Cell. Mol. Life Sci. 65 (2008) 8 – 15
1420-682X/08/010008-8
DOI 10.1007/s00018-007-7425-z

Cellular and Molecular Life Sciences

The logic and regulation of cell cycle exit and reentry

D. Pajalunga, A. Mazzola, A. Franchitto, E. Puggioni and M. Crescenzi*

Dept. of Environment and Primary Prevention, Istituto Superiore di Sanità – Viale Regina Elena, 299, 00161 Roma (Italy), Fax: +39 064990-3650, e-mail: marco.crescenzi@iss.it

Online First 20 November 2007

Abstract. Tissue repair and regeneration are very complex biological events, whose successful attainment requires far more than mere cell division. However, almost unavoidably they entail cell proliferation as a fundamental premise. Full regeneration or repair cannot be achieved without replacing cells lost to disease or injury, replacement that can only take place via proliferation of surviving cells. This review endeavors to outline the molecular bases of exit from and reentry into the cell cycle. In recent years, the decision to proliferate or not has been seen as mostly the concern of cyclins and cyclin-dependent kinases. This account tries to show that cell cycle inhibitors are as important as the positive regulators in the making of this decision. Finally, the authors wish to suggest that the molecular knowledge of the cell cycle can be harnessed to the benefit of many aspects of regenerative medicine. (Part of Multi-author Review)

Keywords. Cell cycle, quiescence, terminal differentiation, proliferative senescence, cyclins, cyclin-dependent kinase inhibitors.

Regulation of the cell cycle

A discussion of cell cycle exit and reentry requires knowledge of the core molecular machinery that drives and regulates the cell cycle itself. Thus, we provide a brief outline of essential aspects of such machinery. The purpose of this sketch is only to supply the minimum background necessary to understand what follows and does not aim for completeness.

The standard picture of cell cycle regulation relies in large part on experiments performed with G0-synchronized cells released into their first cell cycle after a period of quiescence. It should be borne in mind that the first cell cycle is not entirely regulated like the following ones [1]. This distinction, important as it is, goes beyond the scope of the present review and will be largely ignored. The decision to divide is made in G1, after which, in the absence of derangements, damage, or stress, the rest of the cell cycle is a highly coordinated but nonetheless automatic process [2]. Physiologically, a quiescent cell reenters the cell cycle in the presence of appropriate conditions, most important among which is growth factor stimulation. G1 phase, like the rest of the cell cycle, is regulated and orchestrated by a variety of cyclin-dependent kinases (cdks), whose activity critically depends on their forming stable complexes with fitting cyclins. G1-regulating cyclins include Cyclin D1, D2, D3, and E. The main G1 cdks are Cdk4 and Cdk6, which bind D-type cyclins, and Cdk2, which associates principally with Cyclin E and Cyclin A [3, 4].

The following description is best understood by making reference to Figure 1. G0-resting cells stimulated with growth factors progress toward S phase in a march successively promoted by diverse cyclin-cdk complexes. One of the main tasks of such complexes is to phosphorylate the 'pocket' proteins pRb, p107, and p130 at multiple residues, thereby releasing their negative control on transcription factors of the E2F family, whose activity drives the expression of numerous effectors of DNA synthesis [2].

Growth factor stimulation is sensed and mediated by D-type cyclins, particularly Cyclin D1 [5]. Accumulation of the latter is exquisitely dependent on growth factors, which increase Cyclin D1 protein levels through a variety of transcriptional and posttranscriptional mechanisms [2]. Most histotypes express

* Corresponding author.

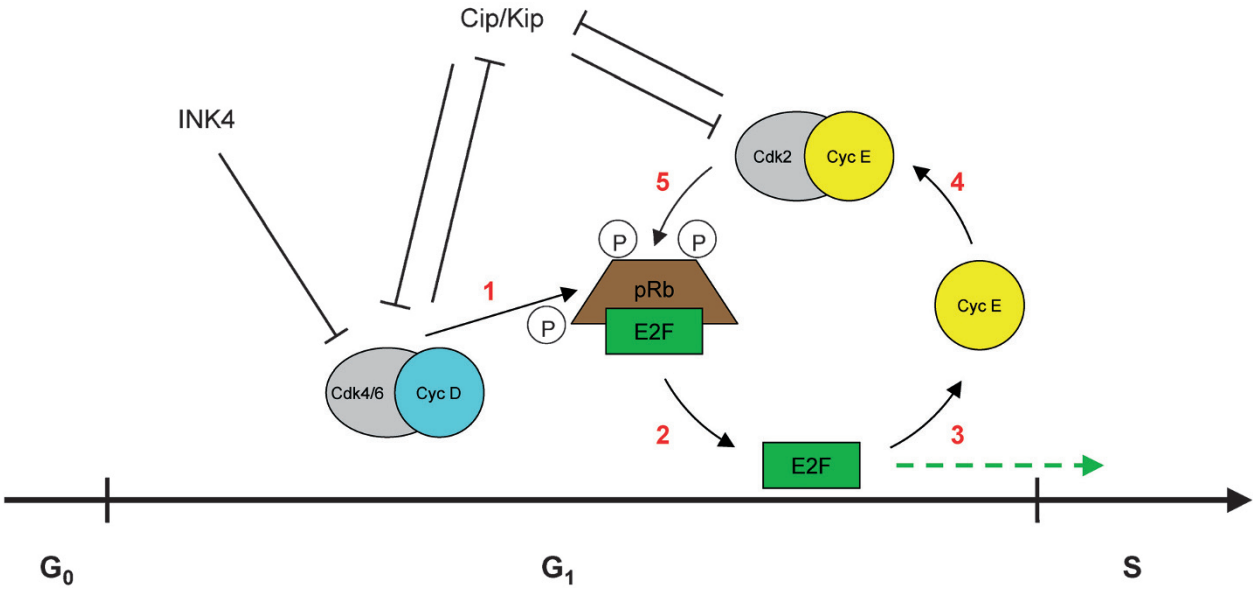

Figure 1. Schematic representation of G1 phase regulation. (1) Cyclin D-Cdk4/6 complexes phosphorylate pRb, inducing (2) partial release of E2F transcription factors, which (3) transcribe and stabilize Cyclin E; the latter (4) heterodimerizes with Cdk2 and (5) the active kinase complex further phosphorylates pRb, determining the release of more E2F. E2F transcribes numerous DNA synthesis effector genes required for DNA replication. Phases 2 through 5 constitute a positive feedback loop that makes irreversible the decision to enter S phase. INK4 inhibitors prevent Cdk4/6 from forming complexes with D-type cyclins. Cyclin D-Cdk4/6 complexes can be inhibited by Cip/Kip CKIs, but also act as sinks for such inhibitors, relieving Cyclin E-Cdk2 from their control. Cip/Kip CKIs are capable of inhibiting Cyclin E-Cdk2 complexes, but at least p27 is phosphorylated and directed to degradation by active Cdk2. It should be emphasized that this is a highly simplified rendition of G1 phase regulation. Many details and qualifications have been purposely omitted.

more than one cyclin D and variable levels of cdk4 and/or cdk6 [6]. Active, heterodimeric complexes are formed by one of the D-type cyclins and either Cdk4 or Cdk6. Cyclin D-Cdk4/6 complexes phosphorylate the pRb family proteins with some residue specificity [7], beginning to release the grip of pocket proteins on E2F transcription factors [8]. At the same time, the increasing number of Cyclin D-Cdk4/6 complexes may subtract inhibitory molecules (see later) from Cyclin E-Cdk2 complexes, thereby contributing indirectly to their activation. The latter complexes are also activated by the initial phosphorylation of the pocket proteins at the hands of Cyclin D-Cdk4/6, which begins to release the E2Fs. This event ignites a positive feedback loop [2] in which free E2F increases Cyclin E transcription and stabilizes cyclin E protein [9], which associates with free Cdk2 to form active complexes that further phosphorylate the pocket proteins, releasing more E2F (Fig. 1). In addition to its role as one regulatory subunit of the Cdk2 kinase, Cyclin E exerts a critical non-kinase function by loading the MCM proteins, a putative helicase complex, onto DNA replication origins [10]. Together, Cyclin E and E2F establish the necessary conditions to enter S phase. By this time, cell cycle progression has become growth factor-independent and will proceed to the eventual cell division.

Cdk activity is regulated by two classes of cyclin-dependent kinase inhibitors (CKIs): the INK4 and the Cip/Kip family [11]. The INK4 family comprises four members named p15, p16, p18, and p19 after their approximate molecular size. Cip/Kip inhibitors include the similarly indicated p21, p27, and p57. INK4 inhibitors bind only Cdk4 and Cdk6, preventing their association with D cyclins. Cip/Kip molecules are capable of binding essentially all kinds of cyclins and cdks separately, but display a higher affinity for their respective heterodimeric complexes. Thus, whereas INK4 molecules can only inhibit the Cdk4/6 kinase, Cip/Kip inhibitors modulate the activity of all cdks and therefore contribute to the regulation of all phases of the cell cycle.

The description of the cell cycle just provided refers to mammalian cells. The regeneration field encompasses a wide variety of organisms and one might legitimately wonder how applicable is this account to species distant from humans. However, the central principles of cell cycle regulation are conserved throughout the two eukaryotic kingdoms [12]; witness the fact that the molecular regulation of the cell cycle in mammals has been understood thanks to the elucidation of its workings in yeast. Thus, this description as well as the conclusions and speculations to be found later in this article are likely to apply to any and all animals and plants.

Exit from the cell cycle

Most cells in the body of an adult vertebrate divide occasionally, rarely, or not at all, spending most of their time in G0 phase. At least three states of temporary or definitive growth arrest (shorthand for the more proper expression, proliferation arrest) can be distinguished: quiescence, a condition of reversible exit from the cell cycle, terminal differentiation, characterized by a perpetual postmitotic state, and proliferative senescence, a different kind of permanent postmitotic arrest.

Quiescence is determined by the lack of requisites for proliferation, among which mitogenic stimulation, anchorage to extracellular matrix, and space [13]. The molecular mechanisms determining quiescence vary according to its causes. For example, lack of growth factors triggers a swift reduction in the expression of D-type cyclins [14]. Such reduction is at least in part responsible for the cell cycle exit that follows growth factor deprivation. Lack of space for cell division, the *in vitro* phenomenon known as contact inhibition, determines growth arrest via an increase in p27 levels [15, 16], with no reduction in cyclin D. Usually, removal of the condition that causes quiescence promptly abolishes its molecular underpinnings and reverts the cell to proliferation. As already described, the functions of cyclins and CKIs in G1 phase modulate the proliferation-restrictive activity of pocket proteins, more often that of pRb. Surprisingly, whereas pRb is not required to enter quiescence [17], its acute ablation in quiescent (and senescent) cells reactivates the cell cycle [18]. Thus, the mechanisms establishing quiescence are partly distinct from those that maintain it.

Terminal differentiation is empirically defined as a state characterized by specialized cell functions (and thus gene expression) and permanent withdrawal from the cell cycle. Expression of G1 cyclins is generally downregulated in terminally differentiated (TD) cells, while that of different CKIs, depending on cell type, is increased [19]. Thus, the balance of cell cycle regulation is markedly tilted toward growth arrest, making TD cells capable of resisting a variety of powerful mitogenic stimuli [20]. However, in such cells the mitotic cycle can generally be reactivated by fusion with proliferating cells [21] or forced expression of selected genes. Interestingly, reconstitution of physiological levels of cdk4 activity is sufficient to drive a variety of TD cells back into the cell cycle [22]. The general rule that G1 cyclins are downmodulated in TD cells has significant exceptions. In one of the best-studied examples, cyclin D3 expression is strongly upregulated during adipocyte differentiation and forms complexes with cdk4 [23]. Quite unexpectedly, these complexes are endowed with kinase activity; however, they seem to be diverted to non-proliferative, tissue-specific functions, as they phosphorylate and cooperate with the PPAR-γ nuclear receptor [24]. TD skeletal muscle cells also show increased cyclin D3 expression and cyclin D3-cdk4 complexes that, however, are devoid of kinase activity [9, 25]. The role of pRb in establishing and maintaining the postmitotic state in TD cells is the opposite of that played in quiescence and senescence: in skeletal muscle cells, pRb is absolutely required for the establishment of the postmitotic state [26], but not for its maintenance [27, 28]. Again, this diversity shows that terminal growth arrest is initiated and preserved by partially non-overlapping mechanisms.

Cell senescence, more accurately termed proliferative senescence, when initially discovered, was regarded as an intrinsic, fixed limit to the number of divisions a cell can undergo [29]. More recent findings, on the other hand, indicate that senescence is a response of the cell to a number of different stress conditions. Among these, telomere attrition, DNA damage, and oncogene activation [30–33]. These apparently disparate triggering insults may well be one and the same, as telomere shortening is sensed as and involves DNA damage [34], and oncogene activation, at least in some cases, causes DNA damage [33]. The observed fixed limit to cell division number can be explained differently, depending on the species and cell type considered. Cells possessing relatively short telomeres and high DNA-repair capabilities (e.g., human fibroblasts), reach a critical lower threshold in telomere length, which triggers senescence. Cells endowed with comparatively longer telomeres and less efficient DNA-repair mechanisms accumulate enough senescence-triggering damage, perhaps mostly oxidative, before their telomeres become too short [35]. In both cases, reaching the senescence threshold requires a relatively constant number of cell divisions in standard culture settings.

Senescent cells, even though unable to enter S phase, express constitutively high levels of cyclin D1 and cyclin E, independent of mitogenic stimulation [36]. These cyclins form complexes with cognate cdks, but such complexes are enzymatically inactive. Senescent cells express large amounts of p21 [37] and p16 [38], and the absence of either inhibitor before the onset of senescence has been found to facilitate immortalization [39, 40]. Together, these findings suggest that, in senescent cells, cdks are held inactive by preponderant levels of CKIs, despite the presence of large amounts of cyclins. Surprisingly, this simple model has not been tested directly until very recently, when suppression of either p21 or p16 has been shown to induce previously senescent cells to proliferate [9].

Altogether, our current understanding of cell cycle exit at the level of the core cell cycle machinery is best summarized as a shift in the balance between positive and negative regulators of proliferation in favor of the latter.

Reentry into the cell cycle

Non-proliferating cells can be induced or forced to reenter the cell cycle by different means. On the basis of the concept that the decision to rest or proliferate is always made as a result of the weighing of positive against negative regulators, we will show that cell cycle reentry can descend from increased cyclin expression, diminished CKI levels, or a combination of the two. By definition, only quiescent cells are equipped with the natural capability to reenter the cell cycle. They readily do so in the presence of proliferation-inducing conditions; because we have described growth arrest as a negative shift in the balance of cell cycle regulators, we might as well say that quiescent cells reenter the cell cycle upon removal of the circumstances that induced their dormancy.

We will refer again here to the paradigm of growth factor-starved, quiescent cells stimulated with serum. Serum growth factor stimulation, mediated by a number of signal transduction pathways, promotes the transcription, stabilization, and nuclear translocation of D-type cyclins. Serum also promotes the assembly of these cyclins and cdk4/6 to form active complexes [2]. During G1 phase, the abundance of p27, high in resting cells, is progressively reduced via cyclin E-mediated phosphorylation of Thr 187 and subsequent proteasomal degradation [41] or another ill-defined, mitogen-activated mechanism acting in early G1 phase [42], possibly mediated by Tyr 88 phosphorylation [43]. In contrast, the expression of p21, present in relatively low amounts in G0, has a transient surge in early G1 phase, peaks several hours after the beginning of serum stimulation, and then declines [44, 45]. As Cip/Kip inhibitors are believed to facilitate cyclin D-cdk4/6 complex formation, the transient increase in p21 levels may serve this purpose, but this is still a matter of controversy [11]. The anti-proliferative action of Cip/Kip inhibitors is also counterbalanced by the cyclin D-cdk4/6 complexes that sequester part of the CKIs, facilitating and perhaps initiating cyclin E-cdk2 activation [46, 47]. It is again evident that cell cycle reentry from quiescence entails a shift to conditions favourable to proliferation, which include higher cyclin expression and lower CKI-mediated inhibition. This is strikingly confirmed by the finding that quiescent cells can be induced to proliferate in the absence of growth factors by the sole removal of appropriate CKIs [9, 48]. This finding suggests that growth factors are chiefly needed to alter the balance of positive vs. negative cell cycle regulators. If the latter are artificially lowered, the cell can dispense with growth factors altogether.

Although TD and senescent cells do not spontaneously reenter the cell cycle, they can be forced – or allowed – to do so. TD cells of disparate kinds can be forced to reenter the cell cycle by expression of DNA-tumor-virus oncogenes, such as SV40 LT [49, 50] or adenovirus E1A [51–53]. However striking, these feats are relatively uninformative, as these oncogenes impinge on a variety of growth-restraining mechanisms and molecules, not all of which are known or understood. More revealing is the finding that reactivation of the dormant cdk4/6 kinase at physiological levels by overexpression of cdk4/6 and/or cyclin D1 brings back into the cell cycle TD cell as diverse as skeletal muscle myotubes, adipocytes, at least some neurons, and cardiomyocytes [22, 54]. These results show that the main reason why TD cells never spontaneously reenter the cell cycle is their tight negative control of the cdk4/6 kinase [22, 55]. Interestingly, in myotubes no amount of cyclin E-cdk2 activity can substitute for cyclin D-cdk4/6, suggesting that, at least in these TD cells, reentry into S phase must proceed from cdk4/6 activation [22]. Very recently it was shown that full cell cycle reactivation can be brought about in myotubes by knockdown of CKIs, p21 being the most important among them [9]. This surprising result strongly supports the concept that the decision to progress through G1 phase and start DNA replication is always – even in TD cells – made by the functional summation of positive and negative regulatory molecules.

It should be noted that, thus far, all attempts to reactivate the cell cycle in TD cells have eventually resulted in cell death [9, 56]. The molecular bases for the death of reactivated TD cells are not presently understood, but will constitute a fascinating and very important topic for future investigations. However, we stress that non-TD, senescent, or quiescent cells can be induced to proliferate sustainably [9, and see below], providing hope for future clinical applications.

The close relationship between cell cycle reactivation and death in TD cells is best seen in neurons, where cell cycle reentry is followed by cell death much more promptly than in other TD cell types [our unpublished observations]. In neurons, not only is cell cycle reactivation tightly linked to cell death, but the reverse relationship also seems to hold: molecules normally devoted to cell cycle regulation are required for programmed cell death, at least in some cases [57]. Cell cycle reactivation in these cells seems to be a prerequisite for cell death and to play a significant role

in the pathogenesis of neurodegenerative diseases [58, 59].

Senescence is traditionally considered as irreversible as terminal differentiation. However, it has been shown that the functional ablation of p53 is capable of inducing senescent cells to proliferate [60]. p53 is a strong transcriptional activator of p21, and indeed, upon removal of p53 from senescent cells, p21 levels dropped [60]. However, it was not ascertained whether the observed reduction in p21 levels was causal to cell cycle reactivation or was merely a concurrent phenomenon. Recently, however, it has been shown unequivocally that senescent cells can be reactivated, and indeed made to proliferate, by ablating either p21 or p16 [9]. Because it is well established that senescent cells possess high levels of CKI-inhibited cyclin-cdk complexes [36], these results show that in senescent as well as quiescent and TD cells, the cell cycle is held still by high amounts of inhibitors. In other words, all non-proliferation states, whether reversible or irreversible, require constant expression of cell cycle inhibitors for their maintenance. This realization leads one to ask what the role of cyclins and cdks is in cells that are never intended to proliferate again. For the time being, this is mostly a matter of speculation.

Relevance for tissue repair and regeneration

The recent recognition that the cell cycle can be reactivated by removing CKIs in any non-proliferating cell, whether quiescent, TD, or senescent, has potentially momentous implications for bioengineering, tissue repair, cell replacement therapy, and regeneration; in summary, all those circumstances in which cell proliferation is limiting. For example, cells difficult to grow in culture can be thought of as spending most of their time in G0 or G1 phase, due to inadequate culture conditions. The fact that quiescent cells can be induced to proliferate in the absence of growth factors by removing CKIs suggests that the need to contrive precise culture conditions for fastidious cell types may be circumvented. In this respect, it is worth noting that many kinds of human cells endowed with "stem-like" properties are very hard to culture and are often not available in the numbers that would be necessary for characterizing and practically exploiting them. In vivo, many tissues are excruciatingly slow at repairing themselves. Well-known examples include tendons, cartilages, bones, endocrine cells, and even skin, whose smallest wound requires a week or more to heal. The ability to promote, accelerate, or altogether allow cell proliferation in these and many other settings, both *in vitro* and *in vivo*, might revolutionize wide areas of biotechnology and biomedicine.

The above prospect finds strong support in a recent series of studies concerning the hematopoietic system of CKI knockout mice. Hematopoietic stem cells (HSCs) are known to proliferate infrequently; in non-human primates they may divide as rarely as once per year [61]. The hematopoietic stem cells of p18 knockout mice showed augmented renewal *in vivo*, enhanced competitiveness when transplanted into wild-type hosts, and strikingly increased serial transplantability [62]. HSCs of mice knockout for p21 or p27 showed different modifications of their behavior [63], indicating that diverse CKIs play subtly distinct roles that in part depend on the specific cell type considered. Finally, the HSCs of old p16 knockout mice were shown to be resistant to aging [64]. In principle, these findings could be exploited to achieve the long-sought goal of expanding human HSCs *ex vivo* [65]. As argued above in the case of quiescent cells *in vitro*, CKI removal might be substituted for or enhance unsatisfactory proliferation-promoting cytokine mixes.

Any enthusiasm for the new possibilities should be mitigated by prudence. In the first place, the feasibility of manipulating the cell cycle in a concretely useful fashion awaits demonstration. Second, decreasing CKI levels might impinge on cell cycle checkpoints and DNA repair functions, whose impairment might result in accumulation of mutations and increased risk of neoplastic transformation. This very legitimate concern is in part alleviated by the fact that CKI suppression is envisioned as temporary and reversible, eventually bringing the manipulated cells back to their original state. However, the possibility that cell cycle alterations might promote tumorigenesis should be throroughly investigated.

One further issue is whether the conclusions drawn from *in vitro* experiments are valid *in vivo*. Very few comparative experiments have been carried out so far, and the issue is still unresolved. Ideally, such experiments should be designed with regenerative medicine in mind. Thus, rather than using transgenic or knockout mice, whose genetic modifications might be compensated for in the course of ontogenesis and anyway do not befit humans, regenerative interventions should be acutely applied to adult animals in a setting that mimics a clinical situation. Some such experiments show, for example, that manipulations capable of reactivating cardiomyocytes *in vitro* prove similarly effective in vivo [66, 67]. Although the available examples are too few to draw sweeping conclusions, they encourage us to suppose that most *in vitro* results concerning the regulation of the cell cycle

will be eventually confirmed by *in vivo* experimentation.

A still elusive goal is achieving sustainable and reversible mitotic reactivation of TD cells in mammals. In several organisms, urodele amphibians being the best known, regeneration involves dedifferentiation and proliferation of TD cells, which contribute to form the regenerated tissues [68]. If mammalian TD cells could be persuaded to undertake similar changes, the potential for regeneration of organs and systems such as the heart, the nervous system, or endocrine glands would be enormous. We have moved several steps forward in this direction. Mammalian TD cells should no longer be regarded as permanently locked in a non-proliferative state: we have learned that such a state can be reverted, and we have devised minimally invasive strategies to do so. We must understand now what causes the reactivated cells to die and how to circumvent this last hurdle.

Acknowledgments. This work has been supported by funds granted to M. C. by the following agencies: Telethon Italy (grant no. GGP05006), the Italian Association for Cancer Research (AIRC), the ISS-NIH Collaboration program, and the Italian Ministry of Health (Stem Cells grant no. CS5 and Ricerca Finalizzata 2005).

1 Stacey, D. W. (2003) Cyclin D1 serves as a cell cycle regulatory switch in actively proliferating cells. Curr. Opin. Cell Biol. 15, 158–163.
2 Sherr, C. J. and Roberts, J. M. (1999) CDK inhibitors: positive and negative regulators of G1-phase progression. Genes Dev. 13, 1501–1512.
3 Sherr, C. J. (1994) G1 phase progression: cycling on cue. Cell 79, 551–555.
4 Sherr, C. J. and Roberts, J. M. (2004) Living with or without cyclins and cyclin-dependent kinases. Genes Dev. 18, 2699–2711.
5 Kerkhoff, E. and Rapp, U. R. (1997) Induction of cell proliferation in quiescent NIH 3T3 cells by oncogenic c-Raf-1. Mol. Cell. Biol. 17, 2576–2586.
6 Ekholm, S. V., Zickert, P., Reed, S. I. and Zetterberg, A. (2001) Accumulation of cyclin E is not a prerequisite for passage through the restriction point. Mol. Cell. Biol. 21, 3256–3265.
7 Kitagawa, M., Higashi, H., Jung, H. K., Suzuki-Takahashi, I., Ikeda, M., Tamai, K., Kato, J., Segawa, K., Yoshida, E., Nishimura, S. et al. (1996) The consensus motif for phosphorylation by cyclin D1-Cdk4 is different from that for phosphorylation by cyclin A/ E-Cdk2. EMBO J. 15, 7060–7069.
8 Weinberg, R. A. (1995) The retinoblastoma protein and cell cycle control. Cell 81, 323–330.
9 Pajalunga, D., Mazzola, A., Salzano, A. M., Biferi, M. G., De Luca G. and Crescenzi, M. (2007) Critical requirement for cell cycle inhibitors in sustaining nonproliferative states. J. Cell Biol. 176, 807–818.
10 Geng, Y., Lee, Y. M., Welcker, M., Swanger, J., Zagozdzon, A., Winer, J. D., Roberts, J. M., Kaldis, P., Clurman, B. E. and Sicinski, P. (2007). Kinase-independent function of cyclin E. Mol Cell 25, 127–139.
11 Pei, X. H. and Xiong, Y. (2005) Biochemical and cellular mechanisms of mammalian CDK inhibitors: a few unresolved issues. Oncogene 24, 2787–2795.
12 De Clercq, A. and Inze, D. (2006) Cyclin-dependent kinase inhibitors in yeast, animals, and plants: a functional comparison. Crit. Rev. Biochem. Mol. Biol. 41, 293–313.
13 Coller, H. A., Sang, L. and Roberts, J. M. (2006) A new fescription of cellular quiescence. PLoS Biol. 4, e83.
14 Guo, Y., Harwalkar, J., Stacey, D. W. and Hitomi, M. (2005) Destabilization of cyclin D1 message plays a critical role in cell cycle exit upon mitogen withdrawal. Oncogene 24, 1032–1042.
15 Polyak, K., Kato, J. Y., Solomon, M. J., Sherr, C. J., Massague, J., Roberts, J. M. and Koff, A. (1994) p27Kip1, a cyclin-Cdk inhibitor, links transforming growth factor-beta and contact inhibition to cell cycle arrest. Genes Dev. 8, 9–22.
16 Hengst, L. and Reed, S. I. (1996) Translational control of p27Kip1 accumulation during the cell cycle. Science 271, 1861–1864.
17 Sage, J., Mulligan, G. J., Attardi, L. D., Miller, A., Chen, S., Williams, B., Theodorou, E. and Jacks, T. (2000) Targeted disruption of the three Rb-related genes leads to loss of G(1) control and immortalization. Genes Dev. 14, 3037–3050.
18 Sage, J., Miller, A. L., Perez-Mancera, P. A., Wysocki, J. M. and Jacks, T. (2003) Acute mutation of retinoblastoma gene function is sufficient for cell cycle re-entry. Nature 424, 223–228.
19 Kranenburg, O., van der Eb, A. J. and Zantema, A. (1995) Cyclin-dependent kinases and pRb: regulators of the proliferation-differentiation switch. FEBS Lett. 367, 103–106.
20 Tiainen, M., Pajalunga, D., Ferrantelli, F., Soddu, S., Salvatori, G., Sacchi, A. and Crescenzi, M. (1996) Terminally differentiated skeletal myotubes are not confined in G_0, but can enter G_1 upon growth factor stimulation. Cell Growth Diff. 7, 1039–1050.
21 Ringertz, N. R. and Savage, R. E. (1976) Academic Press.
22 Latella, L., Sacco, A., Pajalunga, D., Tiainen, M., Macera, D., D'Angelo, M., Felici, A., Sacchi, A. and Crescenzi, M. (2001). Reconstitution of cyclin D1-associated kinase activity drives terminally differentiated cells into the cell cycle. Mol. Cell. Biol. 21, 5631–5643.
23 Phelps, D. E. and Xiong, Y. (1998) Regulation of cyclin-dependent kinase 4 during adipogenesis involves switching of cyclin D subunits and concurrent binding of p18INK4c and p27Kip1. Cell Growth Differ. 9, 595–610.
24 Sarruf, D. A., Iankova, I., Abella, A., Assou, S., Miard, S. and Fajas, L. (2005) Cyclin D3 promotes adipogenesis through activation of peroxisome proliferator-activated receptor-gamma. Mol. Cell. Biol. 25, 9985–95.
25 Cenciarelli, C., De Santa, F., Puri, P. L., Mattei, E., Ricci, L., Bucci, F., Felsani, A. and Caruso, M. (1999) Critical role played by cyclin D3 in the MyoD-mediated arrest of cell cycle during myoblast differentiation. Mol. Cell. Biol. 19, 5203–5217.
26 Schneider, J. W., Gu, W., Zhu, L., Mahdavi, V. and Nadal-Ginard, B. (1994) Reversal of terminal differentiation mediated by p107 in $Rb^{-/-}$ muscle cells. Science 264, 1467–1471.
27 Huh, M. S., Parker, M. H., Scime, A., Parks, R. and Rudnicki, M. A. (2004) Rb is required for progression through myogenic differentiation but not maintenance of terminal differentiation. J. Cell Biol. 166, 865–876.
28 Camarda, G., Siepi, F., Pajalunga, D., Bernardini, C., Rossi, R., Montecucco, A., Meccia, E. and Crescenzi, M. (2004) A pRb-independent mechanism preserves the postmitotic state in terminally differentiated skeletal muscle cells. J. Cell Biol. 167, 417–423.
29 Hayflick, L. and Moorhead, P. S. (1961) The serial cultivation of human diploid cell strains. Exp. Cell Res. 25, 585–621.
30 Herbig, U., Jobling, W. A., Chen, B. P., Chen, D. J. and Sedivy, J. M. (2004) Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53, and p21(CIP1), but not p16(INK4a). Mol. Cell 14, 501–513.
31 Serrano, M., Lin, A. W., McCurrach, M. E., Beach, D. and Lowe, S. W. (1997) Oncogenic ras provokes premature cell senescence associated with acumulation of p53 and $p16^{INK4a}$. Cell 88, 593–602.
32 Bartkova, J., Rezaei, N., Liontos, M., Karakaidos, P., Kletsas, D., Issaeva, N., Vassiliou, L. V., Kolettas, E., Niforou, K., Zoumpourlis, V. C. et al. (2006). Oncogene-induced senes-

cence is part of the tumorigenesis barrier imposed by DNA damage checkpoints. Nature 444, 633–637.
33 Di Micco, R., Fumagalli, M., Cicalese, A., Piccinin, S., Gasparini, P., Luise, C., Schurra, C., Garre, M., Nuciforo, P. G., Bensimon, A. et al. (2006). Oncogene-induced senescence is a DNA damage response triggered by DNA hyper-replication. Nature 444, 638–642.
34 Jacobs, J. J. and de Lange, T. (2005) p16INK4a as a second effector of the telomere damage pathway. Cell Cycle 4, 1364–1368.
35 Parrinello, S., Samper, E., Krtolica, A., Goldstein, J., Melov, S. and Campisi, J. (2003) Oxygen sensitivity severely limits the replicative lifespan of murine fibroblasts. Nat. Cell Biol. 5, 741–747.
36 Dulic, V., Drullinger, L. F., Lees, E., Reed, S. I. and Stein, G. H. (1993) Altered regulation of G_1 cyclins in senescent human diploid fibroblasts: accumulation of inactive cyclin E-Cdk2 and cyclin D1-Cdk2 complexes. Proc. Natl. Acad. Sci. USA 90, 11034–11038.
37 Tahara, H., Sato, E., Noda, A. and Ide, T. (1995) Increase in expression level of p21sdi1/cip1/waf1 with increasing division age in both normal and SV40-transformed human fibroblasts. Oncogene 10, 835–8340.
38 Alcorta, D., A., Xiong, Y., Phelps, D., Hannon, G., Beach, D. and Barrett, J. C. (1996) Involvement of the cyclin-dependent kinase inhibitor p16 (INK4a) in replicative senescence of normal human fibroblasts. Proc. Natl. Acad. Sci. USA 93, 13742–13747.
39 Brown, J. P., Wei, W. and Sedivy, J. M. (1997) Bypass of senescence after disruption of p21CIP1/WAF1 gene in normal diploid human fibroblasts. Science 277, 831–834.
40 Kiyono, T., Foster, S. A., Koop, J. I., McDougall, J. K., Galloway, D. A. and Klingelhutz, A. J. (1998) Both Rb/p16INK4a inactivation and telomerase activity are required to immortalize human epithelial cells. Nature 396, 84–88.
41 Bloom, J. and Pagano, M. (2003) Deregulated degradation of the cdk inhibitor p27 and malignant transformation. Semin. Cancer Biol. 13, 41–47.
42 Malek, N. P., Sundberg, H., McGrew, S., Nakayama, K., Kyriakides, T. R. and Roberts, J. M. (2001) A mouse knock-in model exposes sequential proteolytic pathways that regulate p27Kip1 in G1 and S phase. Nature 413, 323–327.
43 Grimmler, M., Wang, Y., Mund, T., Cilensek, Z., Keidel, E. M., Waddell, M. B., Jakel, H., Kullmann, M., Kriwacki, R. W. and Hengst, L. (2007). Cdk-inhibitory activity and stability of p27Kip1 are directly regulated by oncogenic tyrosine kinases. Cell 128, 269–280.
44 Michieli, P., Chedid, M., Lin, D., Pierce, J. H., Mercer, W. E. and Givol, D. (1994) Induction of WAF1/CIP1 by a p53-independent pathway. Cancer Res. 54, 3391–3395.
45 Liu, Y., Martindale, J. L., Gorospe, M. and Holbrook, N. J. (1996) Regulation of p21WAF1/CIP1 expression through mitogen-activated protein kinase signaling pathway. Cancer Res. 56, 31–35.
46 Perez-Roger, I., Kim, S. H., Griffiths, B., Sewing, A. and Land, H. (1999) Cyclins D1 and D2 mediate myc-induced proliferation via sequestration of p27(Kip1) and p21(Cip1). EMBO J. 18, 5310–5320.
47 Bouchard, C., Thieke, K., Maier, A., Saffrich, R., Hanley-Hyde, J., Ansorge, W., Reed, S., Sicinski, P., Bartek, J. and Eilers, M. (1999). Direct induction of cyclin D2 by Myc contributes to cell cycle progression and sequestration of p27. EMBO J. 18, 5321–5333.
48 Nakanishi, M., Adami, G. R., Robetorye, R. S., Noda, A., Venable, S. F., Dimitrov, D., Pereira-Smith, O. M. and Smith, J. R. (1995) Exit from G_0 and entry into the cell cycle of cells expressing $p21^{Sdi1}$ antisense RNA. Proc. Natl. Acad. Sci. USA 92, 4352–4356.
49 Endo, T. and Nadal-Ginard, B. (1989) SV40 large T antigen induces reentry of terminally differentiated myotubes into the cell cycle. In: Cellular and molecular biology of muscle development, pp. 95–104, Kedes, L. H. and Stockdale, F. E. (Eds.) Alan, R. Liss, New York.
50 Cardoso, M. C., Leonhardt, H. and Nadal-Ginard, B. (1993) Reversal of terminal differentiation and control of DNA replication: cyclin A and cdk2 specifically localize at subnuclear sites of DNA replication. Cell 74, 979–992.
51 Crescenzi, M., Soddu, S. and Tatò, F. (1995) Mitotic cycle reactivation in terminally differentiated cells by adenovirus infection. J. Cell. Physiol. 162, 26–35.
52 Kirshenbaum, L. A. and Schneider, M. D. (1995) Adenovirus E1A represses cardiac gene transcription and reactivates DNA synthesis in ventricular myocytes, via alternative pocket protein- and p300-binding domains. J. Biol. Chem. 270, 7791–7794.
53 Tiainen, M., Spitkovsky, D., Jansen-Dürr, P., Sacchi, A. and Crescenzi, M. (1996) Expression of E1A in terminally differentiated muscle cells reactivates the cell cycle and suppresses tissue-specific genes by separable mechanisms. Mol. Cell. Biol. 16, 5302–5312.
54 Tamamori-Adachi, M., Ito, H., Nobori, K., Hayashida, K., Kawauchi, J., Adachi, S., Ikeda, M. A. and Kitajima, S. (2002) Expression of cyclin D1 and CDK4 causes hypertrophic growth of cardiomyocytes in culture: a possible implication for cardiac hypertrophy. Biochem. Biophys. Res. Commun. 296, 274–280.
55 Sacco, A., Pajalunga, D., Latella, L., Siepi, F., Rufini, A. and Crescenzi, M. (2002) Cell cycle reactivation in skeletal muscle and other terminally differentiated cells. In: Reactivation of the Cell Cycle in Terminally Differentiated Cells, pp. 45–61, Crescenzi, M. (Ed.) Kluwer Academic, New York.
56 Latella, L., Sacchi, A. and Crescenzi, M. (2000) Long-term fate of terminally differentiated skeletal muscle cells following E1A-initiated cell cycle reactivation. Cell Death Differ. 7, 145–154.
57 Kranenburg, O., van der Eb, A. J. and Zantema, A. (1996) Cyclin D1 is an essential mediator of apoptotic neuronal cell death. EMBO J. 15, 46–54.
58 Becker, E. B. and Bonni, A. (2005) Beyond proliferation – cell cycle control of neuronal survival and differentiation in the developing mammalian brain. Semin. Cell Dev. Biol. 16, 439–448.
59 Krantic, S., Mechawar, N., Reix, S. and Quirion, R. (2005) Molecular basis of programmed cell death involved in neurodegeneration. Trends Neurosci. 28, 670–676.
60 Gire, V. and Wynford-Thomas, D. (1998) Reinitiation of DNA synthesis and cell division in senescent human fibroblasts by microinjection of anti-p53 antibodies. Mol. Cell. Biol. 18, 1611–1621.
61 Mahmud, N., Devine, S., M., Weller, K. P., Parmar, S., Sturgeon, C., Nelson, M. C., Hewett, T. and Hoffman, R. (2001) The relative quiescence of hematopoietic stem cells in nonhuman primates. Blood 97, 3061–3068.
62 Yuan, Y., Shen, H., Franklin, D. S., Scadden, D. T. and Cheng, T. (2004) In vivo self-renewing divisions of haematopoietic stem cells are increased in the absence of the early G1-phase inhibitor, p18INK4C. Nat. Cell Biol. 6, 436–442.
63 Cheng, T. (2004) Cell cycle inhibitors in normal and tumor stem cells. Oncogene 23, 7256–7266.
64 Janzen, V., Forkert, R., Fleming, H. E., Saito, Y., Waring, M. T. Dombkowski, D. M., Cheng, T., DePinho, R. A., Sharpless, N. E. and Scadden, D. T. (2006). Stem-cell ageing modified by the cyclin- dependent kinase inhibitor p16INK4a. Nature 443, 421–426.
65 Stier, S., Cheng, T., Forkert, R., Lutz, C., Dombkowski, D. M., Zhang, J. L. and Scadden, D. T. (2003) Ex vivo targeting of p21Cip1/Waf1 permits relative expansion of human hematopoietic stem cells. Blood 102, 1260–1266.
66 Agah, R., Kirshenbaum, L. A., Abdellatif, M., Truong, L. D., Chakraborty, S., Michael, L. H. and Schneider, M. D. (1997) Adenoviral delivery of E2F-1 directs cell cycle reentry and p53-independent apoptosis in postmitotic adult myocardium in vivo. J. Clin. Invest. 100, 2722–2728.

67 Tamamori-Adachi, M., Ito, H., Sumrejkanchanakij, P., Adachi, S., Hiroe, M., Shimizu, M., Kawauchi, J., Sunamori, M., Marumo, F., Kitajima, S. et al. (2003). Critical Role of Cyclin D1 Nuclear Import in Cardiomyocyte Proliferation. Circ. Res. 92, E12–E19.

68 Brockes, J. P. and Kumar, A. (2002) Plasticity and reprogramming of differentiated cells in amphibian regeneration. Nat. Rev. Mol. Cell. Biol. 3, 566–574.