

COMMENTARY

Regulation of mitosis by poly(ADP-ribosylation)

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The spindle is a dynamic, microtubule-based structure responsible for chromosome segregation during cell division. Spindles in mammalian cells contain several thousand microtubules that are arranged into highly symmetric bipolar arrays by the actions of numerous microtubule-associated motor and non-motor proteins. In addition to these protein constituents, recent work has demonstrated that poly(ADP-ribose) is a key spindle component. Of the multitude of poly(ADP-ribose) polymerase proteins encoded in the genome, tankyrase 1 appears to be the primary enzyme responsible for building poly(ADP-ribose) in spindles during mitosis. In this issue of the *Biochemical Journal*, Susan Smith

and co-workers show that the primary target of tankyrase 1 in dividing cells is NuMA (nuclear mitotic apparatus protein), a protein that cross-links microtubule ends at spindle poles. The impact of poly(ADP-ribosylation) on the biochemical function of NuMA remains murky at this time, but these new results represent the first step to clearing the view as to how poly(ADP-ribosylation) regulates cell division.

Key words: microtubule, mitosis, nuclear mitotic apparatus protein (NuMA), poly(ADP-ribose), spindle, tankyrase.

The essential function of the mitotic phase of the cell cycle is accurate chromosome segregation. Failure in this process leads to aneuploidy, which contributes to the initiation and progression of cancer. This essential cellular function is fulfilled by a complex microtubule-based structure called the spindle. In mammalian cells, several thousand microtubules are devoted to spindle assembly. These microtubules are organized into symmetric bipolar arrays by the actions of motor proteins of the dynein and kinesin families and various non-motor microtubule-associated proteins [1]. For example, the kinesin-5 motor protein Eg5 plays a critical role in directing bipolar spindle assembly, since monopolar spindles result when its activity is inhibited. An example of a non-motor protein involved in spindle assembly is NuMA (nuclear mitotic apparatus protein) [2]. NuMA's name reflects its cell cycle distribution, where it resides in the nucleus during interphase and associates with the mitotic apparatus in mitosis. NuMA is a large protein (≈ 235 kDa) with globular head and tail domains separated by a very long coiled-coil domain. During mitosis, NuMA associates with spindle microtubules through its C-terminal globular domain, and concentrates at spindle poles. NuMA can cross link microtubules, a function that is essential for spindle organization because perturbation of NuMA disrupts microtubule focusing at spindle poles, leading to splaying of microtubule ends.

In addition to the protein constituents of spindles, it was recently shown that spindles are enriched in PAR [poly(ADP-ribose)] [3]. Spindle-associated PAR plays a functional role in spindle organization, because spindles assembled in frog-egg extracts lose bipolarity when treated with a PAR antibody or if PAR levels are experimentally reduced. This mitotic function reflects a new role for PAR, since it was originally implicated as a regulator of genome integrity as a nuclear protein post-translational modification induced in response to DNA damage [4]. Through covalent attachment to glutamic acid residues, the negative charge imparted by PAR dramatically alters the structure and function of substrate proteins, implying that it acts as a regulatory mechanism akin to phosphorylation.

As a post-translational regulatory mechanism, it is not surprising that PAR levels are tightly controlled. PAR is synthesized by a family of enzymes called PARPs [poly(ADP-ribose) polymerases] [5]. Members of this gene family share a conserved catalytic domain responsible for the transfer of ADP-ribose from NAD⁺ on to proteins. However, PARP family members diverge in regions outside the catalytic domain, and have disparate cellular localizations. As their name implies, PARPs are capable of processively transferring more than one ADP-ribose on to a substrate, and the extent and branching of the PAR network is controlled by the antagonistic action of PARG [poly(ADP-ribose) glycohydrolase]. Thus cells can rapidly and reversibly PARsylate proteins in response to appropriate stimuli by selectively activating PARP and PARG enzymes. DNA damage was the stimulus that led to the identification of PAR, but new data show that PAR levels respond to cell cycle stimuli. In this issue of the *Biochemical Journal*, in cultured cells without DNA damage, Chang et al. [6] show that PAR levels peak dramatically during mitosis, suggesting acute activation of PARPs at the onset of mitosis, followed by PARG activation at mitotic exit.

Recently, an RNAi (RNA interference) strategy was used to knock-down expression of all major PARP enzymes in cultured cells to identify those that are responsible for the formation of PAR during mitosis [7]. The results show that tankyrase 1 is the primary PARP responsible for PAR synthesis in mitosis. Tankyrase 1 was originally identified as a protein associated with TRF1, a telomere-associated protein that limits telomere DNA-repeat length [8]. Tankyrase 1 PARsylates TRF1 under *in vitro* conditions, and diminishes the TRF1 affinity for telomeric DNA. This led to the hypothesis that PARsylation promotes telomere growth through negative regulation of TRF1. Consistent with this view, telomere length increased in human cells overexpressing tankyrase 1 [9], and some cells lacking tankyrase 1 appear unable to disjoin telomeres during anaphase [10].

But what role does tankyrase 1 play in mitosis? The consequence of tankyrase 1 deficiency induced using RNAi is inhibition of cell cycle progression, with cells significantly delayed

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in transit through mitosis [6,7]. Most tankyrase-1-deficient cells have disorganized mitotic spindles, suggesting that the mitotic delay is caused by persistent activation of the spindle-assembly checkpoint [7]. The few cells that slip past this checkpoint control may have difficulty in resolving telomeres at early anaphase [10], although that would yield a high frequency of chromatin bridges, which have not been reported. These results demonstrate mitotic spindle organization and consequent mitotic progression rely on PARsylation of specific spindle-associated proteins by tankyrase 1.

To identify tankyrase 1 substrates, Smith and co-workers [6] immunoprecipitated tankyrase 1 from cultured cells and performed *in vitro* PARsylation assays. In addition to auto-PARsylation of tankyrase 1, they observed significant PARsylation of a protein of approx. 230 kDa. The most likely 'suspect' at that size range was NuMA, based on previous data showing that NuMA possessed a tankyrase-interacting domain [11] and that tankyrase 1 and NuMA co-localize at spindle poles [12]. Smith's group confirmed this suspicion through co-immunoprecipitation of tankyrase 1 with NuMA antibodies [6], whereas other data show the co-immunoprecipitation of NuMA with PAR antibodies [7]. The key experiment showed that NuMA failed to be PARsylated in cells deficient in tankyrase 1 induced by RNAi [6,7]. Finally, Chang et al. [6] use careful cell cycle analyses to demonstrate that NuMA is only PARsylated during mitosis, a finding consistent with the compartmentalization of NuMA in the cell nucleus and tankyrase 1 at the Golgi during G₁, S and G₂ cell cycle phases. Taken together, these data demonstrate that NuMA is specifically PARsylated during mitosis, and that tankyrase 1 is the PARP responsible for that cell-cycle-dependent modification. Other mitotic proteins are PARsylated [13] and may very well be substrates for tankyrase 1, but the present data indicate that the major target for tankyrase 1 in mitosis is the spindle protein NuMA.

The obvious question is: what is the functional significance of PARsylation of NuMA? Tankyrase-1-deficient mitotic cells display disorganized spindles, and NuMA has a well-documented role in spindle organization. Also, tankyrase 1 and NuMA co-localize at spindle poles in cultured cells [12]. These observations lead to the simple idea that localization of NuMA at spindle poles is regulated by PARsylation catalysed by tankyrase 1. Consistent with this view, Chang et al. [6] show that partial deficiency of NuMA eliminates the targeting of tankyrase 1 to spindle poles, demonstrating that tankyrase 1 relies on NuMA for spindle targeting [6]. However, the localization of NuMA is not detectably altered in tankyrase-1-deficient cells despite inhibition of NuMA PARsylation under these conditions [6]. Moreover, NuMA appears to retain its essential mitotic function in the absence of tankyrase 1 function because microtubule ends remain focused at spindle poles, albeit in multiple poles instead of just two [6,7]. Thus the simple view does not offer a straightforward explanation for the function of NuMA PARsylation, and more sophisticated analyses are needed to tease apart this problem. One possibility is that PARsylation regulates the dynamic exchange of NuMA on and off mitotic spindles. Recent work demonstrates that multiple

mechanisms regulate NuMA dynamics at spindle poles [14], and PARsylation may regulate NuMA dynamics in a manner that cannot be detected using immunofluorescence analysis of fixed cells. Tankyrase 1 interacts with NuMA at a site adjacent to the microtubule-binding domain in the C-terminal globular domain [11]. This suggests that PARsylation of that region of NuMA could influence its interaction with microtubules. Another possibility is that PARsylation creates covalent NuMA–NuMA (or NuMA and some other protein) cross-links at spindle poles. Such cross-links might suppress release of NuMA from spindle poles, consistent with the observation of a small, non-exchangeable pool of NuMA at poles [14].

In summary, these new findings unveil PARsylation as a new mechanism of cell cycle regulation and reveal both tankyrase 1 as the primary mitotic PARP and NuMA as the major spindle-associated substrate. As with any phenomenon emerging from the convergence of previously unrelated fields, these new results offer as many questions as answers. For example, what other mitotic substrates does tankyrase 1 PARsylate? How are tankyrase 1 and PARG activities regulated during the cell cycle? Which glutamic acid residues in NuMA are the sites of PARsylation? How does PARsylation influence NuMA function in mitosis? Answers to these questions will emerge from the concerted application of both biochemical and cell biological assays.

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