MOLECULAR NETWORKS

The power of prediction

Over the past 50 years or so, many studies of individual genes and proteins have revealed the fundamental aspects of development that we recognize today. But the recent increase of high-throughput screens and the accumulation of gene-expression and protein-interaction data sets indicates that we are not going to study single genes and proteins for much longer. Kristin Gunsalus and colleagues now predict system-level models of molecular machines that function in early development, based on interactome, transcriptome and phenotypic data sets.

But what is a suitable system to develop such predictive models and then evaluate them? The nematode *Caenorhabditis elegans* has paved the way for high-throughput approaches, because processes involved in early embyogenesis, such as cell division and polarity, are amenable to large-scale functional analysis. The authors generated network graphs in which each node represents an early embryogenesis gene and its product(s), and each edge represents a potential functional connection based on 6,572 binary physical interactions between 3,848 *C. elegans* proteins, as well as expression profiling and phenotypic similarity.

Using an algorithm, they then identified densely interconnected regions in a portion of the network — 305 nodes joined by 1,036 edges, each supported by two or three types of functional evidence — and generated two types of model representing the higher-level organization networks that underlie early embryogenesis. The first type contains links that are supported by physical interactions and phenotypic correlations and represents molecular complexes that constitute distinct molecular machines within the cell, such as the ribosome, the proteasome and the anaphase-promoting complex. On the other hand, the second type contains few protein interactions but is dominated by edges that are supported by both phenotypic and expression correlations; examples include genes involved in mRNA and protein metabolism, chromosome maintenance and meiosis.

To assess the predictive value of these models, the authors selected ten previously uncharacterized genes and

MEMBRANE TRAFFICKING

Uncovering the secrets of secretion

In eukaryotes, secreted lipids and proteins move from the *trans*-Golgi network (TGN) to the plasma membrane through a tightly controlled network of carrier vesicles. How such vesicles are generated is not well understood, but Klaus Pfizenmaier and colleagues have taken an important step forward by showing how protein kinase D (PKD) and phosphatidylinositol (PI) 4-kinase IIIβ (PI4KIIIβ) interact to promote this process.

Three PKD isoforms are present in humans (PKD1–3), at least two of which (PKD1 and PKD2) are known to help form carrier vesicles from the TGN membrane, but exactly how this occurs is unclear. The phosphorylated membrane component PI(4)P is also a known mediator of this process and is produced by PI4K enzymes. Pfizenmaier and colleagues therefore looked for a connection between PKD proteins and PI4Ks in vesicle transport from the TGN to the plasma membrane.

There are several PI4Ks in mammalian cells, but PI4KIIIβ is a homologue of a yeast PI4K, Pik1, that is known to function in the Golgi–cell-surface transport pathway in that organism — so the authors concentrated their efforts on this protein. They found that the three PKD isoforms and PI4KIIIβ co-localized at the Golgi complex, and that PI4KIIIβ was phosphorylated *in vitro* by the PKD isoforms, most notably PKD1 and PKD2. The PI4KIIIβ phosphorylation site (Ser294) that was recognized by PKD1 and PKD2 was conserved in yeast Pik1, and an antibody against the phosphorylated site was used to confirm that PKD-mediated PI4KIIIβ phosphorylation also occurred *in vivo*.

But what effect does phosphorylation of Ser294 have on PI4KIIIβ? Mutation of the phosphorylation site (S294A) reduced the lipid-kinase activity of PI4KIIIβ by ~60%. Furthermore, in agreement with the known dominant-negative effects of kinase-inactive PKD mutants, overexpressing kinase-dead PKD1 reduced the lipid-kinase activity of PI4KIIIβ. This also reduced the transport of a fluorescently labelled secretory protein from the TGN to the cell surface. Moreover, overexpressing PI4KIIIβ increased the transport of the labelled protein to the cell surface. Together, these results confirm that both the protein kinase and the lipid kinase are important for this process.

So, the authors propose that PKD functions as a 'bottleneck' at the TGN where it regulates the transport of secretory molecules by phosphorylating proteins that are involved in carrier-vesicle formation. PI4KIIIβ is one of these substrates, and its PKD-mediated phosphorylation increases its lipid-kinase activity. This, in turn, increases the amount of PI(4)P in the TGN membrane, which, in combination with membrane-bound ADP-ribosylation factor (ARF), is thought to mediate carrier-vesicle formation. Now, the next challenge for researchers is to clarify the precise mechanism of carrier-vesicle formation.

Lesley Cunliffe

References and links

ORIGINAL RESEARCH PAPER Hausser, A. *et al*. Protein kinase D regulates vesicular transport by phosphorylating and activating phosphatidylinositol-4 kinase IIIβ at the Golgi complex. *Nature Cell Biol.* **7**, 880–886 (2005)

WEB SITE

Klaus Pfizenmaier's lab: http://www.uni-stuttgart.de/izi/

analysed their potential participation in a molecular machine by visualizing their *in vivo* dynamic subcellular localization using green fluorescence protein (GFP)-tagged proteins. They tested proteins with connections to three different early embryogenesis models of the second type — centrosomal function, cell polarity and a molecular network involved in DNA replication, chromatin architecture and nucleocytoplasmic transport — and generated supporting evidence for the genes as potential new components of these molecular machines.

The authors propose that this integrated network comprising two types of model is a potential reservoir for hundreds of testable predictions about cellular processes in the early embryo. Most importantly, this approach is scalable and could be applied not only to other biological processes, but also to more complex organisms.

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References and links

ORIGINAL RESEARCH PAPER Gunsalus K.C. *et al.* Predictive models of molecular machines involved in *Caenorhabditis elegans* early embryogenesis *Nature* **436**, 861–865 (2005)

CELL DEATH

A balancing act

Following DNA damage, cells can either activate a checkpoint response or undergo apoptosis. These responses need to be carefully balanced and might depend on the level of the damage. Two groups now report in *Cell* the identification of the pro-apoptotic BCL2 family member BID as a dual-function factor with roles in both apoptosis and the intra-S-phase checkpoint following DNA damage.

Sandra Zinkel *et al.* noted pronounced chromosomal instability in a BID-deficient myeloid progenitor cell (MPC) line and showed that these cells had increased sensitivity to DNA-damaging agents, particularly to those causing replicative stress. Using BID-defective mouse embryonic fibroblasts (MEFs), Iris Kamer *et al.* observed instead a decreased sensitivity to several DNA-damaging agents. Although the effects of DNA damage must clearly be cell-type and signal dependent, both sets of observations indicated a role for BID in the DNA-damage response.

Indeed, both groups showed that $BID^{-/-}$ cells failed to accumulate in S phase following replicative stress or treatment with etoposide (a topoisomerase-2 inhibitor), and that re-expression of wild-type BID rescued this defect. Zinkel and colleagues demonstrated that rescue of S-phase accumulation does not require the pro-apoptotic BH3 domain of BID, indicating that its role in S phase is distinct from its pro-apoptotic function. Both groups also showed that BID localizes to the nucleus in response to DNA damage — providing another indication that BID has different functions depending on its subcellular localization.

Western-blot analysis of lysates of cells treated with DNA-damaging agents first indicated to Kamer *et al.* that agents causing double-strand breaks induce phosphorylation of BID. Zinkel *et al.* noted a similar effect but also in response to agents that cause replicative stress. *In vivo*, the DNA-repair kinase ATM (ataxia-telangiectasia mutated) proved to be the main kinase responsible for phosphorylating BID, whereas, *in vitro*, BID is a substrate for both ATM and the related kinase ATR (ATM and RAD3-related). Both consensus phosphorylation sites S61 and S78 are phosphorylated in the mouse BID protein.

So, what happens in S phase in BID-defective cells and how does phosphorylation of BID contribute to the DNA-damage response? To address these questions, Zinkel and colleagues carried out assays to assess replication arrest following treatment with agents that cause replicative stress. $BID^{+/+}$, but not $BID^{-/-}$, MPCs underwent replication arrest in S phase, and this result was confirmed in primary activated T cells. Wild-type and BH3-mutant BID, but not a non-phosphorylatable S78A BID mutant, were able to rescue S-phase arrest, indicating that BID phosphorylation at S78 is required for its role in the intra-S-phase checkpoint. Kamer *et al.* also showed that $BID^{-/-}$ cells expressing a non-phosphorylatable BID mutant are more susceptible to damage-induced apoptosis than those expressing wild-type BID. So, the impaired ability of mutant BID to induce cell-cycle arrest might increase the sensitivity of BID-defective cells to DNA damage.

Both studies show that the role of BID in response to DNA damage is clearly a balancing act between cell-cycle arrest (and possibly subsequent DNA repair) and apoptosis — or between cell survival and cell death — which is presumably determined by the context (including the cell type) and by the level and type of DNA damage.

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References and links

ORIGINAL RESEARCH PAPERS Zinkel, S. S. *et al*. A role for proapoptotic BID in the DNA-damage response. *Cell* **122**, 579–591 (2005) | Kamer, I. *et al*. Proapoptotic BID is an ATM effector in the DNA-damage response. *Cell* **122**, 593–603 (2005)