by mtHsp70 close to the incoming polypeptide chain and thereby can serve as a targeting factor, it becomes less clear why, in addition, a stable interaction of mtHsp70 with Tim44 is necessary. In a comparable translocation system, the posttranslational import into the ER, a protein analogous to Tim44 has not been found so far, and the pore associated J-domain protein Sec63 and the Hsp70 BiP seem to be the only components required to drive the import of polypeptides. A membrane anchor in addition to a J-domain protein may be necessary in mitochondria to guarantee a higher import rate as compared to the post-translational protein import into the ER. Such a hypothesis is supported by the finding that not only one but two Tim44 (and consequently two Pam18/Tim14 and two mtHsp70) are tethered to the TIM23 complex¹¹. In addition, Tim44 may be necessary to link Pam18/Tim14 to the pore and to present mtHsp70 in a favorable orientation to the incoming

polypeptide. Interestingly, Tim44 itself weakly interacts with unfolded proteins and was therefore proposed to correspond to the peptide-binding domain often found in J-domain proteins, in particular, since Pam18/Tim14 does not have such a domain⁶.

The fact that two mtHsp70 molecules are present at the import site poses the question how the action of the two chaperones is coordinated. The function of the presequence translocase-associated motor is even more complicated because it contains an additional essential 16 kDa subunit, called Tim16/Pam16. This mitochondrial matrix protein, which also contains a J-domain (though an incomplete one), cooperates with Pam18/Tim14 in promoting the reaction cycle of mtHsp70 (P. Rehling and N. Pfanner, K. Hell and W. Neupert, and E. Craig, personal communication).

Although these new findings represent a large step forward in the understanding of

protein import into mitochondria, they do not distinguish between the two existing models. Therefore, the Brownian ratchet versus power stroke controversy persists in the mitochondrial import field.

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E3 ligase activity and suppression of breast cancer

BRCA1 and *BRCA2* are hereditary breast and ovarian cancer-associated genes. Despite concerted effort to unravel the cellular and biochemical functions of the proteins encoded by these genes, we still do not know their precise roles. Both *BRCA1* and *BRCA2* have been implicated in DNA repair, and each interacts with Rad51, a central player in homologous recombination. *BRCA1* may also be involved in transcriptional activation mediated by the p53 tumor suppressor. *BRCA2* has no known enzymatic function. *BRCA1* as a heterodimer with BARD1 is an E3 ubiquitin ligase.

Ramin Shiekhattar and colleagues asked whether BARD1 interacts with proteins besides *BRCA1*. Their results (Dong *et al.*, *Mol. Cell* **12**, 1087–1099, 2003) have revealed that the E3 ligase complex is larger than had been appreciated. Beginning with tagged BARD1, they pulled down seven other proteins that constitute a complex termed BRCC (for *BRCA1*–*BRCA2*–containing complex). Three of the proteins are *BRCA1*, *BRCA2*, and Rad51, but the other four components do not resemble any characterized proteins.

Like the *BRCA1*–BARD1 dimer, BRCC is an E3 ligase that can ubiquitinate p53. In mammalian cells, homologous recombination occurs primarily in S phase, and phosphorylated *BRCA1* forms 'repair' foci during this stage. To determine whether *BRCA1* phosphorylation affected the composition of the complex, BRCC was isolated following treatments that induce *BRCA1* foci formation. The E3 ligase activity of the complex was not affected, but the association of Rad51 and p53 with BRCC appeared to be enhanced.

Two of the other components, BRCC36 and BRE, were examined in greater detail for their effect on BRCC structure and function. A C-terminal truncation of *BRCA1*, representative of a cancer-associated mutation, prevented interaction of BRCC36 and BRE with BRCC and destabilized the association of *BRCA2* and Rad51

with the complex. Comparison of the *BRCA1*–BARD1 heterodimer with a four-subunit *BRCA1*–BARD1–BRCC36–BRE complex shows that these additional subunits enhance the E3 ligase activity. This is significant at a cellular level because cells that are depleted of *BRCA1*, BRCC36 or BRE have a less stringent checkpoint arrest after ionizing radiation.

Finally, the authors asked whether BRCC36 might be another protein which, when mutated or dysregulated, gives rise to breast cancer. From an invasive ductal carcinoma section (upper left), cancerous cells were captured by laser microdissection (captured cells, right; tissue after procedure lower left) and used in quantitative PCR to measure BRCC36 mRNA levels. A striking 100% of tumors showed elevated BRCC36 mRNA, with >75% having levels >20-fold elevated.

Thus, the questions regarding the function of the *BRCA1*–associated E3 ligase activity are now even more complicated. With new insights into the nature of the enzymatic activity of the complex come new players to consider. Nevertheless, the results with BRCC36 in ductal carcinomas reinforce the idea that the biochemical activity of this important complex helps suppress oncogenesis in the breast.

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