Repo-Man-PP1

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Coordination of late mitotic events is crucial for the maintenance of genome stability and for the control of gene expression after cell division. Reversible protein phosphorylation regulates this process by de-phosphorylation of mitotic phospho-proteins in a sequential and coordinated manner: this allows an orderly sequence of events to take place during mitotic exit. We have identified Repo-Man/PP1 as a phosphatase complex that regulates temporally and spatially chromatin re-organization and nuclear envelope re-formation during anaphase-telophase.

Following CDK inactivation at anaphase onset, a pool of Repo-Man/PP1 localizes homogeneously to the anaphase chromatin, where it removes the major mitotic phosphorylations on Histone H3 (Thr3, Ser10 and Ser28). We have shown that this de-phosphorylation mediated by Repo-Man/PP1 is essential for the re-establishment of heterochromatin in postmitotic cells.

A second pool of Repo-Man/PP1 targets to the periphery of chromosomes slightly later in anaphase. There, it contributes to the recruitment of Importin β to the chromatin. This fraction of Repo-Man appears to be important in the regulation of nuclear pores and lamina re-assembly since depletion of the complex leads to an abnormal lamina morphology and nuclear shape in G1 cells.

In summary Repo-Man/PP1 represents a molecular coupler between chromatin re-modelling and nuclear envelope re-formation that coordinates these processes during mitotic exit.1

Introduction

Within the cell cycle, mitosis is a key process for the maintenance of genome stability. Progress through mitosis is highly regulated, and reversible protein phosphorylation is one of the key features that allows an orderly and timely execution of cell division.6

Once sister chromatids have separated, the various biochemical and structural changes that have supported the entry into mitosis need to be reversed in order to start a new cell cycle.7 This part of cell division is termed mitotic exit.

During mitotic exit, the compact structure of mitotic chromosomes needs to be dismantled and the chromatin de-condensed to allow transcription to be resumed in G1. At the same time, major rearrangements of the cytoskeleton take place to allow physical separation of the two daughter cells. Finally, in organisms in which the nuclear envelope (NE) breaks down during mitosis (open mitosis), the nuclear-cytoplasmic barrier must re-form. All these events take place in a brief time period (about 10 min in a human cell) and they are coordinated in space and time.4

How the coordination of events during mitotic exit is achieved is an important open question, but the rapidly emerging picture is that this process is driven by a tug-of-war between kinases and phosphatases and their relative affinity for their substrates.2,5-10 Thus, while prometaphase/metaphase is driven by high levels of CDK and low phosphatase activity, mitotic exit is dominated by a burst in phosphatase activity and the decreasing phosphorylation of CDK substrates.
In view of this, it is now of great interest to identify the phosphatases that regulate mitotic exit in space and time and to identify their specific substrates.3,11,12 

**Chromatin Re-Organization During Mitotic Exit**

During early mitosis, chromatin is organized as highly compacted structures known as mitotic chromosomes. At anaphase onset, when the sister chromatids start their journey to the opposite spindle poles, the chromatin undergoes several important changes that are essential for the reformation of a functional interphase nucleus. While several chromosomal proteins (histones and non-histones) are dephosphorylated but remain in place, others leave the chromosomes (e.g., the chromosomal passenger complex (CPC)) and still others are recruited to anaphase chromosomes.

Several studies have suggested that protein phosphatase 1 (PP1) is important for cells to progress from mitosis to G1. For example, PP1 mutants in Drosophila show abnormal sister chromatid segregation and excessive chromosome condensation.13-15 This phosphatase has also been suggested to promote histone dephosphorylation during mitotic exit.16,17 However, the PP1 targeting subunits responsible for these chromatin re-organizations are not known.

Repo-Man was identified as a nuclear protein that is a specific regulatory subunit for PP1,18 which targets to the chromatin and can effectively de-phosphorylate histone H3 and possibly other chromosomal substrates (e.g., the chromosomal passenger complex (CPC)) on mitotic chromosomes.19-21 This is particularly relevant for the phosphorylation of Histone H3 Thr3, as it represents the docking site for the chromosome passenger complex (CPC) on mitotic chromosomes.22-25 Repo-Man is both a targeting subunit and substrate for PP1. This double regulation of the complex appears to be designed to act as a signal amplifier. In fact, small changes in CDK activity can result in enhanced binding of PP1 to the Repo-Man RVTF motif (Repo-Man docking site for PP1) and in Repo-Man de-phosphorylation. The resulting holoenzyme thus has an increased affinity for the chromatin and can effectively de-phosphorylate histone H3 and possibly other chromosomal substrates (Fig. 1C).19

The phosphorylation status of the Repo-Man regulatory subunit rather than

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**Figure 1. Repo-Man/PP1 complex regulation and function in mitosis. (A and B) Analysis of the dynamic behavior of GFP-Repo-Man in prometaphase (A and A') and interphase (B and B') FRAP of HeLa cells transiently expressing GFP-Repo-Man in prometaphase (A and A') and interphase (B and B'). Cells were bleached using 488 nm laser line of a confocal microscope. Images were taken before bleaching and at the indicated time points after the end of the bleached pulse (dotted line) at every 2 sec. The bleached area is indicated by a blue circle. Graphs represent corresponding quantitative data for fluorescence recovery kinetics for the bleached area on chromosomes, (blue line) and unbleached area on chromosomes (red line) and, for the mitotic cells also the unbleached cytoplasm (green line). Fluorescence intensities of GFP-Repo-Man in the bleached region were measured and expressed as recovery rate. The values represent averages +/- SD from 5 (mitosis) and 5 (interphase) cells. (C) CDK and PP1 control the on/off-rate of Repo-Man onto the chromosomes.**

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PP1 itself directs the formation and activity of the Repo-Man/PP1 holoenzyme. This appears to contrast with results of a recent study, in which cell-cycle regulation of PP1 was found to occur at the level of the catalytic subunit, through PP1 phosphorylation and inhibitor-1 binding.\textsuperscript{9} Our data suggest that there may be two different mechanisms for the regulation of PP1 activity in mitosis. It is possible that both are used according to the different substrates targeted.

At anaphase onset, Repo-Man/PP1 stably associates with the chromatin and promotes Histone H3 de-phosphorylation of Thr3,\textsuperscript{26,27} Ser10\textsuperscript{-\textsuperscript{phospho}},\textsuperscript{26,27} and Ser28.\textsuperscript{20,21} The importance of these de-phosphorylations during mitotic exit is not completely understood. Removal of the Thr3ph mark could be involved in the mechanism of CPC transfer to the spindle midzone, however clear demonstration of such a mechanism is still missing.

The removal of the Ser10 mark on histone H3 seems to be involved in the regulation of the binding of heterochromatin Protein 1 (HP1). HP1 recognizes histones with phosphorylated H3S10.\textsuperscript{24} The recruitment of HP1 to mitotic heterochromatin is mediated by Elys/MEL-28,\textsuperscript{49} which can associate to chromatin via its AT-hook.\textsuperscript{49} ELYS/MEL-28 seems to represent an essential component in NPC re-assembly both in vitro and in vivo.\textsuperscript{55}

NPC reformation is regulated at several levels by importin β. In mitosis, Importin β binds to some of the Nups and prevents their association. During anaphase, RanGTP around chromatin directs the release of the Nups from this inhibitory complex with Importin β, therefore directing the spatial positioning of the NPC. As stated above, it is believed that the first NPC assembly step is conducted by the recruiting of single copies of the Nap107-160 complex on the chromatin, mediated by the molecular adaptor ELYS/MEL-28. Importin β negatively regulates the seeding of Nups at these sites and subsequent assembly steps are dependent on specific membrane components.\textsuperscript{39,41,44} Depletion of ELYS/MEL-28 abolishes the assembly of pores at the chromatin periphery and causes the formation of anucleate lamellae (membrane stacks of pores in the cytoplasm). Also fundamental to this process is the de-phosphorylation of nucleoporins, chromatin associated factors and NE membrane proteins.

A variety of evidence in the literature supports the involvement of PP1 and PP2A in nuclear membrane assembly at the M/G1 transition.\textsuperscript{47,49}

PP1 appears to be the major mitotic lamin phosphatase responsible for removal of mitotic phosphates from lamin B\textsuperscript{-R} and the A-kinase anchoring protein AKAP149 recruits PP1 to the nuclear envelope (NE) upon somatic nuclear reformation in vitro.\textsuperscript{47} PP1 targeting to the NE is also a prerequisite for assembly of B-type lamins. In Drosophila, reassembly of the NPC is blocked by the specific PP1/PP2A inhibitor okadaic acid.\textsuperscript{47} Although the identity of the phosphatase responsible for de-phosphorylation of NUPs remains unknown, there are indications that at least NUP153 and NUP 50 can interact with PP1.\textsuperscript{48}

During our studies aimed at identifying the targets of Repo-Man/PP1 complex on anaphase chromatin we discovered a Nap107/160 complex between Repo-Man and importin β. This binding is negatively regulated by Cdk phosphorylation of the N-terminal domain (aa 1–135) of Repo-Man. This interaction appears to be important for targeting at least a fraction of importin β to the periphery of the anaphase chromosomes and it seems to represent a direct structural function of Repo-Man rather than requiring catalytic activity of the Repo-Man/PP1 holoenzyme.

At this stage of mitotic exit, Repo-Man also interacts with NUP50 and NUP153.\textsuperscript{49} However, more work is required to understand how Repo-Man interacts with this subset of nucleoporins and to determine its biological relevance at the transition from mitosis to G1.

The importin β targeting function of Repo-Man may represent an important step in NE re-assembly since it has been shown, at least in vitro, that Importin β levels are critical for proper NE re-assembly.\textsuperscript{34,45} In addition, it has been suggested that the pathway of nuclear pore complex assembly could be regulated at sequential points by transportin and importin β but also that other effectors could exist, particularly for the FG nucleoporins Nup558, Nup153 and Nup50.\textsuperscript{76,85} Our experiments showed that lack of Repo-Man compromises the process of NE reformation: Importin β is not properly recruited to the nuclear rim but form cytoplasmic aggregates that co-localize with NUPs (possibly annulate lamellae) and the nuclear lamina fails to form a smooth structure after cells have completed cytokinesis.

Current knowledge leads us to propose the following model. Very early during mitotic exit, the Nap107/160 complex is recruited to regions at the chromosome periphery via the chromatin binding protein ELYS/MEL-28. This is negatively regulated by importin β and transportin...
(Fig. 2A). In the vicinity of these seedling regions, Repo-Man docks to the chromatin (directly or indirectly) and brings importin β and an early pool of NUP153/ NUP50 (Fig. 2B). In this respect Repo-Man could act either as an assembly factor for these FG NUPs or as a spacer by enriching some regions for importin β and preventing the seeding of more NUP107/160 complex.

Although the binding and recruitment of importin β by Repo-Man seems to be independent of the catalytic activity of the Repo-Man/PP1 holoenzyme, it is plausible to assume that, once the complex is targeted to a localized region of the chromosome periphery, it could be involved in local de-phosphorylation processes either of NUPs or the lamina during the later steps of the nuclear re-assembly process (Fig. 2C).

Further studies will be required to elucidate the global functions of Repo-Man at this stage of mitosis and to identify substrates critical for nuclear re-assembly. In particular it will be important to clarify the mechanisms and roles of interactions between Repo-Man and NUP153/50 during mitotic exit. It will also be important to determine whether the fraction of Repo-Man bound to the nuclear periphery has a role in the organization and function of the interphase chromatin.

Future Prospective

Contrary to what was believed, the emerging view is that protein phosphatase complexes show stringent and selective substrate specificity. The modifications of chromatin during mitotic exit and the re-formation of a nuclear envelope both require the de-phosphorylation of several proteins in a local and timely manner. To date only three major PP1 targeting subunits have been found to be involved in these chromosome modifications in anaphase: Repo-Man, PNUTS and AKAP149. Clearly other targeting subunits must be involved in this complex process and we can hypothesize that the anaphase chromosome will give us more PP1 targeting subunits that will have specific functions regulating the transitional steps at the M/G1 boundary.

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