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From G0 to S Phase: A View of the Roles Played by the Retinoblastoma (Rb) Family Members in the Rb-E2F Pathway

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Abstract Tumor suppressor pRb/p105, pRb/p107, and pRb2/p130 genes belong to the retinoblastoma (Rb) gene family. The members of the Rb gene family and the transcription factor E2F play an essential role in regulating cell cycle and, consequently, cell proliferation. This mini-review describes the mechanisms by which Rb family members and E2F regulate cell cycle progression. J. Cell. Biochem. 102: 1400–1404, 2007. © 2007 Wiley-Liss, Inc.

Key words: tumor suppressor genes; Rb family; pRb2/p130; E2F; cell cycle; gene expression

In the mid-1990s, Dr. Weinberg proposed a model to describe how the products of Rb family regulates cell cycle progression in conjunction with the transcription factor E2F (Fig. 1) [Weinberg, 1995]. The retinoblastoma (Rb) gene family comprises the tumor suppressor pRb/p105 gene and related factors pRb/p107 and pRb2/p130 [Giordano et al., 2007]. These three factors share similar structures and biological functions. All of them are composed of two subdomains simply termed "A" and "B", which are separated by a highly conserved spacer region [Giordano et al., 2007]. The presence of the spacer region allows for the assembly of the two subdomains into a pocket-like structure. For this reason, the three members of the Rb family are known as pocket proteins [Giordano et al., 2007].

The transcription factor E2F binds to the promoter region of many genes, such as DNA polymerase subunits, cyclin A and cyclin E, which are required for S phase entry. Following the binding of E2F to the promoter region, the expression of genes that are necessary for S phase entry takes place and cell cycle progresses from G1 to S phase.

The gene regulation of E2F follows a complex mechanism, which allows for cell cycle to enter S phase and initiate DNA replication only under favorable conditions. For instance, if genomic DNA sustained damages and need repair, cell cycle will not progress to S phase. This may prevent the accumulation of genetic defects within the cellular genome. In this respect, the regulators of E2F activity are the members of Rb family, which are able to bind to E2F and prevent it from interacting with the promoter region of those genes that are critical for S phase entry. While Rb family members mediate E2F activity, a number of other factors regulate the function of Rb family members. For instance, cdk–cyclin complex has the ability to hyperphosphorylate the Rb family members. Following hyperphosphorylation, Rb family members can no longer bind to E2F, which, in turn, is now

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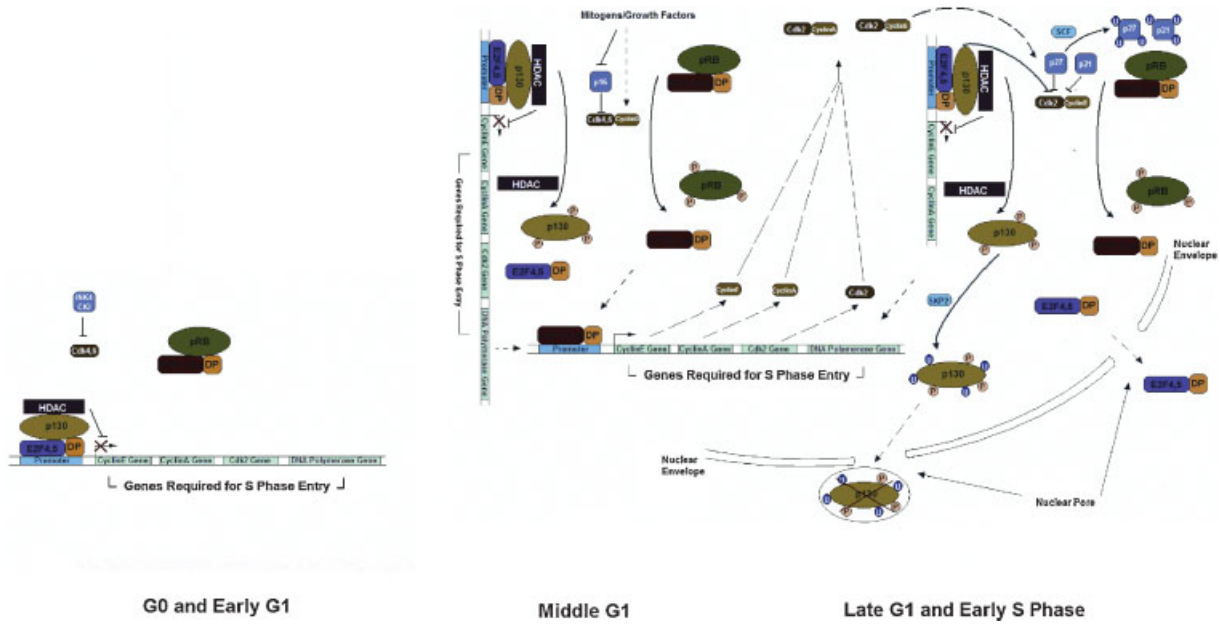

Fig. 1. Possible interpretation of the Rb-E2F pathway in the regulation of cell cycle progression.

able to interact with the promoter regions of those cellular factors that may initiate S phase entry.

Cells have means to regulate cell cycle at different levels by targeting a variety of molecules. Just as Rb family members can be regulated by cdk/cyclin complexes, the cdk/cyclin complexes can be targeted by cdk kinase inhibitors (CKI), which include two categories: the INK4 and Cip/Kip families [Giordano et al., 2007]. Thus, CKI-mediated inhibition of cdk/cyclin kinase activity does not allow for hyperphosphorylation of Rb family members, which can bind to E2F and, consequently, prevent it from initiating S phase entry. At this stage, cells are arrested in G1 phase.

As anticipated, the aforementioned Rb-E2F pathway model was proposed in the mid-1990s and was based on the knowledge available at that time. In that model, firstly, the E2Fs were considered always as activators of those genes that are required for S phase entry; secondly, the Rb family members (pRb/p105, pRb/p107, and pRb2/p130), were considered to fulfill their cell cycle regulating functions by preventing E2Fs from binding to certain promoters; thirdly, the Rb-E2F pathway was retained to take place within an indissoluble period and without a particular distinction of the roles played by the Rb family members and by E2Fs. Since then, more discoveries in cell cycle regulation and cancer biology were made. As a result, the mechanism by which the Rb family members regulate cell cycle became much more complex than previously thought. Additional E2F family members were found. By 2005, at least a dozen members, including isoforms, were described in the E2F family [Cam and Dynlacht, 2003; Stevens and La Thangue, 2003; Blais and Dynlacht, 2004; Frolov and Dyson, 2004; Dimova and Dyson, 2005]. Intriguingly, not all the E2F family members were activators of gene expression. The E2F family members can be divided into two groups: transcriptional activators (E2F1, 2, and 3a) and transcriptional repressors (E2F3b, 4, 5, 6, 7a, and 7b) [Cam and Dynlacht, 2003; Blais and Dynlacht, 2004; Frolov and Dyson, 2004; Dimova and Dyson, 2005]. The transcriptional activators interact with pRb/p105, whereas the repressors E2F4 and E2F5 interact with pRb2/p130 and pRb/p107 [Cam and Dynlacht, 2003; Cobrinik, 2005; Dimova and Dyson, 2005]. Such findings clearly outline that the biological functions of pRb/p105, pRb/p107, and pRb2/p130 cannot be considered fully redundant [Giordano et al., 2007].

In quiescent cells (G0) and cells in early G1 phase, pRb2/p130 is expressed at a high levels [Cobrinik et al., 1993; Smith et al., 1996] and interacts mainly with E2F4 and to a less extent with E2F5 [Hijmans et al., 1995; Vairo et al., 1995]. This complex first binds to the promoter regions of the genes required for S phase entry.

However, this binding will not initiate the transcription of the genes required for S phase entry. On the contrary, it recruits a number of chromatin remodeling factors, such as SWI/SNF [Gunawardena et al., 2004] and HDAC (Histone Deacetylase) [Ferreira et al., 1998; Stiegler et al., 1998; Iavarone and Massague, 1999]. These chromatin-remodeling factors cause histone deacetylation with consequent the chromatin condensation, which is not permissive for transcriptional activity. Naturally, this represses the expression of those genes that are required for S phase entry. In this regard, pRb2/p130 plays a major role, as it is predominantly expressed over pRb/p107 in G0 and early G1 phases [Beijersbergen et al., 1994; Ginsberg et al., 1994; Kiess et al., 1995; Shin et al., 1995; Smith et al., 1996; Raschella et al., 1997; Ferreira et al., 1998; Stiegler et al., 1998; Iavarone and Massague, 1999].

Besides the transcriptional repression mediated by pRb2/p130 and pRb/p107 in G0 and early G1 cell cycle phases, there is another mechanism to suppress the expression of the genes required S phase entry. This is provided by pRb/p105. In contrast to pRb2/p130 and pRb/p107, pRb/p105 is expressed at moderate and steady levels throughout the cell cycle [Buchkovich et al., 1989; Chen et al., 1989; Decaprio et al., 1989; Mihara et al., 1989]. In G0 and early G1 phases, pRb/p105 expression levels are lower than those of pRb2/p130, so, during this period, pRb2/p130 plays a more predominant role than pRb/p105 does. When pRb2/p130 and pRb/p107 interact with E2F4 and E2F5 to bind various promoter regions, pRb/p105 associates with the E2F activators and prevents them from stimulating transcriptional activity of the promoters [Beijersbergen et al., 1994; Ginsberg et al., 1994; Hijmans et al., 1995; Vairo et al., 1995]. So, in G0 and early G1 phases, Rb family members cooperate together to prevent the expression of those genes that are required for S phase entry.

In middle G1 phase, pRb2/p130 still binds to the repressor E2Fs and, this complex, suppresses the initiating of the set of genes for S phase entry, whereas pRb/p105 still binds to activator E2Fs and thus prevents them from binding to the promoter regions. However, the expression of pRb2/p130 in middle G1 phase begins to decrease, while the expression of pRb/p105 tends to remain constant. This indicates a less important role of pRb2/p130 in middle G1 phase than in G0 and early G1 phases. Also the cdk4,6–cyclinD (cyclinD1, D2, D3) complex may hyperphosphorylate Rb family members in G1 phase. However, when cell division is favorable, the kinase activity of cdk4,6–cyclinD complex is suppressed by INK4 CKIs [Roussel, 1999; Vidal and Koff, 2000; Lowe and Sherr, 2003].

The third stage is the late G1 phase. In late G1, cdk2/cyclinE phosphorylates pRb/p105, pRb/p107, and pRb2/p130. As a result of the hyperphosphorylation, on one hand, pRb/p105 no longer binds to the activator E2Fs and, on the other hand, pRb2/p130 and pRb/p107 no longer bind to E2F4/5. The disassembly of the complex formed by pRb2/p130 and E2F4/5 results in the release of the chromatin remodeling proteins.

In contrast to the activator E2Fs, E2F4 and E2F5 lack NLS nuclear localization signals (NLS) [Muller et al., 1997; Chestukhin et al., 2002]. So, without the association with pRb2/p130 and pRb/p107, E2F4 and E2F5 can no longer access the cell nucleus [Magae et al., 1996; Puri et al., 1998]. Of course, this event reduces dramatically the possibility of E2F4 and E2F5 to form repressor complexes with pRb2/p130. In addition to this mechanism, there is a second mechanism that completely eliminates the possibility of forming pRb2/p130–E2F4/5 complexes. This second mechanism is contributed by Skp2, which is the ubiquitin ligase of pRb2/p130 and belongs to the SCF (Skp1, Cullin, F-box protein) family. Skp2 recognizes hyperphosphorylated pRb2/p130, ubiquitin-ligases it and, therefore, causes the quick removal of pRb2/p130 by proteosomes [Tedesco et al., 2002; Bhattacharya et al., 2003; Kalejta and Shenk, 2003]. At this point, the promoter regions become vacant for the activator E2Fs, which, in turn, express the set of gene to initiate the S phase entry.

Paradoxically, to date, it is still not clear whether or not pRb2/p130-repressor E2F complexes and activators E2Fs target the same promoter regions [Takahashi et al., 2000; Wells et al., 2000]. Obviously, this is very debated issue.

While cells are in late G1 phase, CKI, as well as p27, may inhibit the functions of cdk2. In 1997, our laboratory reported that also the spacer region of pRb2/p130 could inhibit the kinase activity of cdk2 [DeLuca et al., 1997]. So, in late G1 cell cycle phase, CKI and pRb2/p130 have the ability to inhibit the kinase activity of cdk2/cyclin E complex. The inhibition

of cdk2/cyclin E kinase activity prevents Rb family members from being hyperphosphorylated. When the conditions are favorable for cell division, cdk2 predominates over p27 and pRb2/p130. At this juncture, p27 is degraded by SCF, whereas the inhibition exerted by cdk2/cyclin E on Rb family members will increase the production of cyclin E. These events allow for cell cycle progression into S phase. The biological balance among p27, pRb2/p130, and cdk2 is extremely important for cell cycle control. As for transformed cells, the situation is different. For instance, in some cancer cells the CKI are constitutively inactivated. Mutations in myc were found in many non-small cell lung cancer (NSCLC) cells. This results in increased SCF activity, which neutralizes CKI functions. The reconstitution of CKI activity by therapeutics can cause suppression of tumor growth. In this respect, a pRb2/p130-derived peptide, termed Spa310, was able to inhibit the cdk2 kinase activity in cancer cells [Bagella et al., 2007; Giordano et al., 2007]. Spa310 is based on the spacer domain of pRb2/p130. This region is not substrate for hyperphosphorylaton by cdk2/cyclin A and cdk2/cyclin E [DeLuca et al., 1997; Classon and Harlow, 2002]. On these grounds, Skp2 should not be able to recognize and, consequently, ubiquitin-ligase Spa310. Another Rb family member that has the ability to inhibit cdk2 activity is pRb/p107 [Woo et al., 1997]. Interestingly, pRb/p107 and pRb2/p130 inhibit cdk2 activity via different mechanisms.

CONCLUSION

The latest interpretations of the Rb-E2F pathway took under consideration the differential Rb family expression patters throughout the cell cycle phases, further characterized the E2F family and viewed cell cycle progression as a series of dynamically intertwined events. As already discussed, these events can be summarized into three main stages. The important discoveries achieved over the last decade in the field of cell cycle regulation is leading to a better understanding of malignant cell transformation and to the development of novel therapeutics against cancer.

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