Apicobasal polarity and cell proliferation during development

Nitin Sabherwal and Nancy Papalopulu

Faculty of Life Sciences, Michael Smith Building, University of Manchester, Oxford Road, Manchester M13 9PT, U.K.

Abstract

Cell polarization and cell division are two fundamental cellular processes. The mechanisms that establish and maintain cell polarity and the mechanisms by which cells progress through the cell cycle are now fairly well understood following decades of experimental work. There is also increasing evidence that the polarization state of a cell affects its proliferative properties. The challenge now is to understand how these two phenomena are mechanistically connected. The aim of the present chapter is to provide an overview of the evidence of cross-talk between apicobasal polarity and proliferation, and the current state of knowledge of the precise mechanism by which this cross-talk is achieved.

Introduction: apicobasal polarity and cell proliferation

Polarization can be defined as the asymmetrical distribution of cellular components. Most, if not all, cells are polarized in one way or another. Cells polarize in order to reduce intracellular disorder and vectorize cellular activities, such as membrane trafficking and cellular secretion. In the context of epithelial tissues,
polarization of cells perpendicular to the plane of tissue results in apicobasal polarity.

Apicobasally polarized epithelial cells show polarity not only in their plasma membrane, but also within their cortex, the cytoskeleton-rich cytoplasm lying underneath the membrane. Cortical polarity results from the polarization of actin cytoskeleton and its associated proteins. Unlike in non-polar cells where plasma membrane constituents are in motion and intermixing continuously, membrane polarization results in the generation of distinct apical and basolateral plasma membrane domains. These domains do not mix due to the presence of AJCs (apical junctional complexes; composed of adherens junctions, and tight or septate junctions), resulting in differences with respect to their protein and lipid content. Therefore the formation of AJCs is thought to be essential for establishing and maintaining polarity, although, in some instances, cells have been reported to establish and maintain polarity in isolation, in the absence of cell–cell contacts [1].

Apicobasal polarity is established and maintained by the antagonistic interactions between apical and basolateral protein complexes, primarily mediated through the phosphorylation reactions of the kinases involved in polarity [2]. The key apical complex is the aPKC (atypical protein kinase C)–PAR (PARtitioning defective) complex, consisting of an atypical serine/threonine protein kinase (aPKC), along with two PDZ [PSD-95 (postsynaptic density 95), Dlg (discs large) and ZO-1]-domain-containing scaffold proteins (PAR-3 and PAR-6). These three proteins form a functional unit, which is essential for the establishment and maintenance of the apical domain. The apical Crumbs complex, consisting of the transmembrane protein Crumbs and its associated cytoplasmic proteins PALS1 (protein associated with lin seven 1) and PATJ (PALS1-associated tight junction protein), is also required for apical membrane establishment. Basolateral kinases PAR-1 and PAR-4 are necessary for basolateral membrane identity. Like PAR-1 and PAR-4, the basolateral Scribble complex [containing Scribble, Lgl (lethal giant larvae) and Dlg (discs large)] is involved in the maintenance of apicobasal polarity by negatively regulating the expansion of the apical membrane. These molecular complexes are highly conserved between various systems and contexts [3].

Like cell polarity, cell proliferation is a fundamental and equally important cellular process. Cell division leads to cell proliferation and tissue growth, and is controlled by the cell cycle. The cell cycle can be defined as a process whereby a cell divides [in M (mitotic)-phase] after its genome has duplicated [in S (synthetic)-phase]. These two phases are alternated with the presence of two gap phases (G₁ after M-phase and G₂ after S-phase), which function as the preparatory phases for the upcoming events in M- and S-phases. The key regulators of the cell cycle are the CDKs (cyclin-dependent kinases), which are positively regulated by cyclins and negatively regulated by CDKIs (CDK inhibitors). Various combinations of CDKs, cyclins and CDKIs constitute the molecular check-points, which serve as the regulatory points for the cells to leave one phase of the cell cycle and enter the next. While the basic machinery of the cell
cycle is fairly well understood and can be viewed as an autonomous entity, it is clear that it receives inputs from the cellular environment both during development and in disease, in the form of extracellular signalling. The G1-phase is the key phase where extracellular signals impinge on the cell-cycle machinery, to instruct the cell to proceed through division or not [4]. In addition, as we will discuss in detail below, the structure of the cell, for example, whether it is polarized or not can affect its division properties.

During development and tissue homoeostasis, the cell cycle needs to be controlled in order to prevent premature or complete depletion of progenitor cells, or conversely, uncontrolled growth. There are two main ways in which the cell cycle may be affected: cells may exit the cell cycle prematurely or remain in the cell cycle longer than the normal schedule. Alternatively, the kinetics of the cell cycle, such as the speed or proportional length of G1, can also be altered. Both of these can have profound effects on cell numbers and the differentiation state of a tissue. Interestingly, these are not mutually exclusive possibilities, as emerging evidence indicates that altered cell-cycle kinetics may affect cell-cycle exit [5].

Model systems for studying polarity in conjunction with cell proliferation

Model systems, from yeast to higher vertebrates, have been invaluable for elucidating the mechanisms of polarity and cell proliferation control. Although each of these processes can be studied separately, in the present chapter we will limit our discussion to the model systems that have been used for elucidating the mechanisms that control polarity and cell proliferation in relation to each other, as these would offer the most promise for understanding the molecular intersection of these two biological processes. These include Drosophila NBs (neuroblasts) and imaginal discs as the invertebrate model systems, and mouse and Xenopus neuroectoderm as the vertebrate models. Work on Caenorhabditis elegans, a model system which has been instrumental in the initial identification of genes involved in polarity, its establishment and maintenance and its role in spindle orientation, will be discussed elsewhere in the volume (see Chapter 1).

Drosophila NBs and imaginal discs

Drosophila NBs are stem cells for the development of the central nervous system and one of the key invertebrate model systems for studying cell polarity, asymmetric cell division and stem cell self-renewal. NBs divide asymmetrically to generate two cells of unequal developmental potential: an apicobasally polarized cell, which is identical with the mother stem cell, and stays in the cell cycle, and a non-polarized cell, called the GMC (ganglion mother cell), which divides only once more to terminally differentiate into two neurons or glial cells [6]. NBs show all the hallmarks of cortical apicobasal polarity, in the form of apical localization of a PAR complex and basolateral localization of the Lgl–Dlg–Scribble complex. A number of critical RNAs and proteins are associated with
this basolateral complex, such as Numb, Prospero and Brat. These are collectively called fate determinants (Figure 1). Both apical and basolateral polarity protein complexes are necessary for their localization to the basal side of the neuroblast. Before cell division, alignment of spindle parallel to the apicobasal axis ensures the correct segregation of these fate determinants in the GMC [6]. The key function of the fate determinants is to inhibit cell division and promote differentiation of GMCS. Numb acts as a Notch inhibitor, therefore the unequal inheritance of Numb generates Notch asymmetry between the daughter NB and the GMC. The GMC has low Notch activity and shows low proliferation potential [7]. Prospero, upon asymmetric division, is relieved from its adaptor

![Figure 1. Key model systems of polarity and proliferation](image)

(A) Drosophila NBs (neuroblasts) are a model of polarized neural stem cells. A NB delaminates from the ventral neuroectoderm but still maintains its apicobasal polarity. It divides asymmetrically to self-renew and give rise to a non-polar GMC (ganglion mother cell), which further divides terminally to differentiate into two neurons. (B) The VZ (ventricular zone) of the mouse embryonic cortex/mouse neuroectoderm contains polarized APs (apical progenitors) which divide both symmetrically to self-renew and asymmetrically to produce either a BP (basal progenitor) or a neuron, along with another AP. BPs mainly divide terminally to differentiate into neurons. Other layers of mouse cortex [SVZ (subventricular zone), IZ (intermediate zone) and CP (cortical plate)] have not been shown in the scheme for the sake of simplicity. (C) Xenopus neuroectoderm presents with a unique system. This ectoderm is bilayered with outer layer cells being apicobasally polar and inner cells non-polar. Inner cells are generated by asymmetric divisions of outer polar cells, which stay as long-term neural progenitors. Only the inner non-polar cells have the capacity to differentiate into primary neurons. Free arrows in the schemes indicate what possible fates the cells can adopt on their division. Another invertebrate model system of polarity and proliferation is the Drosophila imaginal discs, which are sacs of epithelial cells and show all the hallmarks of apicobasal polarity. Schematically they are similar to the Drosophila ventral neuroectoderm and are not shown here. In all of the systems described, polar cells have a different proliferation potential than non-polar cells, and manipulating their polarity alters their proliferation properties.
protein Miranda, enters the GMC nucleus and acts as a transcription factor [8,9]. Nuclear translocation of Prospero acts as a binary switch between a proliferating NB and a differentiating GMC. It represses genes required for stem cell renewal and cell cycle, such as String/Cdc25 (Cdc is cell division cycle), cyclin A, cyclin E and E2F factor, and activates differentiation genes such as even skipped and fushi tarazu [10]. Brat functions similarly, but at a post-transcriptional level; it inhibits the translation of Myc protein, a key regulator of cell-cycle growth [11].

*Drosophila* imaginal discs are another well-characterized experimental system to study cell proliferation and polarity. Several mutational screens for proliferation mutants have been performed using imaginal discs. These are larval epithelial sacs that contribute to adult cuticular structures. Imaginal disc precursors are small groups of embryonic ectodermal cells that are set aside during embryogenesis as clusters of approximately 20–50 cells. They proliferate extensively to their final sizes of 20000–50000 cells and invaginate to form the imaginal discs during larval development. Towards the end of the larval stage imaginal disc cells are committed to specific fates. During metamorphosis, while larval cells enter apoptosis, the imaginal discs undergo major morphogenetic changes to form the adult legs, wings, eyes, antennae, head capsule, halteres and genital organs. Because of their epithelial architecture, proliferation potential and well-described development, imaginal discs (in particular wing and eye discs) serve as important model systems for studying proliferation control of apicobasally polarized epithelial cells. As these cells proliferate during the larval stages, the issues pertaining to maternal contribution are avoided. They are also highly amenable to mosaic analysis as well as measurements of doubling times because of the ease of inducing clones via mitotic recombination [12].

**Vertebrate embryonic neuroepithilium/mouse and Xenopus embryonic neuroectoderm**

The mouse embryonic cortex is the most widely studied vertebrate model to understand the effect of polarity on the balance of proliferation versus differentiation. It contains several cellular layers, namely VZ (ventricular zone), SVZ (subventricular zone), IZ (intermediate zone) and the CP (cortical plate), which are identified on the basis of their locations, the cell types they contain and the markers they express. The VZ layer faces the cortical lumen, whereas SVZ, IZ and CP layers lie underneath the VZ. The VZ is composed of apico-basally polarized progenitors (apical progenitors), showing apical aPKC with tight and adherens junctions at the most apical end of the lateral plasma membrane and integrin receptors in the basal plasma membrane. They also show a pseudostratified epithelial architecture, undergo IKNM (interkinetic nuclear migration) and their nuclei divide on the apical side of cells (Figure 1). Before the onset of neurogenesis, apical progenitors divide symmetrically to generate more apical progenitors and expand their population. With the progression of neurogenesis, they increasingly switch to asymmetric neurogenic division, giving rise to a polar apical progenitor and a non-polar neuronal cell, as well as
asymmetric proliferative divisions, replenishing themselves and also giving rise to basally located non-polar BPs (basal progenitors) (Figure 1). BPs, located in both the VZ and SVZ, divide symmetrically to differentiate into neurons; they rarely self-renew. Neurons generated from apical progenitors and BPs migrate away from the VZ and SVZ to the underlying neuronal layers (IZ and CP) [13]. With the development of cortex, BPs are gradually depleted, whereas the apical progenitors are the only progenitors with unlimited self-renewing capacity [14].

With the progression of neurogenesis from E11–E17 (E is embryonic day), the cell-cycle length of neural progenitors increases significantly, accompanied by a lengthening of the G1-phase [15]. Both apical progenitors and BPs also differ with respect to the G1-phase length [16]. Both gain- and loss-of-function experiments with regulators of the G1 length (cyclin D/CDK4 and cyclin E/CDK2) have shown that G1 lengthening is a cause, rather than a consequence, of neurogenesis [17,18]. It is primarily linked with the generation of BPs by apical progenitors and also associated with a switch from proliferative to neurogenic divisions [17–19]. Shortening of the G1-phase by overexpression of G1 kinase complexes increases symmetrical expansions of both apical progenitors and BPs at the expense of neurogenic divisions. Inversely, increasing G1 length by RNAi (RNA interference) knockdown of the same complexes was shown to promote neurogenesis at the expense of proliferative progenitor divisions [17,18]. These experiments led to the formulation of the ‘cell-cycle length hypothesis’ [19] which postulates that the length of the G1-phase is the determining factor for a cell to stay as a proliferative progenitor, or to undergo differentiation. The hypothesis is that a longer G1 gives enough time to the differentiation determinants to accumulate and mature, acting on the newly post-mitotic cells to push them towards differentiation. Thus apical progenitors and BPs in the mouse cortex differ not only in their polarity status, but also in their proliferation and cell-cycle properties, making this an excellent model system to study the links between polarity and proliferation.

The neuroectoderm of the Xenopus embryo during gastrulation and early phases of neurulation, provides another vertebrate model of polarity and proliferation. Before neural tube closure, the ectoderm is bilayered, consisting of a superficial cell layer of apicobasally polarized cells, overlaying a deep layer of cells, which are non-polar (Figure 1). The polarized cells exhibit all the hallmarks of embryonic epithelial polarity, such as the apical localization of aPKC and basolateral localization of Lgl and PAR-1. The polarity of superficial cells is established and maintained by the classical regulators of polarity, aPKC and Lgl [20]. Cells in these two layers are intrinsically different with respect to their cell fates: while the deep cells are able to differentiate into primary neurons, superficial cells stay as long-term progenitors [21]. Superficial cells can be compared with the apical progenitors in mouse cortex (but show no pseudostratified architecture and no apparent IKNM), whereas the deep cells are akin to the basal progenitors. This simple bilayered architecture of Xenopus neuroectoderm makes it an excellent model system to study the effects of polarity on proliferation.
Evidence that polarity and proliferation are linked

Various observations indicate that polarity and proliferation are linked in normal development and disease. From the evidence presented above, it is clear that during the development of the *Drosophila* and vertebrate nervous system, non-polarized progenitors are closer to cell-cycle exit than polarized progenitors. In the mouse cortex, polarized and non-polarized progenitors also differ in their cell-cycle kinetics, such as the length of G1 [16].

In disease, it is notable that approximately 90% of the human cancers originate from the apicobasally polarized epithelial cells. Indeed, aberrant proliferation and concomitant loss of apicobasal polarity are the most salient feature of human cancers [22].

Molecular data support these phenomenological observations. A genetic screen in *Drosophila* to uncover novel tumour suppressor genes identified genes, which were later found to be integral components of the apicobasal polarity machinery, such as Lgl [23,24]. Components of apicobasal polarity are also known to function as proto-oncogenes or tumour suppressors. Loss- or gain-of-function mutations in these genes are associated with uncontrolled proliferation during cancer progression and stem cell renewal [22,25]. Last, but not the least, polarity molecules also regulate the Hippo pathway, a key sensor of cellular growth and division, to control cell proliferation [26].

Mechanisms linking polarity and proliferation in *Drosophila* models

*Drosophila* has been a key developmental model system to study the mechanistic links between polarity and proliferation. During *Drosophila* neural development, non-polarized GMCs formed after the asymmetric divisions of polarized NBs receive the full complement of basal fate determinants (Numb, Prospero and Brat). GMCs generated from the loss-of-function NB mutants for cell-fate determinants cannot exit the cell cycle, show NB-like properties and keep proliferating, giving rise to ectopic NBs and brain tumours in flies [10,11,27]. These results put the emphasis on proteins of the basolateral complex for cell-cycle exit regulation, as GMCs lack the apically localized aPKC–PAR complex.

The situation is more complex in the polarized self-renewing progenitor, the NB, which contains the full complement of both apical and basolateral polarity proteins. What keeps them in a proliferative/self-renewal and non-differentiation state despite having the basal fate determinants? The answer lies in the differential subcellular localization of fate determinants; for example, Prospero is nuclear and thus active as a transcription factor only in GMCs (Figure 1) [9]. What then regulates the differential localization and activity of fate determinants in NBs versus GMCs? The key molecule regulating this process is the apical polarity kinase.
aPKC, the first protein identified to regulate self-renewal of NBs [28]. The role of aPKC in NB self-renewal involves aPKC phosphorylating and inactivating fate determinants, such as Lgl, Numb and Miranda [29–31]. aPKC also regulates the orientation of the mitotic spindle. Thus an indirect control of aPKC over fate determinants could also be exerted by controlling the distribution of the basolateral complex by virtue of orienting the mitotic spindle [32]. These data suggest that aPKC has an indirect role in preventing NB differentiation by negatively regulating the activity of basolateral fate determinants. But does aPKC have a more direct role in NB proliferation?

Some evidence suggests that the role of aPKC in NB proliferation might result from aPKC actively speeding up the cell cycle. NB mutants with zygotic loss of aPKC show reduced cellular proliferation [28,33]. A similar phenotype was observed for the loss-of-function mutant of Dap160 (dynamin-associated protein 160), a positive regulator of aPKC. Reduced proliferation in Dap160 mutants was attributed to a prolonged cell cycle due to longer interphase [34]. Conversely, NB-specific overexpression of membrane-targeted aPKC or loss-of-function of negative regulators of aPKC [such as the phosphatase Twins/PP2A (protein phosphatase 2A)] induced ectopic NB self-renewal and supernumerary NBs [33,35]. Similar to the gain of apically localized aPKC, loss of basolateral Lgl results in hyperproliferation of NBs [33]. Very importantly, Lgl mutant NBs show ectopic cortical localization of aPKC, and the hyperproliferation phenotype of these mutants can be fully rescued in an Lgl/aPKC double mutant. These data suggest that the role of aPKC in NB proliferation is not only direct, but may be dominant as well, and it is sufficient to turn GMCs into NBs. However, biochemical evidence is needed to understand how aPKC may exert a direct role on proliferation and this will be elaborated further below.

This ‘dominant role of apical aPKC in NB renewal’ hypothesis has recently been vigorously tested. In Mud (Mushroom body defective) NB mutants, apical localization of PAR-3 and aPKC is normal, but spindle alignment parallel with apicobasal polarity is disrupted, leading to ectopic symmetric divisions and proliferation of mutant NBs. Prospero overexpression in Mud mutant NBs results in striking depletion of ectopic NBs. If the role of aPKC was dominant in determining NB cell fate, overexpressing Prospero should have no effect in rescuing the phenotype. This observation led to the idea that it is the ratio of apical/basal polarity markers that determines the NB/GMC identity; while a higher apical/basal ratio promotes NB identity, a higher basal/apical ratio promotes GMC identity [36].

In imaginal discs, similar to NBs, loss of aPKC leads to the loss of polarity and proliferation in the eye disc cells [28,32]. Conversely, loss of basolateral proteins Lgl/Dlg/Scribble, is associated with overproliferation and loss of tissue architecture in the wing imaginal disc cells [24]. Ectopic growth in the imaginal disc tissues by gain of apical aPKC or loss of basolateral Lgl/Dlg/Scribble is more actively associated with positive changes in cell-cycle progression.
Overproliferation in *Drosophila* mutants for Lgl/Dlg/Scribble in the developing eyes, results from the ectopic S-phase entry of the nuclei, which is accounted for by the negative regulation of cyclin E, the key regulator of G_{1}-to-S-phase entry during cell cycle, by Lgl/Dlg/Scribble [37]. This is consistent with the role of the Lgl/Dlg/Scribble polarity protein complex in cancer, which is discussed in Chapter 11.

Imaginal disc cells have also paved the way for elucidating the control of cell proliferation by polarity molecules via regulation of the hyperplastic tumour suppressor Hippo pathway. This is a kinase cascade pathway, which negatively regulates the activity of a downstream oncogenic transcriptional co-activator Yki (Yorkie), by direct phosphorylation and cytoplasmic sequestration [26]. Although primarily cytoplasmic, an active apical pool of Hippo kinase complex has been reported [38,39], which is positively regulated by the upstream apical scaffold protein complex MEK, consisting of Merlin (M), Expanded (E) and Kibra (K) [26,40]. The Hippo pathway is reviewed in Chapter 9; therefore it will be only briefly touched upon in the present chapter in the context of polarity. Overexpression of aPKC or loss of Lgl in the eye epithelial tissue causes cytoplasmic mislocalization of apical Hippo and its co-localization with its negative regulator RASSF (Ras-associated factor), dampening Hippo signalling and enhancing proliferation [38]. How does Lgl/aPKC deregulate the Hippo pathway? Human Kibra is an aPKC direct phosphorylation target [41]. Kibra phosphorylation and deregulation by aPKC could be a possibility, leading to apical loss of Hippo complex. Apical Crumbs also regulates cellular proliferation via regulating the levels and apical localization of Expanded protein. Crumbs directly binds to Expanded and this binding also leads to the phosphorylation of Expanded [42,43], although the kinase involved is not known. It is possible that aPKC is the kinase, phosphorylating apical Expanded.

Interestingly, there are examples of mechanistically separable effects of polarity components on polarity and proliferation. In *Drosophila* eye imaginal discs, Lgl mutant cells in the wild-type surroundings show ectopic proliferation via up-regulated Hippo pathway. Clonal analysis of these mutant cells had shown that they exhibited no effect on polarity, which was attributed to the presence of maternal Lgl protein. However, the same cells, when grown until pupal stage, showed loss of polarity. Together these observations led to the idea that Lgl affected proliferation and polarity at different thresholds; higher levels of Lgl are needed for inhibiting proliferation, whereas lower levels are required for its function in polarity maintenance [38,44]. Separable effects on polarity and proliferation of Crumbs were also shown by the gain-of-function mutants of Crumbs\textsuperscript{incd} (intracellular domain of Crumbs). Separate subdomains within the intracellular domain of Crumbs were shown to be responsible for its function in proliferation via the Hippo pathway, and in polarity through its interaction with the PAR–aPKC complex [45]. Further examples of such functional separation are provided by LKB1/PAR-4 and Dlg [46,47]. These examples add an
additional layer of complexity in deciphering the direct effects of polarity components in proliferation.

**Mechanisms linking polarity and proliferation in the vertebrate neuroepithelium**

As cortical development proceeds in mouse (from E11 to E17), neural progenitors perform more neurogenic than proliferative divisions [13]. During this period, apical polarity markers such as PAR-3, PAR-6 and aPKC show significant reduction in their staining intensity, indicating that proliferation and the amount of apical polarity markers are correlative linked. Loss-of-function of endogenous PAR-3 and PAR-6 blocks cortical progenitor expansion and overexpression of PAR-6 results in clonal expansion of apical progenitors, both *in vitro* and *in vivo*, without having any obvious effect on the expansion of BPs. This expansion is achieved by means of an increase in the number of symmetric proliferative divisions, without affecting their overall cell-cycle length [48].

Nevertheless, since it is also known that polarized and non-polar progenitors in mouse neuroepithelium have different cell-cycle lengths [16], one possibility is that, in some instances, polarity affects cell fate via changes in the cell-cycle length, which is known to be associated with cell-fate changes [19]. However, mechanistic evidence for this is currently lacking. Some answers in this direction might come from the experiments in *Xenopus* ectoderm. Here, inhibiting aPKC leads to enhanced differentiation, whereas the overexpression of membrane-tethered aPKC (aPKC-CAAX) leads to the inhibition of differentiation and enhanced proliferation of the entire ectoderm. This suggested that aPKC is a key regulator of proliferation [49], consistent with results in other organisms. Further experiments in *Xenopus* showed that the effects of aPKC-CAAX on differentiation and proliferation were phenocopied by a nuclear kinase construct of aPKC [49]. This suggested that aPKC itself might shuttle between the membrane and the nucleus to implement its effect on cell fate, possibly via direct phosphorylation of nuclear proteins. Follow-up experiments showed that aPKC overexpression leads to faster cell cycle of the progenitors, without affecting their growth fraction (N. Sabherwal and N. Papalopulu, unpublished work). So in *Xenopus* neuroepithelium, the effects of aPKC on proliferation may be mediated via cell-cycle changes. Such changes by aPKC overexpression might lead to alterations in the proliferative compared with differentiative divisions of both apical progenitors and BPs, as has been observed for mouse cortical progenitors. The mechanistic details of these changes are still under investigation and will be revealed in future publications. Finally in this system, polarity molecules, such as PAR-1, can also control the orientation of division, and their manipulation can also affect the fate of the cells [50].
Conclusions and unanswered questions

Model systems described above have provided a wealth of information on how the polarization state of a cell regulates its proliferation. It regulates the mode of division of cells (symmetric compared with asymmetric) and thereby affects their cell-cycle exit properties. It may regulate their cell-cycle kinetics, which has also been shown to affect the fate of the cells. And lastly, it affects proliferation by means of regulating other pathways such as the Hippo pathway. But a number of questions remain unanswered about the precise nature of the cross-talk between polarity and proliferation. Distinguishing direct from indirect effects remains a big challenge, and the evidence for direct interactions is scarce.

Are these processes co-ordinated or truly linked mechanistically? For example, during mouse cortical development, both overexpression of apical polarity determinants (PAR-3 and PAR-6) and shortening of G1-phase (by cyclin D/CDK4 overexpression) expand progenitors by increasing their symmetric proliferation divisions. Whether polarity components modulate molecules regulating the G1-phase of the cell cycle directly remains to be addressed.

Perhaps some mechanistic clues can be provided from studies of the cell cycle in non-polarized systems, such as cells in culture. For example, in a non-polarized cell culture system, aPKC has been shown to directly phosphorylate and destabilize the CIP (CDK-interacting protein)/KIP (kinase inhibitory protein) family member of cell-cycle kinase inhibitor p21Cip1, in an insulin-dependent manner, linking energy metabolism and the cell cycle [51]. A similar kind of destabilization of p27Kip1, another negative regulator of the cell cycle, was observed when aPKC regulated the oestrogen-induced nuclear transport of ERK (extracellular-signal-regulated kinase) 2 [52]. Studies on lung cancer using human alveolar adenocarcinoma cells in culture have also shown that aPKC functions as an oncogene and transforms the cells via Rac1 activation, leading to the ERK1/2 signalling downstream of it [53,54]. aPKC also directly phosphorylates and stabilizes Src-3 (steroid receptor activator 3), a known oncogene and a regulator of cell proliferation. This causes transcriptional up-regulation of c-Myc and cyclin D1, promoting cellular growth and the cell cycle [55]. Thus aPKC feeds directly into the cell-cycle machinery, both at transcriptional and post-transcriptional levels, and enhances cell proliferation; however, whether this holds true in the case of cell cycle of polarized epithelium remains to be addressed.

Apical aPKC–PAR complex seems to have a leading role in stem cell renewal. Detailing the mechanisms behind the action of this complex remains a challenge for the future. Ultimate proof that cell polarity and proliferation are linked will require a biochemical understanding of their mechanistic links. At present the intersection of the Hippo pathway with the cell cycle offers the most promise in that respect. In the case of aPKC, understanding how it affects the cell cycle will require the identification of phosphorylation targets of aPKC.
involved in self-renewal/proliferation in any of the model systems mentioned in the present chapter.

Summary

- Apicobasal polarity and proliferation of stem/progenitor cells are two fundamental cellular processes that are highly co-ordinated and mechanistically linked during development.
- While a polarized progenitor shows unlimited proliferation potential, a non-polarized progenitor tends to exit the cell cycle and differentiate.
- The polarization state of a cell affects the way it divides, symmetrically or asymmetrically, and this has profound effects on the cell-cycle exit properties of the daughter cells and their subsequent fate.
- Polarity affects the cell-cycle kinetics of cells, which itself is associated with their cell-cycle exit properties and fate.
- Polarity components also affect cell proliferation by means of regulating the Hippo signalling pathway, a key sensor of cell growth and cycle.
- The key polarity molecule promoting stem cell proliferation is aPKC, which is actively involved in all of the mechanisms described above. Identifying the phosphorylation targets of aPKC will shed light on the biochemical mechanistic details behind its action on stem cell proliferation.

References


© The Authors Journal compilation © 2012 Biochemical Society