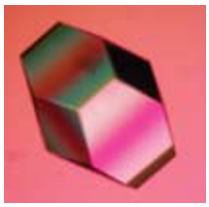
Predicting success



High-throughput protein structure–determination programs, such as those by the Northeast Structural Genomics Consortium (NESG), have made great strides in improving methodologies related to protein expression and structure determination by X-ray crystallography. However, a tremendous bottleneck to high-throughput methods is crystallization—a large proportion of the proteins that are successfully purified simply do not crystallize. Hunt and his collaborators at the NESG performed an analysis of their large-scale results to characterize what, if any, are the strong determinants for protein crystallization. The data-mining results, in which they correlated sequence properties of the proteins such as amino acid frequency, pI, hydrophobicity and hydropathy, and mean side chain entropy, suggested that the most important protein determinant that affects the outcome of a crystallization trial is the presence of well-ordered surface residues. The authors suggest that low surface entropy at the well-ordered sites would be favorable for crystal-packing interactions and found that the prevalence of these sites correlates with successful crystallization. In addition to mining the NESG data set, they also characterized the biophysical properties of many of the proteins. Surprisingly, the authors found that overall thermostability is not a strong determinant for crystallization, except in the cases of unfolded or hyperstable proteins. They also found that proteins capable of forming homogeneous oligomers had a greater chance of crystallization success and that a tendency for polydispersion inhibited successful crystallization. Although these findings are in part confirmatory of what many may have known first hand or anecdotally, this large-scale analysis establishes certain metrics that can be used to determine success probability in crystallization trials. (*Nat. Biotechnol.* advance online publication, doi:10.1038/nbt.1514, 14 December 2008) *MM*

Before the damage is done

Changes in chromatin organization at or around the sites of DNA double-strand breaks (DSBs) are known to be important to activate the DNA-damage response. Now Bustin and colleagues reveal that the chromatin binding high-mobility group protein N1 (HMGN1) modulates a key event in the cellular response to DSBs, the activation of protein kinase ataxia telangiectasia mutated (ATM) by ionizing radiation. Comparison of wild-type and *Hmgn1*$^{-/-}$ mouse embryonic fibroblasts showed that the phosphorylation levels of ATM substrates were markedly reduced in the absence of HMGN1, although DSB sensors were recruited normally to sites of DNA damage caused by ionizing radiation. ATM activation required HMGN1's ability to interact with chromatin, but ATM and HMGN1 did not colocalize, and ATM association with chromatin was actually higher in *Hmgn1*$^{-/-}$ cells, even without any DNA damage; the dynamics of the interaction of ATM with chromatin after irradiation were also altered. These data indicate that, in the absence of HMGN1, there are global changes in the chromatin architecture that alter ATM's association with chromatin and prevent its efficient activation upon DNA damage. As HMGN1 interacts with the nucleosome and can affect histone modifications, the authors looked directly at those and found that there was a global acetylation of histone H3 lysine 14 (H3K14) in response to DNA damage caused by ionizing radiation and that this effect was reduced in *Hmgn1*$^{-/-}$ cells. Strikingly, the requirement of HMGN1 for efficient ATM activation could be bypassed by treating the cells with histone deacetylase inhibitors before irradiation. Thus, this work demonstrates that the status of chromatin organization before any DNA damage occurs can influence ATM activation in response to ionizing radiation. (*Nat. Cell Biol.* advance online publication, doi: 10.1038/ncb1817, 14 December 2008) *IC*

Real-time unfolding

Many secretory and membrane proteins fold in the endoplasmic reticulum (ER). When demand for folding outstrips the ER's ability to cope, unfolded proteins build up, triggering a pathway known as the unfolded protein response (UPR). This pathway reduces the number of unfolded proteins by upregulating the genes that encode chaperones, oxidoreductases and ER-associated degradation components. Many of the pathway components are known, but uncovering their effects on the UPR in real time in living cells has been difficult. The ER has an oxidizing environment that promotes disulfide bond formation. Under ER stress, the UPR promotes oxidative folding, and so Merksamer *et al.* reasoned that redox changes could be used to indicate unfolded protein accumulation. The authors recorded real-time changes in fluorescent protein reporters in the yeast *Saccharomyces cerevisiae*. They used two reporter constructs: a redox-sensitive reporter green fluorescent protein (eroGFP) and a red fluorescent protein fused to four unfolded protein-response elements (UPR-RFP). Using flow cytometry, the authors monitored the real-time effect of different types of ER stress on ER redox status and UPR. They found, as expected, that chemical reduction causes underoxidation of eroGFP and that underglycosylation, inositol deprivation and increased protein secretion also contribute to oxidative stress. The authors propose that the ER's protein folding, modification and quality-control systems are interlinked. This approach has provided a new tool for exploring the ER, and it may have wider uses in other signaling pathways. (*Cell* **135**, 933–947, 2008) *MH*

What BRCA1's E3 ligase doesn't do

Familial susceptibility to breast and ovarian cancer is often mediated by germ-line mutations of the *BRCA1* gene. In the cell, BRCA1 is typically associated with the related BARD1 protein. While each protein displays modest E3 ubiquitin ligase activity *in vitro*, this activity is greatly enhanced upon heterodimerization. Although existing data point to a role for the BRCA1/BARD1 heterodimer in the repair, developmental and tumor suppression functions of BRCA1, it was not clear whether these processes depended on the E3 ligase activity of the heterodimer. To test this, Reid *et al.* generated isogenic embryonic stem cells that express wild type or an E3 ligase-defective form of BRCA1 that can still form heterodimers with BARD1. The fact that they were able to readily generate ES cells lacking the BRCA1 E3 ligase activity suggested that this activity is dispensable for many BRCA1 functions. Previous work showed that spontaneous chromosomal rearrangements increase in ES cells carrying partial deletions of the *Brca1* gene. However, *Brca1* E3 ligase mutant cells showed levels of chromosomal rearrangements, homology-directed repair and formation of Rad51 foci at sites of damage that were comparable to those of wild-type. So what then are the functions of Brca1's E3 ligase activity? Since the ligase mutant is expressed at lower levels than wild type, one possibility is that autoubiquitination acts to stabilize BRCA1. These studies also beg the question of whether the E3 ligase activity of BRCA1 is required for its tumor suppressing role in mammary and ovarian epithelial cells. (*Proc. Natl. Acad. Sci. USA* **105**, 20876-20881, 2008) *BK*

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