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Review

PP2A holoenzymes negatively and positively regulate cell cycle progression by dephosphorylating pocket proteins and multiple CDK substrates

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ABSTRACT

Cell cycle progression is negatively regulated by the retinoblastoma family of pocket proteins and CDK inhibitors (CKIs). In contrast, CDKs promote progression through multiple phases of the cell cycle. One prominent way by which CDKs promote cell cycle progression is by inactivation of pocket proteins via hyperphosphorylation. Reactivation of pocket proteins to halt cell cycle progression requires dephosphorylation of multiple CDK-phosphorylated sites and is accomplished by PP2A and PP1 serine/threonine protein phosphatases. The same phosphatases are also implicated in dephosphorylation of multiple CDK substrates as cells exit mitosis and reenter the G1 phase of the cell cycle. This review is primarily focused on the role of PP2A and PP1 in the activation of pocket proteins during the cell cycle and in response to signaling cues that trigger cell cycle exit. Other functions of PP2A during the cell cycle will be discussed in brief, as comprehensive reviews on this topic have been published recently (De Wulf et al., 2009; Wurzenberger and Gerlich, 2011).

Contents

1. Role of pocket proteins in negatively regulating passage through the restriction point and their inactivation by mitogenically activated CDKs

The cell cycle in metazoans is negatively regulated by members of the pocket protein family (reviewed in Sotillo and Graña, 2010; Wirt and Sage, 2010). In mammalian cells, pocket proteins consist of the tumor suppressor pRB and two closely related paralogs, p107 and p130. Pocket proteins are active in their hypophosphorylated state, which allows binding to a variety of proteins modulating their function. The primary target of pocket proteins in restraining cell cycle progression is the E2F family of transcription factors. Association of

Abbreviations: CDK, cyclin dependent kinase; CKI, cyclin dependent kinase inhibitor; PP1, protein phosphatase 1; PP2A, protein phosphatase 2A; RB, retinoblastoma; EGF, epidermal growth factor; HEAT, Huntington, elongation factor 3, PR65/A, TOR; GANP, germinal center-associated nuclear protein; MCM3, mini-chromosome maintenance protein 3; SG2NA, S, G2 phase nuclear antigen; HUVEC, human umbilical vein endothelial cells; CDC6, cell division cycle 6; FVP, flavopiridol; FGF1, fibroblast growth factor 1; RNAi, RNA interference; Gwl, Greatwall kinase; BMP, bone morphogenic protein; ATRA, all-*trans*-retinoic acid; TGFβ, transforming growth factor β.

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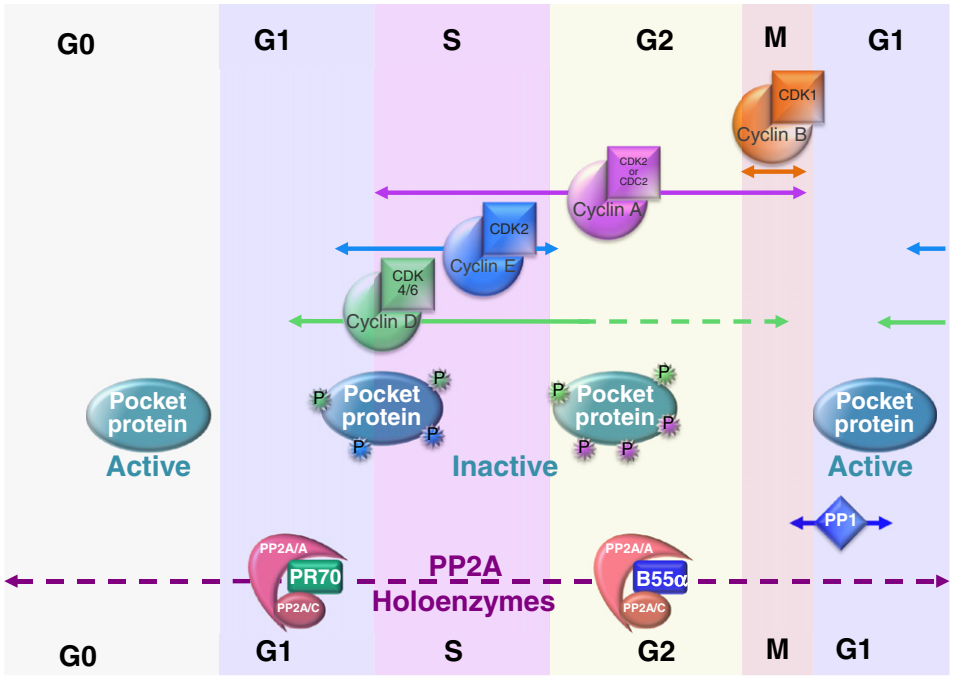

Fig. 1. PP2A heterotrimeric holoenzymes modulate the phosphorylation status of the three pocket proteins throughout the cell cycle, whereas PP1 resets pRB phosphorylation in mitosis (based on Garriga et al., 2004 and Jayadeva et al., 2010). An equilibrium between inducible Cyclin/CDK complexes that are activated as cells progress through the cell cycle and PP2A determines the precise phosphorylation state of each protein during the cell cycle and in quiescent cells. In G0 or early G1, CDK activity is low and pocket proteins are hypophosphorylated (active) as a consequence of PP2A activity. As CDKs become activated (indicated by arrows), pocket proteins become hyperphosphorylated (color coded P(s) according to responsible CDK). In late mitosis, PP1 becomes activated towards pRB leading to its hypophosphorylation (see text). B55α and PR70 trimeric PP2A holoenzymes are known to be involved in the dephosphorylation of pocket proteins and at least B55α PP2A holoenzymes appear to function throughout the cell cycle. Some of these holoenzymes also appear to be induced by specific signals. For instance, oxidative stress induces PR70 association with pRB and its dephosphorylation (see text for details). Recent work has also shown that B55/PP2A activity is low in mitosis to allow for phosphorylation of CDK1 substrates and becomes higher as cells exit mitosis ensuing dephosphorylation of CDK1 substrates and possibly p130 and/or p107.

hypophosphorylated pocket proteins to heterodimers of E2F and DP (E2F dimerization partner) proteins forms repressor complexes that bind E2F-elements on the promoters of E2F-dependent genes required for cell cycle progression causing their silencing. Upon mitogenic stimulation, pocket proteins are phosphorylated by G1 and G1/S Cyclin/CDK complexes. p107 is primarily phosphorylated by D-type Cyclin/CDK complexes, while pRB and p130 are phosphorylated by the coordinated action of D-type Cyclin/CDK and Cyclin E/CDK2 complexes (reviewed in Sotillo and Graña, 2010; Wirt and Sage, 2010). Pocket protein hyperphosphorylation disrupts their interactions with E2F/DP complexes, eliminating repressor complexes coinciding with the expression of E2F dependent genes. Among these genes are those encoding activator E2Fs (E2F1–3), which are recruited to E2F-dependent promoters in the absence of pocket proteins and are associated with their transactivation. Activation of the E2F transcription program in late G1 corresponds with transition through the restriction point (Sotillo and Graña, 2010). Pocket protein inactivation is reversed by protein phosphatase 2A (PP2A), a serine/threonine phosphatase that opposes CDK-dependent phosphorylation of pocket proteins throughout the cell cycle (Garriga et al., 2004; reviewed in Graña, 2008) (Fig. 1).

2. Reactivation of pocket proteins by PP1 and PP2A in mitosis

The three pocket proteins remain hyperphosphorylated and thus inactive, through S, G2 and most of M phase and are concomitantly dephosphorylated as cells exit mitosis and reenter the next G1 phase (Calbó et al., 2002). Using a nocodazole block and release method to synchronize CV-1P cells, it was found that pRb dephosphorylation begins in anaphase and continues until completion in the next G1 phase of the cell cycle (Ludlow et al., 1993). Treatment of late-mitotic phase cell extracts with okadaic acid or protein phosphatase inhibitors 1 and 2 suggested that the phosphatase responsible for pRb dephosphorylation was PP1, as the concentrations needed to inhibit dephosphorylation were much higher than the sub-nanomolar concentrations needed to inhibit PP2A *in vitro* (Ludlow et al., 1993). Independently, a yeast two-hybrid screen identified an isoform of PP1α, designated PP1α2, as a pRB binding protein. This study also showed that PP1α2 associated with pRB from mitosis to early G1 coinciding with the period in which pRB is hypophosphorylated (Durfee et al., 1993). However, it has been proposed that PP1 is not a major regulator of the Rbf1/E2F1 pathway in *Drosophila*, based on PP1 dispensability for E2f1 inhibition by Rbf1 and G1 arrest in the embryonic epidermis and for the expression of E2f1 target genes in S phase in the embryo midgut and larval salivary gland; processes that require Rbf1 (Swanhart et al., 2007). This dispensability might be due to the presence of a different phosphatase or that PP1 cooperates with a redundant phosphatase to activate pRB. An obvious possibility given our results (Jayadeva et al., 2010) and those of the Martelli lab (Magenta et al., 2008), is PP2A. In addition, despite the apparent coordinated dephosphorylation of pocket proteins from mitosis to G1 (Calbó et al., 2002), others have reported that pRB, but not p130 and p107, binds strongly to PP1 (Dunaief et al., 2002). Of note, a peptide present in the C-terminus of pRB has been co-crystallized with PP1, identifying a docking site for PP1 that overlaps with a RxL Cyclin/CDK docking site also at the C-terminus of pRB (Hirschi et al., 2010). It has been proposed that PP1 competition with Cyclin/CDK complexes is sufficient to activate pRB independently of its phosphatase activity. While RxL sites that mediate binding to Cyclin/CDKs are present in the spacer domain of p130 and p107, these binding motifs failed to bind PP1 (Hirschi et al., 2010), further reinforcing the hypothesis that dephosphorylation of