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Downregulation of protein phosphatase 2A activity in HeLa cells at the G2-mitosis transition and unscheduled reactivation induced by 12-O-tetradecanoyl phorbol-13-acetate (TPA)

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Abstract

In the cell cycle the transition from G2 phase to cell division (M) is strictly controlled by protein phosphorylation–dephosphorylation reactions effected by several protein kinases and phosphatases. Although much indirect and direct evidence point to a key role of protein phosphatase 2A (PP2A) at the G2/M transition, the control of the enzyme activity prior to and after the transition are not fully clarified. Using synchronized HeLa cells we determined the PP2A activity (i.e. the increment sensitive to inhibition by 2 nM okadaic acid) in immunoprecipitates obtained with antibodies raised against a conserved peptide sequence (residues 169–182, $Ab^{169/182}$) of the PP2A catalytic subunit (PP2A C). Two different substrates were offered: the phospho-peptide KR(p)TIRR and histone H1 phosphorylated by means of the cyclin-dependent protein kinase $p34^{cdc2}$. The results indicate that in HeLa cells the specific activity of PP2A towards both substrates goes through a minimum in late G2 phase and stays low until metaphase. Treatment of G2 cells with TPA (10^{-7} M) caused a reactivation of the downregulated PP2A activity within 20 min, i.e. the same time frame within which TPA was shown earlier to block HeLa cells at the transition from G2 to mitosis [Kinzel et al., 1988. Cancer Res. 48, 1759–1762]. Activation of PP2A was also induced by TPA in mitotic cells. The low activity of PP2A in mitotic cells was accompanied by a strong reaction of mitotic PP2A C with anti-P-Tyr antibodies in Western blots, which was reversed by treatment of mitotic cells with TPA. The results suggest that the activity of cellular PP2A requires downregulation for the transition from G2 phase to mitosis. Unscheduled reactivation of PP2A induced by TPA in late G2 phase appears to inhibit the progress into mitosis.

Keywords: PP2A; G2/mitosis; Downregulation; Unscheduled reactivation; Phorbol ester; TPA; G2 inhibition

Abbreviations: AT procedure, amethopterin/thymidine synchronization procedure; MPF, M-phase promoting factor; OA, okadaic acid; PP1, protein phosphatase 1; PP2A, protein phosphatase 2A; PP2A C, catalytic subunit of PP2A; TPA, 12-O-tetradecanoyl phorbol-13-acetate

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Introduction

The profound transformation of a cell that occurs as it traverses from interphase to mitosis is regulated at the molecular level to a great extent by a precisely timed cascade of protein phosphorylation/dephosphorylation reactions involving a number of conserved protein kinases and protein phosphatases. Well established is the cyclin-dependent protein kinase $p34^{cdc2}$ alias cdc2 or cdk1 in complex with cyclin B named M-phase promoting factor (MPF) which becomes activated at the G2/M transition (for review, see Dunphy, 1994). Checkpoints in the cell cycle serve to control the completion of individual steps in the cascade prior to progression to the next and thus guarantee the cellular integrity, e.g. by allowing repair processes to take place. The delay of the transition from G2 phase to mitosis caused by a number of chemical and physical influences is a perfect example.

Protein phosphatase 2A (PP2A) represents a highly regulated family of abundant phosphoserine/phosphothreonine phosphatases in eukaryotic cells (Cohen, 1989, 1991) which is indispensable in numerous cellular processes including the cell division cycle (for review see Mayer-Jaekel and Hemmings, 1994). The enzyme activity is regulated in several ways including different regulatory subunits, post-translational modifications, second messengers and inhibitory proteins and it may vary depending on individual substrates and intracellular location (for review, see Janssens and Goris, 2001). The catalytic subunit C (existing in two variants α and β), and a constant regulatory subunit A (or PR65; also existing in two isoforms α and β) represent core dimers to which one of several variable B subunits may be bound forming heterotrimers and giving rise to a number of PP2A isozymes. The expression of PP2A subunits has been found to be constant throughout the cell cycle (Ruediger et al., 1991).

A role of PP2A and the regulation of its activity at the transition from G2-phase to cell division is indicated by the following observations. An extract prepared from frog oocytes which inhibited the entry into meiosis by preventing the activation of MPF (Cyert and Kirschner, 1988) was shown to contain enzymatically active PP2A as the inhibitory principle (Lee et al., 1991, 1994), thus suggesting that an active state of PP2A may counteract entry into cell division. In contrast, the specific inhibition of PP2A-type enzymes in various cells with low concentrations of okadaic acid (OA) has been shown to induce a premature entry into both meiosis and mitosis, suggesting that a less active or inactive state of PP2A may facilitate the transition from G2 phase to cell division (Goris et al., 1989; Felix et al., 1990; Yamashita et al., 1990).

Ruediger et al. (1991) did not observe changes in the activity of PP2A during the cell cycle. Sontag et al. (1995) measured the activity of microtubuli-associated PP2A in highly synchronized cells and found that the activity was diminished if not inhibited during G2 and in mitosis. Turowski et al. (1995) showed that the methylation state of PP2A C is altered during the cell cycle. The published evidence for the involvement of PP2A at a number of biochemical steps during the preparation of the cell for division has been comprehensively reviewed by Janssens and Goris (2001). PP2A is necessary to keep the cdk1/cyclin B complex in its inactive precursor form by regulating cdk-activating kinase (CAK) and Wee1 kinase activity. In addition, it is thought that PP2A operates upstream of cdc25-C (Clarke et al., 1993), the protein phosphatase which is involved in the final activation of cdk1 at the entry into cell division. A PP2A-type enzyme seems to be involved in keeping cdc25-C inactive prior to mitosis by dephosphorylation of activating sites (Clarke et al., 1993). In vertebrates cdc25-C is activated by phosphorylation at these serine and threonine residues by polo-like kinase (Karaiskou et al., 1998) and subsequently by cdk1 itself causing full activation of cdc25-C within a feedback loop responsible for maximal activation of cdk1 for mitosis (Hoffmann et al., 1993). Incubation of enzymatically active cdc25-C isolated from mitotic cells with PP2A in vitro has been shown to cause an inactivation of the former (Clarke et al., 1993) thus raising the possibility that PP2A operates upstream of cdc25-C. Moreover, PP2A represents a major enzyme that dephosphorylates other physiological products of cyclin-dependent protein kinases (Agostinis et al., 1992; Ferrigno et al., 1993; Mayer-Jaekel et al., 1994). Little is known about the actual regulation of PP2A activity prior to and during mitosis up to metaphase. The study of PP2A in mammalian cells has been hindered so far by the failure to stably overexpress functional PP2A by standard gene transfer techniques (Green et al., 1987; Wadzinski et al., 1992) due to an autoregulatory control exerted at the translational level (Baharians and Schonthal, 1998).

To analyze PP2A activity at the G2/M transition and in mitosis we used highly synchronous HeLa cells and determined the phospho-peptide phosphatase activity ex vivo, i.e. in immunoprecipitates obtained with specific polyclonal anti-peptide antibodies raised against the PP2A catalytic subunit and determined the enzyme activity which was sensitive to a low concentration of OA. This fraction is considered to reflect PP2A. As substrates we used a phospho-peptide and phosphohistone H1 previously phosphorylated by means of cdk1 (alias $p34^{cdc2}$).

The elucidation of molecular events in the course of the cell cycle is facilitated by analysis of checkpoints which may also become obvious through the inhibition induced by various chemical and physical means. The well-known inhibitory influence of X-irradiation on the