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REVIEW

Regulation of the G1 phase of the mammalian cell cycle

DONJERKOVIC DUBRAVKA, DAVID W SCOTT

Department of Immunology, Holland Laboratory for the Biomedical Sciences, American Red Cross, 15601 Crabbs Branch Way, Rockville, MD

ABSTRACT

In any multi-cellular organism, the balance between cell division and cell death maintains a constant cell number. Both cell division cycle and cell death are highly regulated events. Whether the cell will proceed through the cycle or not, depends upon whether the conditions required at the checkpoints during the cycle are fulfilled. In higher eucaryotic cells, such as mammalian cells, signals that arrest the cycle usually act at a G1 checkpoint. Cells that pass this restriction point are committed to complete the cycle. Regulation of the G1 phase of the cell cycle is extremely complex and involves many different families of proteins such as retinoblastoma family, cyclin dependent kinases, cyclins, and cyclin kinase inhibitors.

Key words: *Cell cycle, cyclin dependent kinase, cyclin, cyclin kinase inhibitor.*

Retinoblastoma family of proteins

The retinoblastoma family consists of three members: pRb, p107, and p130, that are all important in the cell cycle regulation. Studies from knockout mice showed that pRb null mutation is embryonically lethal, while p107 or p130 knockout mice have no known

* Corresponding author.
Tel: (301) 517-0355; Fax: (301) 517-0344; E-mail: scottd@usa.redcross.org

Abbreviations used in this paper: **pRb**,retinoblastoma protein; **CDK**, cyclin dependent kinase; **CKI**, cyclin kinase inhibitor; **IgM**, immunoglobulin M; **BCR**, B cell receptor; **BHFR**, dihydrofolate reductase; TGF-β, transforming growyh factorβ.

abnormalities. pRb is a 105-110 kD nuclear phosphoprotein that can be phosphorylated by CDK/cyclin complexes on multiple serine and threonine residues (reviewed in[1]) and acts as a tumor suppressor when in an active, hypophosphorylated form (reviewed in[2],[3]). Hypophosphorylated pRb binds E2F transcription factor. After binding to E2F, pRb also recruits histone deacetylase to the promoters of E2F-regulated genes[4-6]. Deacetylation of the core histones results in a more compact chromatin structure that does not allow the transcription machinery to bind to the promoter and the transcription of the E2F-regulated genes is turned off (reviewed in[7]).

E2F has been shown to activate transcription of several genes whose products are important for entering the S phase and for DNA replication, such as genes for: E2F proteins, DP-1, pRb, p107, B-myb, PCNA, topo 1, dihydrofolate reductase (DHFR), c-Myc, DNA polymerase a, thymidylate kinase, cyclin D1, cyclin A[8], cyclin E[8],[9], $p21^{Cip1}$[10], Cdk2[11], Cdc2, and Cdc25C. E2F can activate transcription of these genes only when free; when bound to pRb, E2F is inactive[12]. Lees et al[13] showed that pRb binds to a whole family of E2F transcription factors. So far, known members of the E2F family are: E2F-1, E2F-2, E2F-3, E2F-4 and E2F-5. All these proteins are able to activate transcription of E2F responsive genes. Furthermore, they all heterodimerize with either DP1 or DP2 protein and these heterodimers create active E2F protein (reviewed in[14]).

The status of pRb phosphorylation (determined by the CDK activity) is important for the status of E2F activity because only the hypophosphorylated form of pRb is associated with E2F. In other words, pRb has a growth suppressive role (is active) only when hypophosphorylated[15]. In the resting G0 cells, as well as in early G1 cells, pRb is hypophosphorylated and therefore, active[16-18]. In mid to late G1 phase of the cell cycle, pRb is phosphorylated by CDKs, it becomes hyperphosphorylated and therefore becomes inactive until M phase when it is dephosphorylated by PP1-type phosphatase [19], (Fig 1).

Early G1 phase CDK/cyclin complexes

CDKs are serine/threonine kinases that can phosphorylate multiple substrates (such as pRb family members, p53, E2F, B-myb, other CDKs, Cdc25A phosphatase, $p27^{Kip1}$ CKI, etc.) and in doing so, they can either activate or inactivate their substrates (reviewed in[20]). The catalytic activity of CDKs is controlled by four highly conserved biochemical mechanisms (reviewed in[21]), as illustrated in Fig 2. These mechanisms include: 1) activation by binding the regulatory subunits called cyclins, proteins that are cyclically synthesized and degraded throughout the cell cycle (reviewed in[22]); 2) activation by phosphorylation on threonine residue (reviewed in[23]); 3) inactivation by phosphorylation on threonine and tyrosine residues (reviewed in[23]); and 4) inactivation by binding of the inhibitory subunit called cyclin kinase inhibitor (CKI), proteins that inhibit kinase activity when present in excess in CDK/cyclin complexes

(reviewed in[24]). Thus, in order to be active, CDK has to be appropriately phosphorylated, it has to be in complex with its accompanying cyclin, and must not be in complex with the CKI (more precisely, the ratio of CKI:CDK in the complex has to be low).

Different CDK/cyclin complexes phosphorylate pRb in different phases of the cell cycle. In early to mid G1 phase, Cdk4/cyclin D and Cdk6/cyclin D complexes are responsible for pRb phosphorylation (reviewed in[25]). There are three D-type cyclins (D1, D2 and D3, reviewed in[26]) that physically interact with pRb and inactivate it[27],[28]. They assemble into holoenzymes with either Cdk4 or Cdk6. D-type cyclins have a very short half-life (~ 30 min), and their expression is growth factor inducible[29]. D-type cyclin synthesis begins during the G0 to G1 transition, and they are synthesized as long as growth factor stimulation persists. D-type cyclins reach their peak level (in concentration) at late G1 phase (near the G1-S boundary). As soon as mitogens are withdrawn, D-type cyclins are degraded very rapidly. If their degradation occurs during G1 phase, cells will not enter S phase. If it occurs later in the cycle, it has no effect on the cell cycle progression.

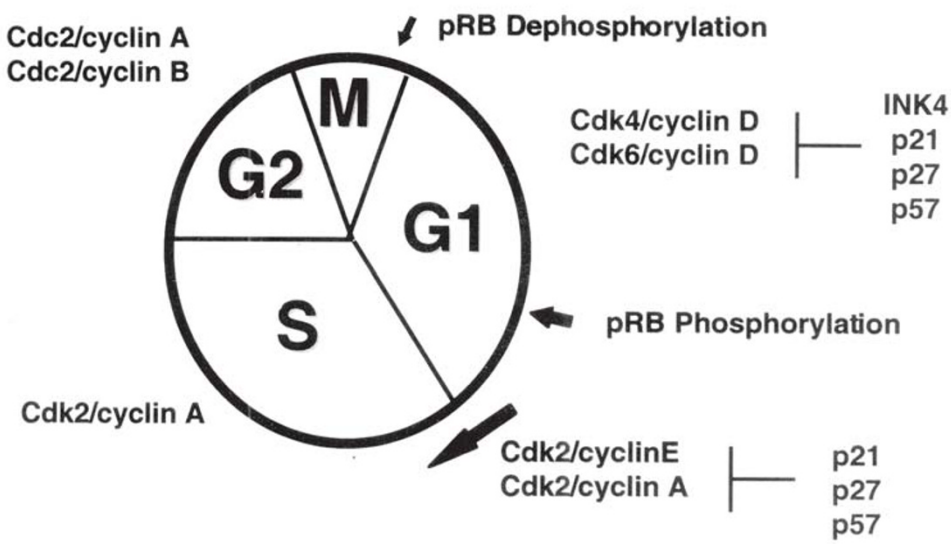

Fig 1.
Regulation of the mammalian cell cycle.
The mammalian cell cycle is regulated by the pRb family of nuclear phosphoproteins that can be phosphorylated on multiple serine and threonine residues. Hypophosphorylated pRb proteins are growth suppressors. Cdk4/cyclin D complexes phosphorylate pRb in mid G1, while Cdk2/cyclin A and Cdk2/cyclin E phosphorylate pRb at the G1 to S transition. During S phase, Cdk2/cyclin A complex is active, while in G2 phase, Cdc2/cyclin A and Cdc2/cyclin B complexes have catalytic activity. An excess of CKIs in CDK/cyclin complexes inactivates these complexes which results in growth arrest.

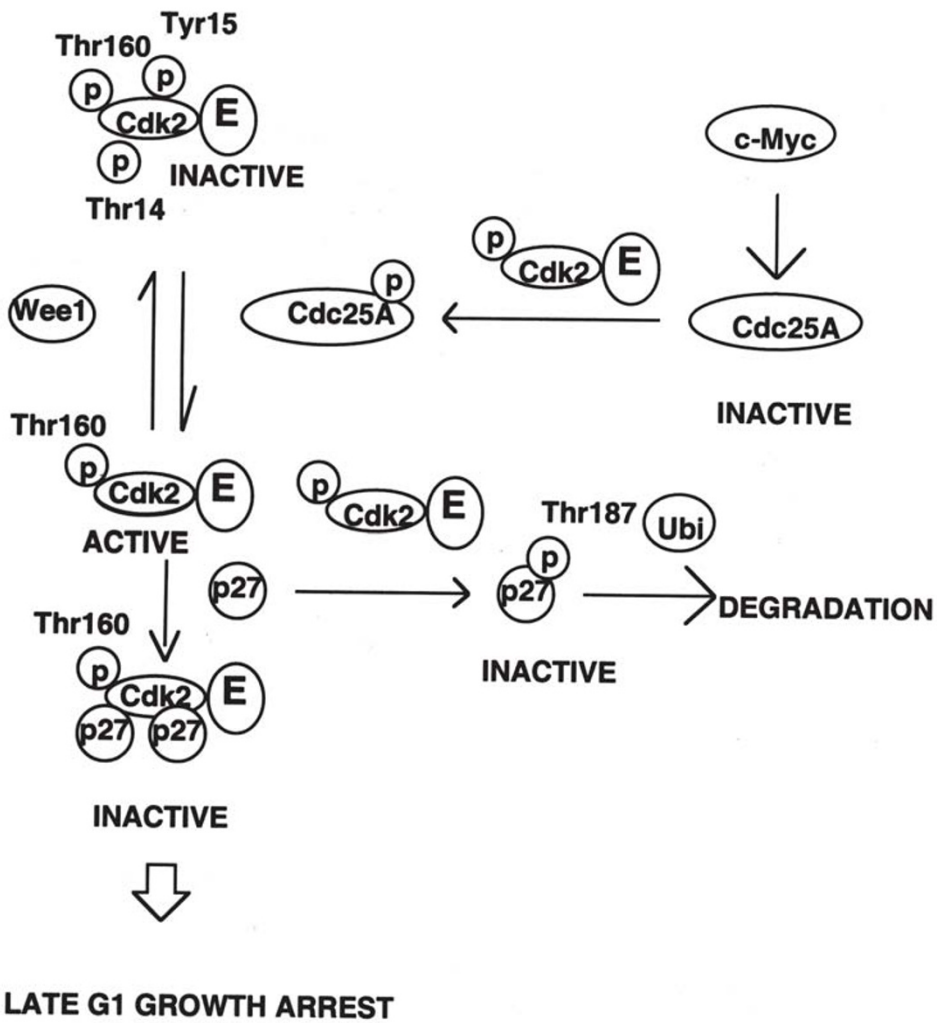

Fig 2.
Regulation of Cdk2/cyclin E kinase activity.
In order to be active, Cdk2 has to be appropriately phosphorylated and complexed with the regulatory subunit, cyclin E. Phosphorylation of Cdk2 on Thr 14 and Tyr 15 by Wee1 kinase inactivates the complex, while dephosphorylation of these residues by Cdc25A phosphatase activates the Cdk2/cyclin E complex. If the inhibitory subunit such as CKI $p27^{Kip1}$ is present in the Cdk2/cyclin E complex in excess, this complex becomes inactive. Cdk2/cyclin E activates Cdc25A (a positive regulator of Cdk2) and inactivates $p27^{Kip1}$ (a negative regulator of Cdk2) via phosphorylation. Additionally, Cdc25A is positively regulated by c-Myc on transcriptional level.

In order to be active, CDKs have to be appropriately phosphorylated. Cdk4 undergoes phosphorylation on the single threonine residue (Thr 161). The kinase responsible for this is CAK (CDK-activating kinase). It is an enzyme composed of catalytic subunit Cdk7

and cyclin H[30]. This phosphorylation has a stimulatory effect on the kinase activity. Phosphorylation of Thr14 and Tyr15, on the other hand, has an inhibitory effect and inactivates the kinase[31].

Additionally, Cdk4 and Cdk6 kinase activity can be modulated by the negative regulators (CKIs). These kinases can be inhibited by all known cyclin kinase inhibitors. CKIs can be divided into two families: INK4 family and Kip/Cip family.

INK4 family of CKI

INK4 proteins form a family of 15-20 kDa proteins that have repeated, conserved, ankyrin motifs which are considered to participate in protein-protein interactions[32]. The first described member of the INK4 family is p16 (also called INK4a). $p16^{INK4A}$ inhibits Cdk4 and Cdk6 activity by binding in competition with D type cyclins. $p16I^{NK4A}$ also inhibits phosphorylation of Cdk4 and Cdk6 on Thr161 by CAK[33]. Importantly, p16 is a well documented tumor suppressor gene that is frequently mutated in many human cancers (reviewed in[34]). INK4A locus has two overlapping genes, each regulated by its own promoter (reviewed in[35]). One promoter produces a transcript that encodes $p16^{INK4A}$, while the other promoter produces a transcript that encodes p19ARF (alternative reading frame) in mice and $p14^{ARF}$ in humans. Since the reading frames of the two transcripts are different, the amino acid sequences of the two proteins are completely unrelated. $p19^{ARF}$ has been shown to bind directly to p53 inhibitor Mdm2 and to neutralize its function, thereby promoting p53 transcriptional activity (reviewed in [36]).

Hannon and Beach[37] isolated another member of the INK4 family, p15 (also called INK4b). The sequences of $p15^{INK4B}$ and $p16^{INK4A}$ are 44% identical in the first 50 amino acids, and even 97% identical in the next 81 residues. $p15^{INK4B}$ expression is induced approximately 30 fold in human keratinocytes, following TGF-β treatment.

Two additional INK4 family members p18, (also called INK4C) and p19 (also called INK4D) were subsequently identified[38]. Overexpression of $p18^{INK4C}$ induces cell cycle arrest and terminal differentiation in B cells[39], while overexpression of $p19^{INK4D}$ in NIH3T3 cells leads to G1 growth arrest due to Cdk4/cyclin D1 inactivation. Finally, $p19^{INK4D}$, as well as $p16^{INK4A}$, rely on the presence of functional pRb to arrest the cycle, since overexpression of these two tumor suppressors does not lead to growth arrest in pRb deficient cells[40].

Late G1 phase CDK/cyclin complexes

While Cdk4 and Cdk6 in complex with D-type cyclins are responsible for the G1 progression, Cdk2 and recently identified Cdk3 in complex with E-type cyclins (reviewed in[41]) and with A-type cyclins are thought to act at the G1 to S transition (reviewed in [42]). Cdk2 and Cdk3 activities increase rapidly in mid to late G1 phase and peak close

to the G1 to S transition (reviewed in[43]). Both Cdk2 and Cdk3 form complexes with cyclins A and E as well as with more recently identified cyclins A1[44] and E2[45]. Furthermore, both Cdk2 and Cdk3 are inhibited by $p21^{Cip1}$ and $p27^{Kip1}$ CKIs[46].

Hinds et al[47] showed that constitutively expressed cyclins A and E can overcome pRb mediated suppression of proliferation. In cells overexpressing cyclin A or E, pRb becomes hyperphosphorylated much earlier than usual. As in the case of D type cyclin overexpression, overexpression of cyclin E accelerates entry into S phase[43]. Cyclin E dependent kinase activity is maximal at G1 to S transition. Once the cell enters S phase, cyclin E becomes degraded.

Cyclin A, on the other hand, binds to and activates Cdk2 in G1 to S transition as well as in S phase. It also binds to and activates Cdc2 in G2 and M phase[49]. Cyclin A and its associated kinase activity are not detected in early and mid G1. It first appears at late G1, accumulates through S and G2, and disappears at mitosis. Cyclin A expression is regulated on the transcriptional level, as shown by Henglein et al[50]. Cyclin A promoter is repressed during early to mid G1 and is activated in late G1 phase. As in the case of cyclin E, overexpression of cyclin A accelerates phosphorylation of pRb[51] and promotes entry into S phase[48].

Very strong evidence for the role of cyclin A/Cdk2 complex in G1 to S transition comes from the research conducted on B lymphoma cell lines. These cells undergo late G1 cell cycle arrest, and subsequently apoptosis, when treated with anti-immunoglobulin M (anti-IgM) antibody. In such anti-IgM arrested cells, most of the pRb is in hypophosphorylated (active) form. Joseph et al[52] showed that hypophosphorylation of pRb is due to the inhibition of Cdk2/cyclin A kinase activity.

Although Cdk2/cyclin E and Cdk2/cyclin A complexes are clearly involved in pRb phosphorylation[53], Cdk2/cyclin E also appears to have at least one additional, still unidentified, pRb independent role in G1 to S transition. The experimental evidence for this is as follows. Firstly, inducible expression of cyclin E in fibroblasts accelerates G1 to S progression without affecting the kinetics of pRb phosphorylation[54]. Secondly, unlike D-type cyclins, cyclin E is essential for cell cycle progression in pRb-deficient cells[55]. Thirdly, ectopic expression of E2F-1 bypasses growth arrest by D-type cyclin inhibitor p16, but not by Cdk2/cyclin E inhibitor p27Kip1[56]. Fourthly, ectopic expression of cyclin E bypasses p16- or pRb-mediated cell cycle arrest independently of pRb phosphorylation[56],[57]. Finally, $p21^{Cip1}$ (another CKI of Cdk2 complexes) overexpression suppresses growth and E2F activity in pRb deficient cells[58]. The identity of the pRb independent mechanism by which Cdk2 complexes promote cell growth is at present unclear.

Cdc25A phosphatase

In the case of Cdk2, its active catalytic subunit has to be phosphorylated on Thr 160 and must not be phosphorylated on Thr 14 and Tyr 15[59], (Fig 2). Cdk2 is phosphory-

lated on Thr 160 in vitro by CAK. CAK (reviewed in[60],[61]) is a multi subunit enzyme that consists of cyclin H and Cdk7[30]. Thr160 can be dephosphorylated by KAP phosphatase in the absence of cyclin, thereby rendering Cdk2 inactive even upon subsequent binding to appropriate cyclin[62]. Cdk2 is phosphorylated on Thr 14 and Tyr 15 in vitro by Wee1/Mik1 related protein kinases. Cdk2 is activated in vitro by Cdc25A, a dual specificity phosphatase that is expressed predominantly in late G1 and that dephosphorylates Tyr 15 and possibly Thr 14 residues[59]. Downregulation of Cdc25A leads to growth arrest in late G1[63]. In vitro assays show that Cdc25A activity is increased in S phase when it is phosphorylated by Cdk2/cyclin E[63], (Fig 2). cdc25A is transcriptionally regulated by c-Myc. Two functional binding sites for the c-Myc/Max heterodimer were found in the cdc25A promoter in vitro[64]. Cdc25A is negatively regulated in human keratinocytes by TGF-β[65]. More precisely, cdc25A transcription is inhibited by E2F-4/p130 complex that recruits histone deacetylase to the E2F site of the cdc25A promoter in response to TGF-β[66].

Finally, similar to Cdk4 and Cdk6, Cdk2 can also be negatively regulated by CKIs. However, in contrast to Cdk4 and Cdk6, which are inhibited primarily by INK4 family members, Cdk2 (and Cdk3) are inhibited by members of the Kip/Cip family of CKI (p21, p27, and p57).

p21^{Cip1}

p21 is a 21 kDa protein, also called Cip1 or Waf1, and is the first characterized member of the family[67]. Inhibition of CDK activity by p21^{Cip1} seems to require the binding of more than one p21^{Cip1} molecule[68]. Cyclin/CDK complexes containing a single p21^{Cip1} molecule are still catalytically active. On the other hand, those containing multiple p21^{Cip1} molecules are not catalytically active. Furthermore, p21^{Cip1} (as well as p27^{Kip1}) has separate binding sites for CDK and cyclin, and both sites need to be intact in order for CKI to bind to CDK/cyclin complexes[69].

The expression of p21^{Cip1} gene is regulated by p53 tumor suppressor protein. p21^{Cip1} promoter has a p53 binding site[70]. However, p53 is not the only transcription factor that regulates p21^{Cip1} expression, since p21^{Cip1} is expressed in p53-deficient cells. Because it is regulated by p53, p21^{Cip1} appears to be essential in inducing p53-mediated growth arrest as a response to the DNA damage. Therefore, in the cells lacking functional p53, the failure to induce p21^{Cip1} after DNA damage could result in elevated genetic instability (as is the case in tumor cells).

p21^{Cip1} (as well as other Kip/Cip family members) are thought to inhibit Cdk2 activity by preventing Thr160 phosphorylation by CAK[33]. Recently however, a novel function for Kip/Cip family members was proposed[71]. Harlow and colleagues suggested that in addition to their roles as inhibitors, Kip/Cip family members also act as adapter proteins that assemble CDK/cyclin complexes and target them to the nucleus. These studies were confirmed by Cheng et al[72] who showed that Kip/Cip CDK "inhibitors"

are actually essential activators of Cdk4 and Cdk6. Murine fibroblasts deficient in both $p21^{Cip1}$ and $p27^{Kip1}$ fail to upregulate D-type cyclins upon mitogen stimulation. They also fail to assemble CDK/cyclin D complexes and to direct D-type cyclins to the nucleus. All these effects are reversed by restoring CKI function. Finally, Lees and coworkers[73] also showed that formation of Cdk4/cyclin D complexes is promoted by members of the Kip/Cip family of CKI. INK4 family members, on the other hand, bind Cdk4 and, in doing so, inhibit the formation of Cdk4/cyclin D complexes.

A current dogma is that Cdk2/cyclin/$p21^{Cip1}$ complexes can transition between active and inactive states, through the changes in the stoichiometry of $p21^{Cip1}$ subunit in the complex[68]. Most recently, however, this dogma has been challenged by Hengst et al[74]. Using analytical ultracentrifugation of Cdk2/cyclinA/$p21^{Cip1}$ complexes, these researchers demonstrated that complete inhibition of CDK/cyclin complexes is achieved by one single molecule of $p21^{Cip1}$ present in the complex. Furthermore, they showed that $p21^{Cip1}$ saturated complexes contain only one stably bound inhibitor molecule.

$p27^{Kip1}$

$p27^{Kip1}$ is a 27 kDa protein that is 42% identical with $p21^{Cip1}$ at its N-terminus[75], [76]. It has 198 aa with the cyclin binding domain residing between aa 27-43, and CDK binding domain between aa 60-79. The crystal structure of Cdk2/cyclin A/$p27^{Kip1}$ complexes[77] reveals that $p27^{Kip1}$ binds to both cyclin A and Cdk2 and also inserts itself deep inside the catalytic cleft, mimicking ATP. Both intact cyclin and CDK binding sites are needed for the stable association of $p27^{Kip1}$ with the Cdk2/cyclin A complex. Indeed, mutants of $p27^{Kip1}$ harboring changes in either binding domain are deficient in inducing E2F/p130 accumulation and inhibition of transcription of E2F-regulated genes such as cyclin A, cyclin E and DHFR[78]. As in the case of $p21^{Cip1}$, it is thought that the stoichiometry of $p27^{Kip1}$ in CDK/cyclin complexes determines whether the kinase activity is inhibited or not.

$p27^{Kip1}$ deficient mice display enhanced growth, multiple organ hyperplasia, retinal dysplasia, pituitary tumors and female sterility[79-81]. These mice have enlarged thymi and spleens, and an increased numbers of thymocytes and mature T cells. However, the number of B cells in these mice is normal and so are functions of B cells. In serum starved $p27^{Kip1}$ deficient fibroblasts, Cdk2 activity is still inhibited[82]. Additionally, $p27^{Kip1}$ deficient oligodendrocyte precursor cells do eventually differentiate, but they go through several more cycles before doing so, as compared to $p27^{Kip1}$ wild type cells[83]. Together, these data suggest the existence of alternative pathway(s) of Cdk2 inactivation and growth arrest when the $p27^{Kip1}$ pathway is disabled or is missing.

Levels of $p27^{Kip1}$ are increased in a variety of cells arrested in G1 by different stimuli, such as murine B-lymphoma cells arrested upon BCR crosslinking[84], macrophages arrested by cAMP[85], fibroblasts arrested by lovastatin[86] or by serum withdrawal [54], and Mv1Lu mink epithelial cells arrested by TGF-β[87],[88].

$p27^{Kip1}$ regulation seems to differ slightly from one cell type to another. $p27^{Kip1}$ seems to be predominantly regulated on the protein level. Redistribution of $p27^{Kip1}$ among different CDK/cyclin complexes is one way of regulating this CKI. In some systems, the availability of $p27^{Kip1}$ (and $p21^{Cip1}$) depends on their subcellular localization. For example, in normal anchorage-dependent fibroblasts, detachment of the cells results in accumulation of the Cdk2/cyclin E/$p27^{Kip1}$ complexes in the nucleus[89]. In contrast, in anchorage-independent, transformed fibroblasts, $p21^{Cip1}$ and $p27^{Kip1}$ are sequestered away from the nucleus by cytoplasmic CDK/cyclin complexes. Another interesting mechanism of posttranscriptional regulation of $p27^{Kip1}$ was reported by Millard et al[90]. In human leukemic HL-60 cells arrested with phorbol ester PMA, $p27^{Kip1}$ accumulation is due to an increase in the amount of $p27^{Kip1}$ mRNA in polyribosomes, which results in an increased translation rate. An increased translation rate of $p27^{Kip1}$ has also been reported in lovastatin-arrested HeLa cells as well as in density-arrested fibroblasts[91]. In this same experimental system, a decreased rate of $p27^{Kip1}$ degradation was observed. Indeed, regulation of $p27^{Kip1}$ protein degradation seems to be the most important mechanism by which this important CKI is regulated. It is known that $p27^{Kip1}$ degradation occurs via ubiquitination ([92]; reviewed in[93]), because $p27^{Kip1}$ is phosphorylated on Thr 187 by Cdk2/cyclin E and this phosphorylated form is then targeted for ubiquitination and degradation[94-97]. Pagano and coworkers[98] demonstrated that ubiquitination of $p27^{Kip1}$ requires prior phosphorylation of $p27^{Kip1}$ on Thr187 as well as trimeric complex formation among $p27^{Kip1}$, Cdk2 and cyclin A or E. As expected, proteasome inhibitors lead to $p27^{Kip1}$ accumulation[99]. $p27^{Kip1}$ ubiquitination and degradation seems to be restricted to the cytoplasmic compartment (reviewed in [100]). Tomoda et al[101] reported a novel protein Jab1 that binds $p27^{Kip1}$ in the nucleus and shuttles it to the cytoplasm where it becomes ubiquitinated and degraded. Although ubiquitin-mediated proteolysis is a major mean of $p27^{Kip1}$ degradation, most recent findings argue that this is not the only mean of $p27^{Kip1}$ degradation. Levkau et al[102] reported the C-terminal cleavage of both p21Cip1 and $p27^{Kip1}$ by caspase 3 and caspase 7 in human umbilical vein endothelial cells (HUVECs) undergoing growth factor deprivation-induced apoptosis. Additionally, it seems that Cdk2 is not the only kinase that can phosphorylate $p27^{Kip1}$. Kawada et al [103] found that $p27^{Kip1}$ can be phosphorylated by Ras/Raf-induced MAP kinase in vitro and that such phosphorylated $p27^{Kip1}$ is unable to bind to and inhibit Cdk2.

There are several lines of evidence that suggest c-Myc as a negative regulator of $p27^{Kip1}$. For example, induction of c-MycER fusion protein by 4-OH tamoxifen in Rat1 fibroblasts leads to Cdk2/cyclin E activation. This is a result of: i) inhibition of $p27^{Kip1}$ binding to the Cdk2/cyclin E complexes[104], ii) $p27^{Kip1}$ release from the Cdk2/cyclin E complexes[97], and iii) $p27^{Kip1}$ degradation[105]. Furthermore, retroviral expression of $p27^{Kip1}$ induces G1 arrest in parental Rat1 cells, but not in Rat1 cells that ectopically express c-Myc[106]. Additionally, co-expression of Ras and c-Myc leads to cyclin E-associated kinase activity, S phase induction and, mostly important, $p27^{Kip1}$ loss[107]. In several experimental systems, there is an inverse correlation between c-Myc and

$p27^{Kip1}$ expression. For example, there is a correlation between c-Myc overexpression and $p27^{Kip1}$ downregulation in mammary epithelial cells[108], while there is an increased p27Kip1 expression in Rat1 cells deficient in c-Myc[109]. There is also an experimental evidence that c-Myc negatively regulates $p27^{Kip1}$ in lymphocytes. IL-2 induces c-Myc in T cells[110] and complete stimulation of T cells (TCR engagement and IL-2R engagement) downregulates $p27^{Kip1}$[111]. In B cells, mIgM crosslinking induces c-Myc[112] and complete stimulation of B cells (BCR engagement and CD40 engagement) downregulates $p27^{Kip1}$[113].

In murine B- lymphoma cells, there is also an inverse correlation between c-Myc and $p27^{Kip1}$ levels. The decrease in c-Myc strongly correlates with anti-IgM induced $p27^{Kip1}$ accumulation, late G1 arrest and apoptosis in anti-IgM sensitive murine B-lymphoma cells. In this experimental system, the loss of c-Myc, when accompanied by an increase in cytosolic free calcium, both of which are induced by mIgM crosslinking, is able to induce $p27^{Kip1}$ accumulation, growth arrest and apoptosis[114].

Because of its growth inhibitory effects via Cdk2 inhibition, $p27^{Kip1}$ is a potential candidate tumor suppressor protein. Abnormally low levels of $p27^{Kip1}$ protein are frequently found in many human cancers, and these low levels correlate with the aggressiveness of the tumor and with the high mortality rate of the patients. However, it has not been possible to establish a causal link between $p27^{Kip1}$ and tumor suppression because only rare instances of homozygous inactivating mutations of $p27^{Kip1}$ gene have been found in human tumors. Fero et al[115] showed that $p27^{Kip1-/-}$ mice and $p27^{Kip1+/-}$ mice are predisposed to tumors in multiple tissues when challenged with g-irradiation or chemical carcinogens. However, the remaining wild-type allele in $p27^{Kip1+/-}$ mice is neither mutated nor silenced. Therefore, $p27^{Kip1}$ is a multiple tissue tumor suppressor in mice, but it is haplo-insufficient for tumor suppression.

In recent years a large number of studies implicated $p27^{Kip1}$, as well as other CKIs, as a prognostic factor in various human cancers (reviewed in[116],[117]). For example, $p27^{Kip1}$ is a prognostic marker in breast and colorectal cancer[118], as well as in prostate adenocarcinoma[119].

$p57^{Kip2}$

Finally, p57 (also called Kip2) is the most recently discovered Kip/Cip family member [120]. It is structurally the most complex member of the family. $p57^{Kip2}$ deficient mice have altered cell differentiation and proliferation. They have many phenotypic characteristics seen in Beckwith-Wiedemann syndrome, a pleiotropic hereditary disorder that is characterized by overgrowth and predisposition to cancer, and that is associated with translocations in p57 gene[121]. Unlike $p27^{Kip1}$, $p21^{Cip1}$, and $p18^{INK4C}$ CKIs which are not imprinted, (i.e. biallelic expression is observed in both fetal and adult tissues [122]), $p57^{Kip2}$ is normally imprinted with the preferential expression of the maternal allele.

CONCLUSION

Regulation of the cell cycle in eucaryotic cells is very complex and is conserved among species, from yeast to humans. Over the last decade, our understanding of the mammalian cell cycle regulation increased dramatically. The discovery of the important cell cycle regulators such as the pRb family of proteins, CDKs, cyclins, CKIs, etc. provides the potential for novel therapeutic targets in treatment of diseases where the balance between cell proliferation and cell death is disrupted.

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