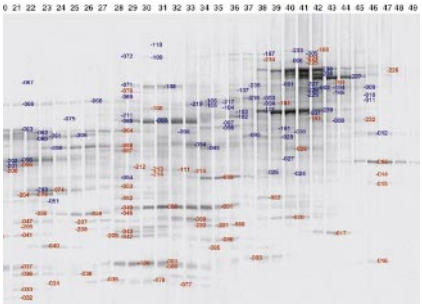
Comprehensive Mapping of the Nuclear Pore Complex

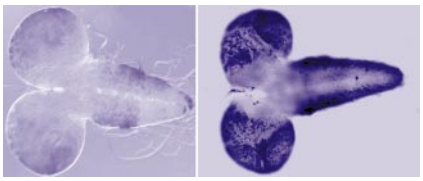
In an impressive demonstration of the combined power of high-throughput proteomics and cell biological analysis, Rout et al. (page 635) describe an exhaustive analysis of the composition and architecture of the yeast nuclear pore complex (NPC). The data present a comprehensive and definitive inventory of the core elements of the NPC, providing not only the identity of each protein component, but establishing their positions in the high resolution NPC structure published last year by Chris Akey's laboratory. Even more importantly, the paper provides a dramatic illustration of just how cell biology is emerging as the playing field on which genomic and proteomic data will be turned into biological insights.



Beginning with a subcellular fraction that is highly enriched in NPCs, the scientists performed two-dimensional separations with HPLC followed by SDS-PAGE. Each band was then analyzed by trypsin digestion and mass spectrometry, and the corresponding open reading frames were identified in a yeast genome database search. The putative nucleoporins were then epitope tagged and their localization tested by immunofluorescence and subcellular fractionation, narrowing the pool to ~30 proteins that fit the authors' definition of nucleoporins. Immunoelectron microscopy using the tagged nucleoporins allowed the team to map the architecture of the NPC, and the stoichiometric relationships between the nucleoporins were determined by quantitative immunoblotting of SDS-PAGE–separated samples. Based on their results, Rout et al. propose that Brownian motion accounts for the translocation of molecules through the NPC, and that the system's directionality is established by asymmetric nucleoporins and the asymmetric distribution of soluble transport factors.

A Bcl-2 Homologue in Drosophila

Beginning on page 703, Colussi et al. report cloning the first known Bcl-2 homologue in insects. The gene product, named Debcl, is most similar to mammalian pro-apoptotic Bcl-2 family members of the Bax subfamily. Genetic and biochemical experiments show that Debcl acts in a caspase-dependent manner and can interact with mammalian or viral pro-survival Bcl-2 proteins, providing further evidence that the pathways to cell death are conserved from invertebrates to mammals.

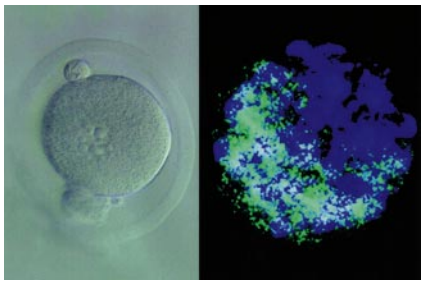


Though several components of the *Drosophila* cell death machinery have been identified, no Bcl-2–like protein has been found previously in the fly. By searching the *Drosophila* DNA sequence database, the researchers identified two putative Bcl-2 homologues, characterizing one in detail. Debcl shares significant sequence homology with mammalian Bax, a pro-apoptotic member of the Bcl-2 family, and its expression correlates with cell death during *Drosophila* development. Analysis of transgenic flies overexpressing *debcl* shows that Debcl kills by a mechanism that is caspase dependent. Since most programmed cell death during *Drosophila* embryogenesis can be suppressed by preventing expression of *debcl*, the new gene product appears to be a key regulator of cell death during fly development. Biochemical experiments show that Debcl can physically interact with many pro-survival Bcl-2 proteins, suggesting that Debcl may act by antagonizing the activity of *Drosophila* pro-survival Bcl-2 proteins.

Spatial Separation of Parental Genomes

Using two different approaches, Mayer et al. (page 629) demonstrate that maternal and paternal genomes remain topologically separated in early preimplantation mouse embryos. The findings have widespread implications for basic research in genomic imprinting and mammalian cloning, and may help illuminate the pathogenesis of human imprinting disorders.

To distinguish between maternal and paternal chromatin, the team first fed a solution of BrdU to male mice for several weeks before mating them with females. Paternal DNA in fertilized eggs and early embryos was visualized by immunofluorescence with anti-BrdU antibodies. The BrdU staining remains highly localized until at least the four-celled stage, demonstrating that the parental genomes remain separated.



In a second set of experiments, the researchers crossed the laboratory mouse, *Mus musculus*, with the wild European mouse, *M. spretus*, using differences in sequence and copy number of centromeric satellite DNAs to distinguish between maternal and paternal chromatin. Results from these experiments also show a striking separation of parental chromatin in early embryos. In more advanced embryos from interspecific

crosses, only 5–10% of the cells show segregation of the two centromere sets. Interestingly, ~10% of somatic cells from adult hybrid animals also appear to show segregated parental chromatin, suggesting that the diploid chromosome complement may be separated into two haploid sets in some somatic cell types or cell cycle stages.

New Ligand for α-Dystroglycan in Postsynaptic Membranes

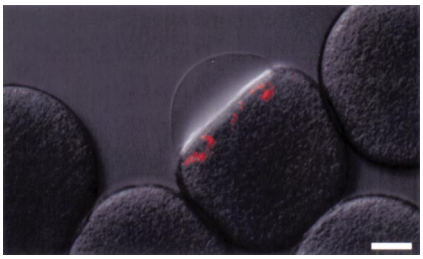
In work that may open a new path to developing therapies for muscular dystrophies, Bowe et al. (page 801) describe the identification of an interaction between α-dystroglycan and the proteoglycan biglycan. Interestingly, biglycan binds to the carboxy-terminal third of α-dystroglycan, a portion with no previously identified structural motifs or functional interactions.

Though the role of α-dystroglycan in extracellular matrix interactions has been characterized extensively, less is known about the protein's potential function in postsynaptic differentiation. The team developed a ligand blot overlay assay to search for novel dystroglycan-binding molecules in synaptic membranes from the *Torpedo* electric organ. Biochemical purification and analysis showed that one such molecule is the *Torpedo* homologue of the small leucine-rich repeat proteoglycan biglycan, and that the interaction occurs in the carboxy-terminal portion of α-dystroglycan. Binding is dependent on the biglycan chondroitin sulfate side chains, suggesting that the interaction might be regulated in vivo by posttranslational modification. Biglycan expression is elevated in muscle tissue from the dystrophic *mdx* mouse, raising the possibility that biglycan has a role in muscular dystrophy. Using the assay developed for this work, the researchers have subsequently identified additional proteins that bind to biglycan.

Coupling Exocytosis and Compensatory Endocytosis

Smith et al. (page 755) analyzed compensatory endocytosis, the process believed to be responsible for compensating for the increase in cell surface area after exocytosis. Their data show that compensatory endocytosis is only observed at sites of prior exocytosis, providing initial support for a new mechanistic model for exocytosis–endocytosis coupling.

Ion channels have long been known to regulate the calcium influx that triggers exocytosis, and voltage-gated calcium channels have been implicated in regulating compensatory endocytosis. In sea urchin eggs, fertilization activates IP3 receptors, and the subsequent release of calcium from internal stores triggers the exocytosis of secretory vesicles. Fertilization also depolarizes the membrane potential, opening P-type voltage-gated calcium channels, and the resulting influx of calcium through these channels triggers compensatory endocytosis. Using toxin inhibition of retrieval activity and immunolocalization, Smith et al. found that P-type calcium channels appear on the cell surface only after egg activation. The channels are found on the secretory vesicle membranes, which fuse with the plasma membrane immediately after fertilization. Since the channels are only found at the exocytic sites on the egg surface, compensatory endocytosis is restricted to these sites.



Based on these results, the researchers propose a model for exocytosis–endocytosis coupling in which prior exocytosis is required in addition to membrane depolarization because the P-type channels that regulate compensatory endocytosis are themselves regulated by exocytotic and endocytotic activity.

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