Cell-Cycle Control in Embryonic Stem Cells

Pierre Savatier and Anna Malashicheva

Introduction

Mouse embryonic stem (ES) cells are the in vitro counterpart of the epiblast cells of the early postimplantation embryo. ES cells are highly pluripotent in that they can generate all cell types of the adult organism, which reflects the central role of the epiblast as the founder tissue of the whole embryo in rodents. Mouse ES cells display unusual proliferative properties. Their derivation does not rely on any immortalizing agent, they cannot enter a quiescence state, they do not undergo senescence, and they can proliferate without apparent limit. They also can multiply in the absence of serum and are not subject to contact inhibition or anchorage dependence. These are features of transformed cells, and, indeed, ES cells are tumorigenic in that they produce teratocarcinomas when injected into adult mice. Human ES cells are also immortal and can form tumors in vivo, suggesting that, besides pluripotency, infinite life span and unrestricted growth are intrinsic features of all ES cells. These are remarkable phenomena in that ES cells are genotypically wild type, as demonstrated by their ability to fully integrate into the developing embryo and to generate healthy adults. ES cells in this respect contrast all other types of neoplastic cells, the growth of which appears to be associated with genetic lesions in growth-controlling protooncogenes and tumor suppressor genes. Some fundamental differences in the expression or regulation of cell-cycle control genes—more specifically, in those regulating the G1-S transition in response to mitogenic signals—are likely to underlie the growth properties of mouse ES cells. Here, we review current data on the control of the mitotic cycle of mouse ES cells and epiblast cells, and we describe the possible relationships among cell-cycle control, self-renewal, and pluripotency.

Mouse ES Cells Lack Control of G1 Associated with a Functional RB Pathway

The proliferation of mammalian cells is controlled largely during the G1 phase of their growth cycles. The decision to initiate a new round of DNA synthesis is dependent upon phosphorylation and functional inactivation of the retinoblastoma protein (RB) (Fig. 5–1). Hypophosphorylated (G1-specific) RB inhibits the expression of genes required for S-phase entry by sequestering the E2F family of transcription factors. During progression through the G1 phase, RB is sequentially phosphorylated by complexes of cyclins and cyclin-dependent kinases (CDKs). Of prime importance are D-type cyclins (cyclin D1, D2, or D3), forming complexes with CDK4 and CDK6, and cyclin E, forming complexes with CDK2. Primary phosphorylation by cyclin D-CDK4/6 complexes displaces histone deacetylases from RB. This derepresses transcription of the cyclin E and the cdc25A genes. Cdc25A phosphatase removes inhibitory phosphates from CDK2. The resulting cyclin E-CDK2 complex then completes RB phosphorylation, leading to E2F release, target gene activation, and S-phase entry. The relevance of the cyclin D-CDK4/6 → RB-E2F circuitry—also called the RB pathway—in G1 control is illustrated by its disruption in many types of human tumors. A second pathway involves the c-myc protooncogene, which directly stimulates transcription of the genes encoding cyclin E and cdc25A to generate cyclin E-CDK2 kinase. The Myc and RB pathways are now thought to be two parallel and cooperative G1–S control pathways converging on cyclin E-CDK2 kinase, the activity of which determines entry into S phase.

The cyclin D-CDK4/6 → RB-E2F pathway appears to be operative in virtually all cell types. In contrast, the control of the ES cell cycle is likely to be markedly different. ES cells display a very short G1 phase of approximately 1.5 hours, during which hypophosphorylated—G1-specific—RB is virtually undetectable. RB is thus likely to be rephosphorylated immediately after mitosis in ES cells. An important issue, therefore, is whether ES cells are subject to G1 regulation by RB. Several pieces of evidence support the notion that the cyclin D-CDK4/6 → RB-E2F pathway does not regulate the ES cell cycle:

1. Cyclin D1 and cyclin D3 are expressed at low levels in ES cells; cyclin D2 is not expressed. CDK4-associated kinase activity is barely detectable. Differentiation induced by the withdrawal of leukemia inhibitory factor (LIF) or by the formation of embryoid bodies results in strong expression of the three D-type cyclins and robust CDK4-associated kinase activity. The relatively low levels of D-type cyclins in ES cells compared to their differentiated derivatives reflects the situation in epiblast cells, which do not express appreciable D-type cyclins until gastrulation commences.
2. ES cells are refractory to the growth inhibitory activity of p16<sup>ink4a</sup> and p16<sup>ink4a</sup> is a specific inhibitor of CDK4 and CDK6 that acts by preventing the association of D-type cyclins with the CDKs. Resistance to p16<sup>ink4a</sup>-mediated growth inhibition is a common feature of cancer cells whose RB pathway has been disrupted. It was recently shown that cyclin D3-CDK6, the most abundant cyclin D-CDK4/6 type complex in ES cells, are resistant to the growth inhibitory activity of p16<sup>ink4a</sup>. Withdrawal of LIF and subsequent differentiation is accompanied by sensitization to p16<sup>ink4a</sup>-mediated growth inhibition, indicating that RB control of G1 is imposed during differentiation.

3. The inactivating disruptions in all three retinoblastoma family members (RB and its two cognates, p107 and p130) do not seem to compromise ES cell proliferation, but they reduce differentiation in experimental teratocarcinomas. This further indicates that RB dependence is only acquired as ES cells undergo differentiation.

4. ES cells share striking similarities in proliferative behavior with embryonic fibroblasts harboring the triple knockout (TKO) of the three retinoblastoma gene family members (Rb<sup>−/−</sup>, p107<sup>−/−</sup>, and p130<sup>−/−</sup>). Both TKO murine embryonic fibroblasts (MEFs) and ES cells fail to arrest in G<sub>1</sub> at confluence. In normal fibroblasts, this phenomenon, known as contact inhibition, is accompanied by increased p27<sup>kip1</sup> (an inhibitor of cyclin E-CDK2 kinase) levels, decreased cyclin D1 levels, and an accumulation of hypophosphorylated RB leading to G<sub>1</sub> arrest. ES cells and TKO MEF also escape replicative senescence and are immortal. In normal cells, replicative senescence and G<sub>1</sub> arrest are associated with an accumulation of hypophosphorylated RB arising from inhibition of CDK4 and CDK6 kinases by p16<sup>ink4a</sup> and from inhibition of CDK2 by p27<sup>kip1</sup>. Finally, both ES cells and TKO MEFs fail to arrest in G<sub>1</sub> following DNA damage. In normal cells, growth arrest in G<sub>1</sub> is strictly dependent on a functional RB pathway.

Together, these data strongly support the notion that ES cells lack RB control in G<sub>1</sub>. This control is likely to be restored when ES cells commit to differentiation. Lack of RB control suggests that the Myc pathway could play a critical role in the promotion of the G<sub>1</sub>–S transition of ES cells.

**Mouse ES Cells Lack Control of G1 Associated with Ras/ERK Signaling**

Extracellular regulated kinase (ERK)-type mitogen-activated protein kinases (MAPKs) regulate a variety of cellular responses and have well-documented roles in the control of proliferation in most, if not all, nontransformed somatic cell types. The ERK pathway is engaged through the recruitment of the Grb2 adaptor–SOS guanine nucleotide exchange factor complex to activated receptors. This promotes activation of Ras, which then initiates a cascade of transphosphorylations that culminates in ERK activation. Active ERKs undergo
nuclear translocation, which enables them to up-regulate the activity of growth-promoting transcription factors such as Elk, Ets, Myc, and serum response factor (SRF).

Several lines of evidence support the notion that ES cells do not rely on Ras/ERK signaling for proliferation control (Fig. 5-2):

1. Grb2-deficient ES cells proliferate and self-renew normally, but they display strongly impaired differentiation. Reintroduction of either a Grb2-SOS chimeric protein or an activated form of Ras into Grb2−/− ES cells restores normal differentiation, indicating that Ras dependence is only acquired at the onset of differentiation.29

2. In ES cells, the Grb2-SOS complex is recruited to the activated gp130 receptor using the protein phosphatase SHP-2. Elimination of the SHP-2 binding site from gp130 receptor blocks coupling to Ras and inactivates ERK signaling, yet it does not impair proliferation.29 Similarly, specific restriction of ERK activity by pharmacological inhibition of MEK, the upstream activator of ERK, does not alter the cell-cycle kinetics.30 Therefore, ERK activity appears to be fully dispensable for ES cell proliferation. Suppression of ERK activity enhances self-renewal, suggesting that Ras/ERK signaling has a prodifferentiative effect on the ES cell.29

3. The SRF transcription factor, a direct target of activated ERKs, is an important regulator of somatic cell proliferation in response to serum stimulation. SRF regulates the expression of growth-promoting transcription factors like c-fos and Egr-1,31 C-fos, with c-jun and ets, is a critical regulator of cyclin D1 expression.32-34 Thus, SRF and its targets are thought to play an important role in activating the cyclin D-CDK4/6 → RB-E2F pathway in response to growth-factor stimulation. SRF-deficient ES cells have a normal proliferation rate despite the severely impaired serum-dependent expression of c-fos and Egr-1.31 Thus, the SRF function is dispensable in the ES cell.

Together, these data provide evidence that the cascade that operates in most cells—which begins with extracellular mitogens, transduces signals through the Ras/ERK pathway, and leads to regulation of cyclin-CDK complexes and RB phosphorylation—is not engaged in ES cells.

The Ras/ERK pathway seems to be dedicated largely to driving RB phosphorylation in somatic cells.35 Hence, inhibitors of this pathway have a strong cytostatic effect on the proliferation of normal cells but only a minimal effect on
tumorigenic cells whose growth has escaped RB control. Therefore, the resistance of ES cells to disruption of Ras/ERK signaling further indicates that the ES cell lack RB control in the G1 phase.

**PI3K Signaling Contributes to Regulation of the ES Cell Cycle**

Increased levels of 3'-phosphorylated phosphoinositides are frequently associated with growth factor and cytokine signaling. This occurs through receptor-mediated translocation of PI3K to the cell membrane. The PI3K products P(3,4)P2 and P(3,4,5)P3 activate several signal transducers, including the serine-threonine kinases phosphoinositide-dependent kinase 1 (PDK1) and protein kinase B (PKB/Akt). The tumor suppressor PTEN, which removes the phosphate from the 3' position of 3'-phosphoinositides, is a negative regulator of this pathway. Coordinate localization of the lipid-bound kinase at the membrane facilitates PDK1-mediated phosphorylation of PKB, which then modulates the expression of key regulators of the cell cycle. In particular, PKB decreases the rate of degradation of cyclin D1, whereas it increases the rate of degradation of p27kip1, an inhibitor of the G1-S transition. Both result in promotion of G1 transit in response to mitogenic stimulation.

Data show that ES cells differ from other cell types both in the regulation of PI3K activity and in the downstream signaling cascades that it activates (Fig. 5–2). Specific pharmacological inhibition of PI3K activity in ES cells increases the proportion of cells in G1. This is accompanied by down-regulation of cyclin D1 expression. Moreover, the genetic disruption of p85α results in G1 growth retardation and up-regulation of the p27kip1 inhibitor. Conversely, ES cells lacking PTEN exhibit an accelerated transit through G1, which appears to be caused by an increase in the rate of degradation of the p27kip1 inhibitor. Therefore, ES cells appear to be dependent on PI3K activity for proliferation. Interestingly, this activity is not impaired by serum starvation in ES cells. This suggests that PI3K activity does not depend on mitogenic stimulation, a finding consistent with the evidence that serum starvation does not induce ES cell growth arrest. One possible activator of PI3K is the product of the Ras-like gene, Eras. Eras is expressed in mouse ES cells, where it interacts with PI3K but not with Raf. Eras-null ES cells display attenuated PI3K signaling, as well as impaired growth that is rescued by forced expression of active PI3K. Eras is therefore a novel pathway that activates PI3K in ES cells.

PI3K-dependent signals that influence the proliferation of ES cells have not been defined. PKB would seem to be a likely candidate, but ES cells lacking the upstream activator PDK1 are viable. These ES cells exhibit negligible activation of PKB and fail to activate other targets of PDK1. These data point to the possibility that PI3K may influence ES cell growth through a PDK1/PKB-independent pathway. The PKB-related protein serum and glucocorticoid-induced kinase 1 (SGK1) might fulfill this role.

**ES Cell-Cycle Machinery Is Constitutively Active**

**EXPRESSION OF CYCLIN D1 IS NOT DEPENDENT ON MITOGENIC STIMULATION IN ES CELLS**

Cyclin D1 is an essential regulator of the G1-S transition in response to growth factor stimulation in somatic cells. As we have described, ES cells express a low cyclin D1 level. Furthermore, regulation of cyclin D1 expression differs between ES cells and other cells (see Fig. 5–2). First, the Ras/ERK pathway, central to the transcriptional activation of cyclin D1 expression in somatic cells, does not contribute to the regulation of cyclin D1 expression in ES cells, a finding consistent with the evidence that ERK signaling does not promote ES cell proliferation. Second, the steady-state level of cyclin D1 protein is critically regulated by PI3K signaling in ES cells like in other cells. PI3K-dependent regulation of the cyclin D1 protein level occurs mostly through the regulation of the glycosynthase kinase 3β-dependent rate of degradation. Unlike somatic cells, however, PI3K activity seems to be uncoupled from persistent mitogenic stimulation in ES cells. Thus, neither PI3K activity nor cyclin D1 expression is down-regulated after serum starvation. Together, these data lead us to the conclusion that expression of cyclin D1 is disconnected from mitogenic signals transduced by tyrosine kinase receptors in ES cells. Constitutive, albeit low, expression of cyclin D1 could contribute to constitutive phosphorylation of RB. Alternatively, the functional significance of cyclin D-CDK4 complexes in ES cells may be to sequester p27kip1 and to prevent this inhibitor from acting on cyclin E-CDK2 kinase.

**ABSENCE OF CELL-CYCLE-REGULATED CDK2 ACTIVITY IN MOUSE ES CELLS**

Cyclin-CDK complexes become activated at precise points of the cell cycle in somatic cells. The cyclin D-CDK4/6 complexes are formed during the G1 phase that precedes mitosis. They are maintained throughout the subsequent G1 phase, and their level drops abruptly when cells enter the S phase. The cyclin E-CDK2 complexes are present during the late G1 phase. Activity of these complexes drops at the beginning of the S phase. Then CDK2 forms new complexes with cyclin A to regulate DNA replication. The cyclin A-CDK2 complex is progressively replaced by cyclin B1-CDC2 complexes during transit through the G2 phase. The level of those latter complexes collapse before reentry into G1. Precise temporal control of these cyclin-CDK kinase activities is crucial in establishing transitions from one cell-cycle phase to the next and in ensuring that cell-cycle events are executed in the correct order. The cell-cycle regulation of cyclin D, E, A, and B1 levels plays a critical role in this process (Fig. 5–3).

The periodic expression of cyclin-CDK complexes and the subsequent regulation of cell-cycle progression appears to be operative in all cell types. The ES cell-cycle clock is likely to be markedly different. ES cells display vastly elevated cyclin E-, cyclin A-, and cyclin B-associated kinase activities, and both cyclin E and cyclin A are expressed at levels well...
5. Cell-Cycle Control in Embryonic Stem Cells

![Figure 5-3. Cyclin-CDK expression patterns during cell-cycle progression in somatic cells and mouse ES cells.](image)

Beyond those seen in other primary and transformed mouse cell lines. Furthermore, cyclin E-CDK2- and cyclin A-CDK2-associated kinase activities show no obvious periodicity during cell-cycle progression. Only cyclin B1-CDC2-associated kinase activity is clearly cell-cycle dependent, being most active in G2-M.30

These data provide evidence that cyclin E-CDK2 and cyclin A-CDK2 kinases are constitutively active in the ES cell. This observation provides a satisfactorily explanation to the previous finding that ES cell are able to proliferate actively despite low cyclin D-associated CDK activity. Hence, high cyclin E activity in breast cancer cell lines can completely substitute for cyclin D-associated CDK activities.30 Similarly, enforced expression of cyclin E renders somatic cells resistant to growth inhibition by p16INK4a,51 whereas constitutive cyclin E-CDK2 activity in fibroblasts is associated with anchorage-independent growth,52 another property exhibited by mouse ES cells.

**E2F TARGET GENES ARE NOT CELL-CYCLE REGULATED IN MOUSE ES CELLS**

Upon release from the block imposed by the hypophosphorylated (G1-specific) form of the RB family members, the free E2F transcription factors up-regulate the expression of a several genes involved in cell-cycle progression (i.e., *cyclin E*, c-myc, B-myb, and Cdc2) as well as in DNA replication. The activation of these E2F target genes is therefore tightly linked to the temporal activation of cyclin D-CDK4/6 and cyclin E-CDK2; together, they are crucial in controlling the kinetics of transit through G1- and S-phase entry in somatic cells.5,51 ES cells again appear different in that E2F4, the predominant form of E2F activity in ES cells,54 is almost exclusively in the free, active form and that strong E2F4 DNA-binding activity is present throughout the cell cycle. Furthermore, ES cells do not display cell-cycle-dependent expression of the E2F target genes.30

As the CDK activities associated with the G1-S transition are ectopically active and the target genes of these activities are constitutively expressed throughout the cell cycle, the ES cell mitotic cycle appears to be constitutively primed for DNA replication. It has been proposed that ES cells lack the G1 checkpoint (also called the restriction point),30 defined as the point in G1 when a cell becomes irreversibly committed to S phase and no longer requires growth factor stimulation. Loss of the G1 checkpoint is often associated with transformation in cancer cells.11

**Mouse ES Cells Lack p53-Dependent Checkpoints**

Following DNA damage or nucleotide depletion, the cell cycle is arrested in G1 and in G2. Arrest in G1 prevents aberrant replication of damaged DNA, and arrest in G2 allows cells to avoid segregation of defective chromosomes. Both checkpoints crucially rely on the p53 transcription factor, which becomes stabilized upon DNA damage and regulates the transcription of several genes, among which is the p21WAF1/CDKN1A CDK inhibitor capable of silencing cyclin E-CDK2 and cyclin B-CDC2. This leads to the accumulation of the growth suppressive form of RB10, 11, 15, 27, 55 (Fig. 5-1).
ES cells do not undergo cell-cycle arrest at the G1 and G2 checkpoints in response to DNA damage or nucleotide depletion, although they synthesize abundant quantities of transcriptionally active p53.24 Several factors may account for the inability of ES cells to arrest growth at the G1–S or G2–M transitions. First, the p53 protein in ES cells is mainly, if not exclusively, cytoplasmic, and it translocates inefficiently to the nucleus upon DNA damage. Second, ectopically expressed nuclear p53 appears unable to activate p21\(^{waf1/cip1}\) expression and to trigger cell-cycle arrest. Hence, ES cells have an effective mechanism for rendering them refractory to p53 function.24 In addition, growth arrest at the G1–S transition following DNA damage relies crucially on the RB pathway,15, 20, 26 and, as we have explained, this pathway is not functional in ES cells.

Therefore, ES cells appear to lack the p53-dependent G1 and G2 checkpoints that characterize normal somatic cells. The question arises of how these cells maintain genome integrity in the absence of cell-cycle checkpoints. After DNA damage, ES cells undergo p53-dependent apoptosis,25 so there is a suggestion that the function of p53 in ES cells is to trigger apoptosis, thereby efficiently eliminating all cells with damaged DNA.

**Unrestricted Proliferation and Self-Renewal**

As we have described, ES cells have an unorthodox cell cycle in which most of the G1 control pathways operative in other cell types are absent. Uncoupling the G1 phase of the cell cycle from extrinsic stimuli helps to explain the rapid proliferative rate of ES cells as well as their ability to form teratocarcinomas when placed in a heterologous environment in vivo.2 Hence, loss of functional RB and p53 pathways, constitutive PI3K activity, or ectopic cyclin E-CDK2 kinase activity—four features of the ES cell mitotic cycle—is often associated with tumor growth.7, 8, 50, 56

It is tempting to speculate that constitutive transit through G1 may actively sustain the undifferentiated state. Indeed, P19 embryonal carcinoma cells display increased sensitivity to retinoic acid-induced differentiation during the G1 phase,27 suggesting that ES cells are particularly vulnerable to differentiation inducers while they progress from mitosis to the next S phase. Hypophosphorylated (G1-specific) RB contributes to differentiation in certain cell types, where it participates in complex formation with differentiation-promoting transcription factors such as MyoD, myogenin, NF-IL6, and C/EBP.56–61 Constitutive phosphorylation of RB would therefore protect the ES cell from differentiation-promoting factors.

The STAT3 transcription factor may contribute to activation of the G1–S transition. On the one hand, STAT3 is known to be a critical regulator of ES cell self-renewal in response to activation of the gp130 receptor by LIF.62, 63 On the other hand, STAT3 is known to promote entry into the S phase in some types of differentiated cells, and it activates expression of specific cell-cycle regulators including junB, Myc, and Pim-1.53–65 JunB and Myc are transcriptional activators of the cyclin A and cyclin E gene, respectively.10, 66 Pim-1 is a serine-threonine kinase that phosphorylates and activates Cdc25A, thereby potentiating the accumulation of active cyclin E-CDK2 and cyclin A-CDK2 kinases.67 Therefore, the LIF/gp130/STAT3 pathway may suppress differentiation by up-regulating expression of key regulators of the G1–S transition.2

**Cell-Cycle Control in the Early Postimplantation Mouse Embryo**

The lack of control over the G1–S transition in ES cells—and the gain of this control during differentiation—is likely to reflect a similar phenomenon in the early postimplantation embryo. Epiblast is the founder tissue of the whole embryo in rodents. Epiblast cells are highly proliferating (\(t_{1/2} = 9–11\) hours) and potentially tumorigenic.54, 68 The onset of gastrulation (6.5 days postcoitus) is associated with an increase in the proliferation rate of epiblast cells as well as with the start of differentiation within the embryo proper. Hence, most cells of the gastrulation embryo (pluripotent epiblast, mesoderm, and endoderm cells) have a cell cycle of 7–7.5 hours70, 71 and a G1 phase of 1.5–2 hours. But the cells of the primitive streak divide every 3–3.5 hours with a further shortening of all three phases of the cell cycle: G1 (<30 min.), S (2–2.75 hours), and G2 (<20 min.).72 Therefore, the commitment of epiblast progenitors to become mesoderm and endoderm is associated with a very short cell cycle during transit through the primitive streak (Fig. 5–4).

Epiblast cells of the pregastrulation embryo do not express D-type cyclins. Expression of cyclin D1 is activated first in the whole epiblast of the gastrulating embryo. Expression of cyclin D2 (superimposed on that of cyclin D1) is activated next and coincides with the transit of epiblast progenitors through the primitive streak.14 Thus, activation of cyclin D1 and cyclin D2 expression coincides first with the increase in the growth rate of the epiblast cells taking place at the onset of gastrulation (cyclin D1) and second with the further increase in the growth rate occurring when epiblast cells migrate through the primitive streak (cyclin D2). The activation of cyclin D1 expression takes place in the pluripotent epiblast prior to commitment to ectoderm, mesoderm, and endoderm lineages, suggesting that the gain of cyclin D1-associated functions is a prerequisite for differentiation.

It is tempting to speculate that activation of cyclin D1 expression in the pluripotent epiblast of the gastrulating embryo reflects the acquisition of G1–S regulation by tyrosine kinase receptors and the downstream Ras cascade. This is corroborated by the genetic disruption of Grb2 or SRF, both of which result in severe developmental defects. Grb2-deficient ES cells are unable to contribute to epiblast development in the gastrulating embryo.73 SRF-deficient embryos display severe gastrulation defects, evidenced by the lack of primitive streak formation and of any mesodermal cells.73 Thus, pluripotent ES cells are likely to become Ras dependent at the onset of gastrulation.
As we have explained, ES cells lack the DNA damage checkpoints and sustain extensive apoptosis after treatment with DNA damaging agents. A similar situation is observed in the gastrulation embryo, where irradiation does not induce growth arrest. Rather, epiblast cells undergo extensive apoptosis. This apoptotic response is not observed in the extraembryonic tissues and is observed only at very low levels in the mesoderm and endoderm. It seems, therefore, that commitment of pluripotent epiblast cells to differentiation into mesoderm or endoderm coincides with a major change in the fate of DNA-damaged cells. It is tempting to speculate that this commitment is associated with the gain of DNA damage checkpoints in the G1 and G2 phases of the cell cycle.

Summary

The epiblast cells of the early postimplantation embryo and the ES cells share extensive similarities regarding their cell-cycle features. Both display an unusual cell-cycle distribution with few cells in the G1 and G2 phases, they do not rely on Grb2-dependent signaling and on SRF-dependent transcription, and they do not seem to undergo cell-cycle arrest—a prerequisite to DNA repair—after DNA damage. Together, these results point to some fundamental differences in the regulation of the mitotic cycle in mouse ES cells. This might simply reflect the requirement of rapidly producing sufficient cell numbers to initiate gastrulation. As we have explained, it is possible that the cell-cycle properties of ES cells might also be involved in sustaining the undifferentiated state. If this is the case, then these properties should be shared with the ES cells of other species. It will be instructive to determine whether primate ES cells exhibit the cell-cycle features of their rodent counterparts, particularly in light of a report that human ES cells have a markedly reduced proliferation rate compared to mouse ES cells.

ACKNOWLEDGMENTS

Anna Malashicheva is a recipient of a fellowship from the Ligue Nationale contre le Cancer.

REFERENCES


5. Cell-Cycle Control in Embryonic Stem Cells


