What is clear from all of these results and those of Kowarik et al. (2002) is that protein folding during cotranslational translocation in the endoplasmic reticulum is likely to have a profound influence on the biogenesis of polytopic membrane proteins and the assembly of the complexes they are found in. Clearly, we have only the initial inklings of either the regulatory possibilities or the disease relevance of these processes.

David W. Andrews
Department of Biochemistry
McMaster University
1200 Main Street West
Hamilton, Ontario L8N 3Z5
Canada

Selected Reading

Hamman, B.D., Chen, J.C., Johnson, E.E., and Johnson, A.E. (1997). Cell *89*, 535–544.

Hegde, R.S., and Lingappa, V.R. (1997). Cell *91*, 575–582.

Kim, S.J., and Hegde, R.S. (2002). Mol. Biol. Cell, in press, E02–05–0293.

Kowarik, M., Küng, S., Martoglio, B., and Helenius, A. (2002). Mol. Cell *10*, 769–778.

Liao, S., Lin, J., Do, H., and Johnson, A.E. (1997). Cell *90*, 31–41.

Meacock, S.L., Lecomte, F.J.L., Crawshaw, S.G., and High, S. (2002). Mol. Biol. Cell. Published online September 24, 2002. DOI: 10.1091/mbc.E02-04-0198.

Young, J.C., and Andrews, D.W. (1996). EMBO J. *15*, 172–181.

Dam1 Is the Right One: Phosphoregulation of Kinetochore Biorientation

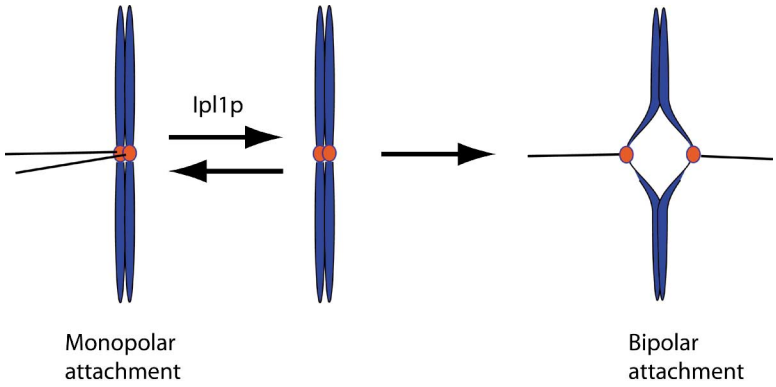
Chromosomes have to establish the proper attachment to the spindle before segregation—a process that requires Ipl1p Aurora kinase. Recent work has identified Dam1p, a member of the DASH complex, as the key Ipl1p substrate responsible for kinetochore/microtubule interaction.

The process of mitosis is, in essence, about faithful segregation of sister chromatids. How do cells manage to push and pull their chromosomes, lining them up in such a way as to ensure that each daughter gets the identical set of chromosomes? Broadly speaking, this process requires proper interactions between kinetochores—centromeric protein complexes—and spindle microtubules (MT). More specifically, each sister kinetochore must establish a connection with MTs from the opposite pole—a state known as biorientation—prior to chromosome segregation (see Figure).

Recent studies focusing on the budding yeast *Saccharomyces cerevisiae* have generated insight into the molecular mechanisms of biorientation. In budding yeast, chromosomes almost always maintain attachment to the nuclear MTs emanating from the spindle pole(s) (except perhaps for a brief period of time during centromeric DNA replication). Sister kinetochores may be monopolarly attached to the same spindle pole immediately after DNA replication, or monopolar attachment may accidentally occur while biorientation is being established (Janke et al., 2002; Tanaka et al., 2002). Either way, in order to convert from mono- to bioriented, the kinetochore-MT interaction has to be weakened/abolished, so that one or both kinetochores are released from the pole. Once free, the kinetochores reassociate with the spindle MTs; presumably, such a "capture and release" cycle takes place until biorientation is achieved (Tanaka, 2002) (see Figure).

One of the key players in establishing biorientation is Ipl11p, an Aurora protein kinase (Biggins et al., 1999; Tanaka et al., 2002). The kinetochores in *ipl1* mutant cells always attach to the old pole prior to chromosome segregation. In Ipl1+ cells, kinetochores attach to the new and old poles with equal frequency, presumably after detaching from the old pole (Tanaka et al., 2002). Thus, Ipl1p kinase may function to facilitate biorientation by promoting the turnover of kinetochore-spindle pole attachment. Its in vivo function may be counteracted by the phosphatase, Glc7p (Sassoon et al., 1999). A recent paper in the October 18th issue of *Cell* (Cheeseman et al., 2002) takes a significant step forward in understanding the mechanism of biorientation by demonstrating that Dam1p, a member of the kinetochore DASH complex, is a crucial physiological substrate of Ipl1p. This complex (also called the Dam1p complex or DDD complex) is an integral part of the kinetochore and is composed of nine subunits, as reported in the current paper (see below). The whole DASH complex binds to MTs directly, and evidence suggests that it is delivered onto kinetochores via MTs. It has been shown that Ipl1p controls Dam1p phosphorylation in vivo, possibly directly (Kang et al., 2001; Li et al., 2002).

Through a series of experiments, identical in vivo and in vitro Ipl1p phosphorylation sites were found in three members of the DASH complex: Dam1p, Ask1p, and Spc34p. Systematic mutations (S to A) of all four Ipl1p phosphorylation sites of Dam1p, but not those in Ask1p and Spc34p, caused cell lethality, indicating an essential role for Dam1p phosphorylation. These phosphorylation site mutations also phenocopied the inactivation of Ipl1p in terms of chromosome missegregation. In addition, cells with alterations (S to D) designed to mimic constitutive phosphorylation of Dam1p showed evidence of lagging chromosomes. Since lagging chromosomes are often indicative of weak kinetochore-MT attachments, this experiment lends support to the possibility that Ipl1p phosphorylation relaxes the kinetochore-MT connection. Finally, these same constitutive phosphorylation mutants were able to partially suppress the defects of *ipl1-2* but were synthetically lethal with the phosphatase mutation *glc7-10*. Taken together, these data suggest that Dam1p is a key substrate of Ipl1p and that its phosphorylation is essential for biorientation.



Schematic Model of Biorientation Establishment

Monopolarly attached sister kinetochores have to be released from the microtubules, so that establishing new attachment is possible. Ipl1p kinase facilitates the release of the monopolarly attached kinetochores from the microtubules. The "capture and release" cycle continues until the correct biorientation is established.

So, how does Dam1p phosphorylation promote turnover of the kinetochore-spindle pole connection prior to the establishment of biorientation? Although the Dam1 complex is capable of binding to MTs directly, the current paper points out that Dam1p phosphorylation does not reduce DASH complex binding to MTs in vitro or change the complex's composition. Instead, the authors suggest that Dam1p phosphorylation weakens/abolishes the association between the DASH complex and the rest of the kinetochore. Presumably, dephosphorylation by Glc7p enables reassociation, and the process continues until biorientation is established. If this is the case, then it supports a model in which Ipl1p moderates kinetochore-MT attachment by regulating the integrity of the kinetochores, rather than their direct affinity with MTs. This model might also provide a role for additional Ipl1p substrates in the kinetochore, such as Ndc80p, as pointed out in this paper. Other potential substrates may include Cse4p—the centromere-specific histone H3—and Ndc10p—a protein that directly participates in centromeric DNA binding. On the basis of this model, phosphorylation by Ipl1p should be taken to weaken the attachment between the DASH complex and the rest of the kinetochore, allowing reorientation. However, we still have to reconcile an apparently conflicting observation that DASH complex association with centromeric DNA is weakened in an *Ipl1* mutant background. Maybe, as the Cheeseman et al. paper suggests, phosphorylation by Ipl1p plays an additional role in promoting new kinetochore assembly after chromosome replication.

Besides its focus on biorientation, the current paper also provides important information about three other subjects. First, the purification procedure used to isolate the various complexes identified five new proteins showing kinetochore localization. In addition, some previously identified kinetochore proteins were isolated as part of a single large complex (with 12 subunits in total). This has an important implication for the study of kinetochore architecture in that it argues for viewing the kinetochore as a more systematically integrated whole, rather than piece by piece. Second, previous studies of Dam1p have ascribed two roles to the protein, one involving the kinetochore and the other dealing with spindle integrity (Cheeseman et al., 2001; Jones et al., 2001). The current paper demonstrates that mutation in the Ip1p sites only affects Dam1p's role in the kinetochore, arguing for separately regulated functions of the protein. Finally, the current paper presents a consensus site for Ipl1p kinase. Since Ipl1p is part of a large family of conserved Aurora kinases, this information will prove helpful to future studies involving phosphoregulation by their activity.

Andrew M. Courtwright and Xiangwei He
Department of Biochemistry and Molecular Biology
Graduate School of Biomedical Sciences
University of Texas Health Science Center at Houston
Medical School
6431 Fannin, 6.200
Houston, Texas 77030

Selected Reading

Biggins, S., Severin, F.F., Bhalla, N., Sassoon, I., Hyman, A.A., and Murray, A.W. (1999). Genes Dev. *13*, 532–544.

Cheeseman, I.M., Enquist-Newman, M., Muller-Reichert, T., Drubin, D.G., and Barnes, G. (2001). J. Cell Biol. *152*, 197–212.

Cheeseman, I.M., Anderson, S., Jwa, M., Green, E., Kang, J., Yates, J.R., III, Chan, C., Drubin, D.G., and Barnes, G. (2002). Cell *111*, 163–172.

Janke, C., Ortiz, J., Tanaka, T.U., Lechner, J., and Schiebel, E. (2002). EMBO J. *21*, 181–193.

Jones, M.H., He, X., Giddings, T.H., and Winey, M. (2001). Proc. Natl. Acad. Sci. USA *98*, 13675–13680.

Kang, J., Cheeseman, I.M., Kallstrom, G., Velmurugan, S., Barnes, G., and Chan, C.S. (2001). J. Cell Biol. *155*, 763–774.

Li, Y., Bachant, J., Alcasabas, A.A., Wang, Y., Qin, J., and Elledge, S.J. (2002). Genes Dev. *16*, 183–197.

Sassoon, I., Severin, F.F., Andrews, P.D., Taba, M.R., Kaplan, K.B., Ashford, A.J., Stark, M.J., Sorger, P.K., and Hyman, A.A. (1999). Genes Dev. *13*, 545–555.

Tanaka, T.U. (2002). Curr. Opin. Cell Biol. *14*, 365–371.

Tanaka, T.U., Rachidi, N., Janke, C., Pereira, G., Galova, M., Schiebel, E., Stark, M.J., and Nasmyth, K. (2002). Cell *108*, 317–329.