

## Views and Commentaries

# Fine Tuning of Kinetochores Function by Phosphorylation

**Silke Hauf**

\*Correspondence to: Silke Hauf; Institute of Molecular Pathology ; Dr. Bohr-Gasse 7; A – 1030 Vienna Austria; Tel.: +43.1.79.730.625, Fax: +43.1.798.71.53, Email: hauf@imp.univie.ac.at

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The movement of sister chromatids to opposite poles of a cell during anaphase is one of the most striking events during the eukaryotic cell cycle. The force for this movement is mediated by microtubules that are attached to chromatids by a specialized proteinaceous structure, the kinetochore (from greek 'kinetos' moving, and 'khoros' place). The kinetochore is not merely a passive link between chromosomes and the mitotic spindle, but is actively directing and controlling chromosome movement during mitosis.<sup>1,2</sup> Kinetochores also play a pivotal role in the mitotic or 'spindle assembly' checkpoint. This checkpoint controls that anaphase is only initiated once all chromosomes have become properly attached to the mitotic spindle. Kinetochores have the ability to sense the absence or presence of attachment, and unattached kinetochores generate a signal that ultimately delays anaphase.<sup>3</sup> It has recently become clear that kinetochore function, like many other processes in mitosis, is regulated by phosphorylation. Work by Cheeseman and colleagues,<sup>4</sup> and by Li and Elledge<sup>5</sup> has begun to identify the relevant sites of phosphorylation within the yeast kinetochore, and the kinases that generate those.

The variety of functions exerted by the kinetochore is reflected by the fact that it is built up by as many as ~40 proteins.<sup>6</sup> The functions of many of these are just starting to be elucidated. Recent work in budding yeast has identified protein subcomplexes that might act as modules that confer specific functions. One of the complexes that is likely to directly connect kinetochore components and microtubules, and could regulate their interaction, is the Dam1 (aka DASH or DDD) complex.<sup>4,7,8</sup> This huge, 1MDa complex is constituted of at least 9 subunits—among them Dam1p, Duo1p, Dad1p, Ask1p, Spc34p, and Spc19p, all of which are encoded by essential genes. Components of the Dam1 complex interact with both microtubules and kinetochores, and temperature-sensitive mutants show spindle and chromosome segregation defects. It had been noted that several Dam1 complex subunits displayed electrophoretic mobility shifts indicative of phosphorylation, and Dam1p itself was found to be phosphorylated by the mitotic kinase Ipl1p.<sup>9</sup> Ipl1 belongs to the aurora family of kinases, and in budding yeast is required to promote bi-polar attachment of chromosomes to the mitotic spindle,<sup>10</sup> and to sustain the mitotic checkpoint under certain conditions.<sup>11</sup> Ipl1p had furthermore already been found to phosphorylate the kinetochore protein Ndc10p.<sup>12</sup> To identify crucial Ipl1p targets at the kinetochore, Cheeseman and colleagues<sup>4</sup> systematically analyzed phosphorylation sites in the Dam1 and two other kinetochore complexes by mass spectrometry. Within the Dam1 complex, Ask1p, Dam1p, Spc34p, and Spc19p were all found to be phosphorylated at several sites in vivo. However, only a subset of these sites, particularly the ones in Dam1p and Spc34p, were phosphorylated by Ipl1p in vitro. Interestingly, mutating serine or threonine in these Ipl1p sites to alanine resulted in a phenotype that strikingly resembles that of *ipl1* mutants, indicating that the Dam1 complex is a key Ipl1p target in vivo. [Note: Although the function of the mitotic kinase Ipl1 seems to be conserved throughout evolution,<sup>13</sup> a functional orthologue of the Dam1 complex has surprisingly not been identified in higher eukaryotes to date.]

The analysis by Cheeseman et al.<sup>4</sup> identified six in vivo phosphorylation sites in Ask1p, only one of which was phosphorylated by Ipl1p in vitro. Mutation of this site to alanine, however, did not confer any growth defect, indicating that Ask1p is not a crucial Ipl1p target. In this issue, Li and Elledge now report that Ask1p is also phosphorylated in vivo by the key mitotic kinase Cdc28p.<sup>5</sup> Cdc28 is the kinase controlling entry into mitosis, and has to be inactivated to allow exit from mitosis.<sup>14</sup> Li and Elledge noticed that Ask1p is phosphorylated in vivo during the S, G<sub>2</sub>, and M phase of the cell cycle, coinciding with the activity of Cdc28p. This prompted them to search for Cdc28 consensus sites within Ask1. Two sites were identified, one of which had been found to be phosphorylated in vivo by Cheeseman and colleagues, and was not phosphorylated by Ipl1p in vitro.<sup>4</sup> Mutation of serine at this site or in both sites to alanine reduced the mitotic mobility shift observed by

SDS-PAGE, and temperature-sensitive alleles of CDC28 had a similar effect. Ask1p furthermore proved to be an excellent *in vitro* substrate for Cdc28p, indicating that Cdc28p might directly phosphorylate Ask1p *in vivo*. However, the double alanine mutant is viable, and does not show any overt phenotype. Growth defects became obvious when the alanine mutations were combined with a temperature-sensitive allele of ASK1. Ask1 phosphorylation by Cdc28p therefore is not essential, and it remains to be seen how it contributes to Ask1 function. As Ask1p phosphorylation already occurs during S phase, and is maintained throughout G<sub>2</sub> and M, it is conceivable that it has a rather structural role in Dam1 complex formation or in binding of the complex to kinetochores. In contrast, phosphorylations that regulate bi-polar attachment or checkpoint functions at the kinetochore, are rather expected to occur during mitosis only.

Ipl1 and Cdc28 most probably are not the only kinases that modulate kinetochore function. Dam1 genetically interacts with the checkpoint kinase Mps1,<sup>15</sup> making the latter also an excellent candidate for influencing the Dam1 complex. Also, other checkpoint kinases like Bub1, and in higher eukaryotic cells also BubR1, could phosphorylate as yet unknown substrates at the kinetochore. In vertebrates, both Bub1 and BubR1 localize to kinetochores that have not yet attached to the spindle in a bi-polar fashion. Furthermore, a phosphoepitope recognized by the 3F3/2 antibody is present at kinetochores while the checkpoint is active, also indicating that checkpoint signaling involves phosphorylation.<sup>16</sup> The kinase that generates the 3F3/2 epitope *in vivo* has not been identified.

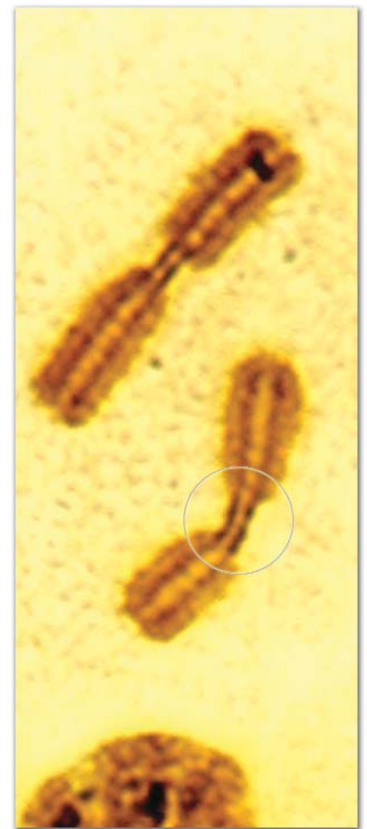
Despite some progress, understanding kinetochore function seems a distant prospect. Even the knowledge about all phosphorylation sites, and all the kinases that generate those phosphorylations, would not tell us how the kinetochore works. To ultimately comprehend the kinetochore, it will be crucial to know how the kinases that modulate its functions are regulated. For example, how is Ipl1's activity confined to those kinetochores that are not yet attached in a bi-polar fashion? We do not have any insight yet, in how the kinetochore detects errors in attachment, and how it translates them in biochemical signals. Also, the functional consequences of phosphorylations at the kinetochore are unknown. It is conceivable that phosphorylation induces conformational changes that alter a protein's function. Phosphorylation could also change the affinity of protein-protein interactions, and because of its reversible nature it could both loosen and tighten interactions at the kinetochore.

The studies by Cheeseman, Li and colleagues have now started to elucidate the role of phosphorylation in kinetochore function. However, considering the multitude of functions that the kinetochore exerts and its complexity, it is likely that phosphorylation is only one mode of regulation and even more are yet to be discovered.

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Figure 1. Silver stained chromosomes of the Indian muntjak. The kinetochores assemble on the central constriction of chromosomes (encircled in gray). Micrograph courtesy of Juan F. Giménez-Abián.



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