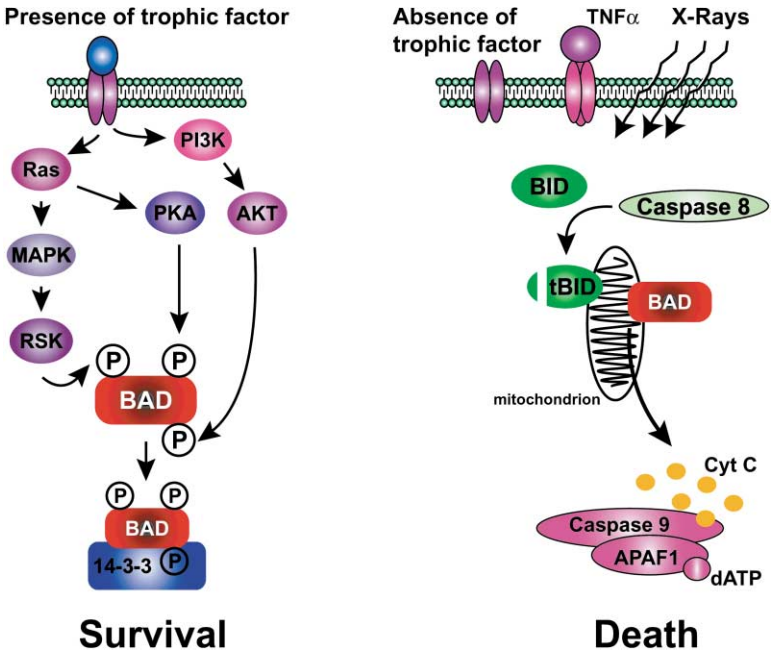

Survival Signaling via BAD Phosphorylation

Growth factor-dependent BAD phosphorylation regulates the cellular response to environmental stress by setting the threshold for cytochrome c release from the mitochondria into the cytoplasm. Cells in which BAD is phosphorylated are more resistant to intrinsic and extrinsic cellular stress. (Illustration courtesy of Janine Zieg, Children's Hospital, Boston.)

elegant genetic proof that phosphorylation of endogenous BAD is an essential mechanism of survival factor-mediated inhibition of apoptosis.

This work sets the stage for a new line of research. First, it provides a precedent for similar studies including other proteins that are targets of survival kinases, such as FOXO3a. This will allow for the assessment of the relative contributions of these factors for cell survival. Second, BAD has been shown, in vitro and in cell culture, to be phosphorylated by multiple survival-promoting kinases. However, whether the phosphorylation of BAD by these kinases is physiologically relevant is unknown. Datta et al. discuss that the phenotypes of *Akt1*$^{-/-}$ and *BAD*$^{3SA/3SA}$ animals are very similar, suggesting that Akt1 could be an important regulator of BAD activity in vivo. Thus, comparison of the knockout phenotypes of other kinases with the *BAD*$^{3SA/3SA}$ phenotype will help to determine the physiological significance of BAD phosphorylation by these kinases. Third, the *BAD*^{3SA} mutant provides a tool to address questions ultimately related to malignancy. For instance, oncogenic activation of the Akt kinase may promote tumorigenesis by inactivating BAD. It is now possible to investigate this directly by analyzing oncogenic activation of Akt in *BAD*$^{3SA/3SA}$ mutant animals. Thus, future work will be very informative in dissecting various roles of survival signaling regarding development, tissue homeostasis, and malignancies.

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Protein Origami for Beginners

In the October issue of *Molecular Cell*, Kowarik et al. examine cotranslational translocation and folding during the synthesis of secretory and integral membrane proteins. Their results suggest that these potentially competing processes are regulated in surprising ways and reveal novel insights into the mechanisms by which proteins are assembled into membranes.

The most obvious consequence of cotranslational protein folding is that the folding pathway of a protein is largely determined by the initial folding of its amino ter-

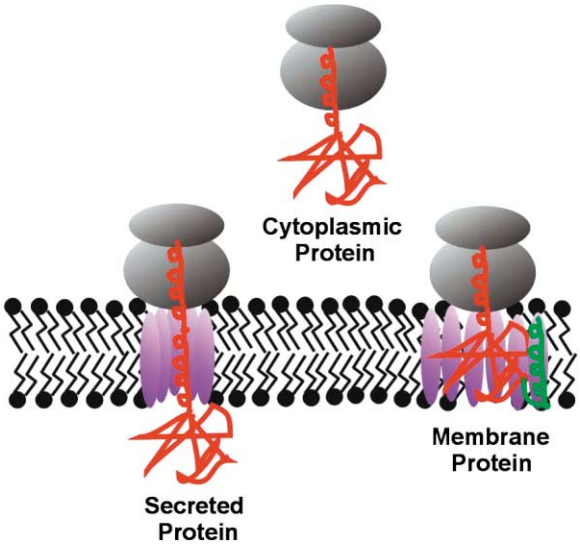
minus. As a result, one can envision circumstances when additional regulation would be necessary. For example, it may be necessary to stop and restart the folding process in order to achieve the proper conformation of a protein containing multiple domains. During synthesis of the α subunit of the signal recognition particle receptor, an mRNA-encoded pause in translation permits the amino-terminal membrane assembly domain to fold independently of the carboxyl-terminal GTPase domain, thereby facilitating assembly of the protein on the endoplasmic reticulum (Young and Andrews, 1996). Significantly, translation was arrested at the point where the amino-terminal domain had just exited from the nascent chain tunnel in the ribosome. By artificially pausing translation using truncated mRNAs encoding a model cytoplasmic protein, Kowarik et al. (2002) have demonstrated directly that folding occurs as soon as a domain capable of independent folding exits the ribosome (see Figure).

Things become more complicated when one considers secreted and integral membrane proteins. These proteins are guided across the membrane of the endoplasmic reticulum cotranslationally through an aqueous pore referred to as the protein-conducting channel (PCC). The PCC is the central component of a larger dynamic structure called the translocon, comprising all of the components that access the nascent polypeptide as it crosses the bilayer.

How then is cotranslational protein folding managed when secretory and integral membrane proteins are also cotranslationally conducted through the PCC? In the simplest scenario, the PCC might function as an extension of the tunnel in the ribosome through which the nascent polypeptide passes. According to this model, and consistent with structural analysis of the PCC, the nascent polypeptide is in an enclosed space insufficient to sustain complex protein folding. Instead, the protein may form local secondary structures, such as an α helix, as it passes through the PCC. Exit of the nascent polypeptide from the PCC would then be analogous to exit from the ribosome by a cytoplasmic protein and would allow folding to occur as soon as a protein domain enters the endoplasmic reticulum lumen. For the relatively simple model secreted protein examined by Kowarik et al. (2002), this appears to be the case. The test protein folds in the cytoplasm once the single domain plus 36 amino acids are synthesized (the extra amino acids are required to span the tunnel in the ribosome). When a signal sequence is added and it is translocated across the endoplasmic reticulum membrane, the protein domain requires a 64 amino acid spacer from the peptidyltransferase center to span both the tunnel in the ribosome and the PCC before folding can be completed (see Figure).

The amino-terminal signal peptide used to target the nascent protein to the PCC is removed as the nascent protein exits from the translocon, and thus, it is not surprising that it plays no detectable role in protein folding. Based on these results, it seems that even for secreted multidomain proteins, the PCC can be viewed as an extension of the nascent chain tunnel in the ribosome.

However, when the nascent protein begins with a transmembrane (TM) domain instead of a cleaved signal peptide, Kowarik et al. (2002) demonstrate that more complex protein folding is accommodated (perhaps



Cotranslational Folding of Secreted and Transmembrane Proteins

The nascent protein (red) adopts very limited structure as it passes through the tunnel of the ribosome, but folds as soon as it exits into the cytoplasm. For secreted proteins, the protein-conducting channel (purple) can be considered an extension of the tunnel in the ribosome. Complex protein folding occurs only as the protein enters the endoplasmic reticulum lumen. However, when a protein begins with a transmembrane sequence (green), protein folding can occur within the protein-conducting channel. Accommodation of the additional space occupied by the folding protein requires reorganization of the protein-conducting channel.

even initiated) within the PCC. The observation that entire protein domains can fold within the translocon has immediate implications for our view of the PCC. First, it suggests that the diameter of the PCC is not fixed, a view first championed by the Johnson laboratory based on fluorescence spectroscopy of translocation intermediates (Hamman et al., 1997). Second, it provides a potential explanation for how the translocon can be a site at which protein topology and folding is not just managed but actively regulated, as has been espoused by Lingappa and his collaborators (Hegde and Lingappa, 1997).

Is the presence of a TM domain sufficient to signal to the translocon that it must become more accommodating? Recent data suggest a primary role for the TM domain, but consistent with the cotranslational nature of these events, it appears that the transmembrane sequence first signals its impending arrival at the PCC while still inside the ribosome (Liao et al., 1997). Moreover, once in the PCC, the different TM domains of polytopic proteins have been shown to interact with distinct translocon components (Meacock et al., 2002). Finally, during synthesis at the endoplasmic reticulum, the prion protein (PrP) segregates into fully translocated and transmembrane fates. Direction to one or the other destinies is critically dependent on the precise timing of signal-mediated initiation of the translocation of the N terminus of the protein (Kim and Hegde, 2002). The results of Kowarik et al. (2002) suggest that cotranslational protein folding within the translocon may be the explanation underlying the necessity for precise timing. Indeed, the primary effect of disease-associated mutations in PrP may be to alter the rate or pathway of cotranslational protein folding rather than having a direct effect on topology.

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.

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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.