COMMENTARY

Mitotic phosphorylation: breaking the balance of power by a tactical retreat

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Profound changes in the phosphorylation state of many proteins occur during mitosis. It is well established that many of these mitotic phosphorylations are carried out by archetypal mitotic kinases that are activated only during mitosis, shifting the equilibrium of kinases and phosphatases towards phosphorylation. However, many studies have also detailed the phosphorylation of proteins at mitosis by kinases that are constitutively active throughout the cell cycle. In most cases, it is uncertain how kinases and phosphatases that appear to be constitutively active can induce phosphorylations specifically at mitosis. In this issue of the *Biochemical Journal*, Escargueil and Larsen provide evidence of an interesting alternative mechanism to attain specific mitotic

phosphorylation. A mitosis-specific phosphorylation site in DNA topoisomerase II α , which is recognized by the MPM-2 antibody, is phosphorylated by protein kinase CK2. The authors found that phosphorylation of this site is suppressed during interphase due to competing dephosphorylation by protein phosphatase 2A. Interestingly, protein phosphatase 2A is excluded from the nucleus during early mitosis, allowing CK2 to phosphorylate topoisomerase II α . It is possible that similar mechanisms are used to regulate the phosphorylation of other proteins.

Key words: CK2, DNA topoisomerase II, mitosis, MPM-2, phosphorylation, protein phosphatase 2A (PP2A).

The entry into mitosis is accompanied by a dramatic increase in the level of protein phosphorylation. Historically, this realization played a pivotal role in precipitating the discovery that MPF (M phase-promoting factor) is composed of a protein kinase (CDC2) and its regulatory subunit (cyclin; reviewed in [1]). Many studies have detailed the direct involvement of phosphorylation events in direct involvement in mitotic processes like nuclear envelope breakdown and chromosome condensation. Apart from CDC2, other protein kinases such as PLK1 are also activated during mitosis. In addition, several constitutively active kinases such as protein kinase CK2 and protein kinase C are believed to be active in both mitosis and interphase. It is the collective efforts of these protein kinases that produce the repertoire of mitotic phosphorylations. Understanding how different mitotic phosphorylations are regulated is critical for comprehending the complete picture of mitosis.

An invaluable tool for studying mitosis has been a monoclonal antibody called MPM-2. Generated long before the advent of phosphorylation site-specific antibodies, MPM-2 was originally raised against lysates of mitotic HeLa cells [2]. MPM-2 recognizes phosphopeptides present in mitotic proteins from a wide variety of species, and has been widely used as a marker for the mitotic state. Although the precise spectrum of epitopes that MPM-2 recognizes is not fully defined, it is clear that a major type of MPM-2 recognition sequences contains phosphorylated Thr/Ser residues followed by a Pro, which are phosphorylation sites for CDC2. However, there are examples of epitopes recognized by MPM-2 that do not fit this consensus.

Whether or not a protein is phosphorylated at a certain point in the cell cycle is a result of competition between the responsible kinase(s) and phosphatase(s). Our most accustomed notion is that an increase of level or activity of the protein kinase shifts the balance towards phosphorylation. This mechanism is important for the major mitotic protein kinase CDC2, which is inactive during interphase and activated during mitosis by a combination of cyclin binding and phosphorylation. In brief, cyclin B is

synthesized during the G₂ phase and forms an inactive complex with CDC2. The complex is held in an inactive state by virtue of the phosphorylation of two inhibitory sites in CDC2. The stockpile of cyclin B–CDC2 complex is then activated by the phosphatase CDC25, driving the cell into mitosis. Mitosis is terminated when cyclin B is degraded by the anaphase-promoting complex/cyclosome complex (reviewed in [3]).

The paradigm provided by CDC2 is likely to be relevant for protein kinases that are regulated robustly during the cell cycle. However, it is unlikely to account for the mitosis-specific phosphorylations delivered by more constitutively active or abundant protein kinases. Conceptually, another way to induce mitotic phosphorylation is by a reduction in the level or activity of the protein phosphatase during mitosis. Finally, it is also conceivable that a substrate can become available for the kinase (or unavailable for the phosphatase) specifically during mitosis.

In this issue of the *Biochemical Journal*, Escargueil and Larsen [4] provide evidence of an alternative mechanism to attain specific phosphorylation during mitosis. Specifically, these authors propose that an MPM-2 site in TopoII α (topoisomerase II α), which was shown previously to be phosphorylated by CK2, is dephosphorylated by PP2A (protein phosphatase 2A). This occurs throughout interphase, thus suppressing the formation of the MPM-2 site in TopoII α . Interestingly, PP2A is translocated from the nucleus during early mitosis. As CK2 continues to associate with TopoII α in the nucleus unabated, this allows the phosphorylation of TopoII α on the MPM-2 site during mitosis.

DNA topoisomerase II is an essential enzyme that catalyses the passage of one double-stranded DNA molecule through another by creating a transient double-strand break, passing the intact double strand through this break before ligating the break. The α isoform is expressed predominantly during mitosis, tightly associated with condensed chromatin during metaphase, and disappears during G₁ phase. Indeed, TopoII α has been implicated for proper chromatin condensation during prophase, as well as

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for the disentanglement and segregation of sister chromatids during anaphase. The role of TopoII α in chromatin condensation, however, has been challenged by more recent data because genetic knockdown or knockout of TopoII α causes only minimal perturbation in chromosome condensation (reviewed in [5]).

TopoII α undergoes a myriad of phosphorylations during mitosis, including those catalysed by CDC2 and protein kinase C [6,7]. TopoII α is in fact the major mitotic chromosomal protein recognized by MPM-2 [8]. Intriguingly, the protein kinase that is responsible in generating the MPM-2 site in human TopoII α (Ser¹⁴⁶⁹) is CK2, a kinase that is believed to be constitutively active during much of the cell cycle [9]. Moreover, the putative MPM-2 site does not contain a Ser/Thr-Pro motif, but lies within a cluster of serine and aspartic acid residues (the first Ser in the sequence Ser-Thr-Ser-Asp-Asp-Ser-Asp-Ser).

It is well established that CK2 forms a complex with and phosphorylates TopoII in multiple organisms, including yeast, *Drosophila* and mammals (reviewed in [10]). Several sites in the highly diverse C-terminal region of human TopoII α , including Thr¹³⁴², Ser¹³⁷⁷ and Ser¹⁵²⁵, are phosphorylated by CK2 during interphase. Phosphorylation of TopoII by CK2 increases the catalytic activity of TopoII in yeast and *Drosophila*. However, whether CK2 affects the activity of TopoII α in mammalian cells is more contentious: conclusions that phosphorylation by CK2 can activate [11,12] or has no effect [13,14] on the activity of TopoII α have both been reported in the literature. There is also no evidence that phosphorylation of the Ser¹⁴⁶⁹ MPM-2 site alters the activity of TopoII α , further underscoring the uncertainty of whether CK2 regulates TopoII α in mammalian cells [9].

Although the function of MPM-2 phosphorylation in TopoII α is not known at the present, the study by Escargueil and Larsen [4] does introduce an interesting concept of how mitotic phosphorylation can be controlled. The authors found that TopoII α is co-localized with both CK2 and PP2A during interphase (at least partially). The authors argue further that PP2A is able to dephosphorylate the Ser¹⁴⁶⁹ MPM-2 site during interphase (but is less effective on other CK2 phosphorylation sites). During early mitosis, PP2A is translocated from the nucleus to the cytoplasm, giving CK2 an upper hand in phosphorylating Ser¹⁴⁶⁹. This model satisfyingly solves the problem of how a protein can be phosphorylated during a specific time in the cell cycle, when both the relevant kinase and the phosphatase appear to be constitutively active. Unlike many other protein kinases, CK2 does not require phosphorylation of residues within its activation loop for activity. Moreover, both CK2 and PP2A are ubiquitously expressed (it has been estimated that PP2A comprises up to 0.25 % of the total cell protein), and their activities do not display significant response to physiological inputs. However, it is likely that redistribution of PP2A may not be the complete story of TopoII α regulation, as it has been shown previously that the phosphorylation of TopoII α by CK2 is also stimulated by CDC2 *in vitro* [9].

It would be exceedingly interesting to see whether the mechanism proposed for the regulation of the TopoII α MPM-2 site represents a general strategy used by eukaryotic cells. Further work is required to ascertain whether other proteins phosphorylated during mitosis are regulated in a similar manner. In part due to the smaller number of phosphatases relative to kinases (approx. 160 phosphatases compared with approx. 560 kinases in the human genome), as well as to the more promiscuous substrate specificity of the phosphatases (at least *in vitro*), an often implicit assumption is that protein kinases are in general the more important of the two powers when it comes down to the regulation of the phosphorylation states. It remains possible that they are more salient regulators than the corresponding phosphatases. This

view is shifting, however, as many experiments indicate that the relatively smaller number and low modest variety and specificity of phosphatases are compensated by other levels of regulation, such as subcellular regulatory subunits and localization. One example is the interaction between PP2A and the shugoshin family of proteins at the centromeres. The localization of PP2A to the centromeres is important for protecting the phosphorylation and cleavage of cohesin subunits (thus sister chromatid separation) before the onset of anaphase (reviewed in [15]). In contrast, the cohesin complexes at the chromosome arms are not protected by PP2A and are phosphorylated before anaphase. Another example is CDC14, a phosphatase involved in mitotic exit. In many organisms, CDC14 is sequestered during interphase in the nucleolus, and is released in early and late anaphase by the FEAR and MEN pathways respectively (reviewed in [16]).

Outstanding questions that arise from the work of Escargueil and Larsen [4] include the precise mechanism that controls the translocation of PP2A from the nucleus during mitosis. In this connection, it is important to see whether the apparent translocation of PP2A also affects the phosphorylation states of other targets of this phosphatase. It would be interesting to see whether PP2A is regulated similarly in other mitotic-like states, such as mitotic catastrophe. For TopoII α , the role of mitotic phosphorylation by CK2 awaits further investigation. As the stability of TopoII α is highly regulated between mitosis and G₁ phase, one can speculate that the phosphorylation of the MPM-2 epitope or other mitotic sites may regulate the protein stability. Another possibility is that phosphorylation of TopoII α is involved in recruiting other proteins to the chromatin. Defining the MPM-2 site(s) in TopoII α more precisely should pave the way for addressing these questions. For instance, whether mutation of Ser¹⁴⁶⁹ to non-phosphorylatable residues in full-length TopoII α can completely abolish MPM-2 phosphorylation and affect TopoII α functions *in vivo* has not yet been addressed.

In summary, these new findings suggest an attractive mechanism to explain how some phosphorylations may occur specifically during mitosis when both the kinase and phosphatase appear to be constitutively expressed.

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