1*; these pathways may provide the direct link between cell-cycle arrest and terminal differentiation.

Placing cell-cycle arrest and terminal differentiation under the control of a single developmental regulator, such as *Drosophila* Prospero, seems like an efficient and effective way of making these two processes occur concomitantly when necessary. There are, however, almost certainly many other cell-type-specific mechanisms for coordinating these two events that are still to be discovered. Moreover, with increasing complexity of the developmental system, the simple Prospero type of mechanism may not suffice. This is hinted at in a recent paper describing isoforms of human Numb protein [10]. Numb was originally identified in *Drosophila*, where it acts as a cell-fate determinant in the peripheral nervous system during asymmetric cell division, much like Prospero. Verdi *et al.* [10] found that distinct isoforms of human Numb regulate either differentiation or proliferation, but not both simultaneously. Thus, a basic mechanism for coupling differentiation and cell cycle may have been modified during evolution to provide new means of regulation in more complex animals. Only through further experimentation in many different experimental systems will we discover how different regulatory mechanisms create direct links between the cell cycle and differentiation during development.

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