A view of antibody maturation

Using X-ray crystallographic snapshots of antibodies with increasing affinities for a protein antigen, Yili Li, Roy Mariuzza (University of Maryland Biotechnology Institute, Rockville, MD), and colleagues suggest that protein–protein interactions are optimized by increasing hydrophobic stickiness and improving the fit between proteins.

Protein interactions are optimized by evolutionary changes that enhance the binding energy between the relevant molecules. The immune response offers a unique opportunity to study these changes in a practical time span. During affinity maturation, B cells produce antibodies with increasing affinity for the antigen—a sort of rapid evolution of molecular evolution resulting from somatic mutation of the antibody genes. Mariuzza’s group examined the structural differences between four antibodies against a lysozyme antigen to determine how the antibodies improved their antigen-binding abilities.

They found that the number of hydrogen bonds and van der Waal contacts, often thought to be the most critical interactions at protein–protein interfaces, did not correlate with improved binding. Instead, hydrophobic interactions were key. As the antibody’s ability to bind the antigen improved, an increasing amount of hydrophobic surface was buried at the interface. The alterations also improved shape complementarity, thus filling energetically unfavorable cavities in the interface.

The residue changes that increased hydrophobic interactions and improved complementarity occurred not in the center of the contact interface, but rather at the edges. “At the center, interactions are already optimized by the germ line-encoded antibody,” says Mariuzza. “There’s no need to change those through somatic mutation. You must improve the parts that are less than ideal. That’s why optimization occurs at the periphery.” Thus, to engineer antibodies with higher affinities to target proteins, researchers should perhaps focus on mutating peripheral contacts.


GPR-1/2 support unequal division

Polarity in worm embryos, which is set early on—even single cell stage embryos already know their front from back. Recent research by the laboratory of Pierre Gönczy (Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland) is identifying how this polarity is translated into differences in cell behavior.

Polarity in worm embryos, which is set by the PAR proteins, produces an unequal first mitotic division, and thus a small posterior and large anterior blastomere. A previous screen by Gönczy identified two proteins, GPR-1 and GPR-2, necessary for this unequal division. Although direct interactions between PARs and GPR-1/2 have not been demonstrated, Kelly Colombo, Gönczy, and colleagues now demonstrate an asymmetric GPR-1/2 distribution that depends on PAR proteins. Higher levels of GPR-1/2 in the posterior are proposed to activate two Gα subunits. The group used RNAi and spindle severing experiments to show that these two Gα’s and GPR-1/2 are required for asymmetric spindle elongation, in which the posterior spindle pole moves further and more quickly than the anterior pole, thus placing the division plane closer to the posterior end.

The resulting larger anterior blastomere divides about two minutes before its posterior counterpart. In a second paper Gönczy, Michael Brauchle, and Karine Baumer show that this time lag is due in part to differential activation of a DNA replication checkpoint.

Inactivation of checkpoint proteins such as ATL-1 decreased the mitotic lag between blastomeres to about 75 s. “Usually checkpoints are used to take care of DNA replication problems. But in this case, it’s used for developmental purposes,” says Gönczy. The sizes of the blastomeres may account for the difference in checkpoint activation. When the group equalized the blastomere sizes by inactivating GPR-1/2, they again decreased the time difference to 75 s. With less cytoplasm, the posterior blastomere may be allocated fewer molecules of a limiting replication factor, and would thus have difficulties completing S phase, thus triggering the checkpoint.


Published June 2, 2003
The cross-system cytokine is IL-4, which is required in immune cells for macrophage fusion. Not one to throw away a good thing, Nature evidently coopted the system for muscle cells. As in immune cells, IL-4 expression in nascent myotubes is driven by a member of the NFAT transcription factor family. The authors identified the residue that is modified during apoptosis as a serine in the NH2-terminal tail of H2B. The antibody reacted with chromatin in dying cultured human cells and in clusters of cells undergoing apoptosis during tail resorption in developing frogs, indicating that the modification is well conserved.

The authors also show that the kinase responsible for H2B’s death stamp is Mst1, which is cleaved at the onset of apoptosis by caspase-3. Mst1 phosphorylates H2B in vitro, and the cleaved form moves to the nucleus just before H2B phosphorylation in vivo. Expression of a truncated Mst1 induced H2B phosphorylation and DNA condensation and even led to cell death in the absence of proapoptotic insults. Although it is not clear how phosphorylation and condensation are linked, the authors found that phosphorylated H2B tends to aggregate in denaturing conditions and thus may be intrinsically sticky. Alternatively, phosphate-modified H2B may recruit some as-yet-unidentified protein that condenses DNA.


**A cytokine that packs a punch**

Muscle cells undergo an unusual developmental program in which several partially differentiated cells called myoblasts fuse to form a multinucleated myotube. This nascent myotube undergoes further maturation and growth, which requires the addition of nuclei by fusion of more mononucleated myoblasts with myotubes. Valerie Horsley, Grace Pavlath, and colleagues (Emory University, Atlanta, Georgia) have found that nascent myotubes promote fusion, and thus their own growth, by secreting a cytokine normally associated with immune cells.

Myotubes lacking either IL-4 or the NFAT factor were smaller and had fewer nuclei than wild-type cells. Recovery from muscle injury was also diminished by the lack of IL-4 or the IL4α receptor.

Myoblasts are the targets of IL-4 action, which may promote fusion by inducing myoblast expression of adhesion molecules such as integrins (as in macrophages) or VCAM. Alternatively, IL-4 may act as a chemokine, as it does for osteoblasts, to stimulate migration of myoblasts toward myotubes. Whatever the mechanism, stem cell therapies for disorders such as muscular dystrophies may be improved by expression of IL-4 to increase the fusion capacity of the muscle stem cells.


**Cyclin B knows its place**

In mammalian cells the work of the cell cycle is divided between two workhorses: Cdk2/cyclin E for S phase and Cdk1/cyclin B for mitosis. Now, Jonathan Moore, Jane Kirk, and Tim Hunt (Cancer Research UK London Research Institute, London, UK) show that this apparent specificity is achieved by limiting access to substrates. By denying entrance to the nucleus, cells prevent Cdk1–cyclin B from jumpstarting S phase at inopportune times.

In frog egg extracts, S phase is induced in nuclei by Cdk2–cyclin E, but not by Cdk1–cyclin B. This difference has often been construed as specificity in cyclin substrate preferences, but the new results show that cyclin/Cdk pairs are in fact surprisingly promiscuous enzymes. Hunt’s group simply replaced the nuclear export signal from cyclin B with a nuclear localization signal and found that this altered Cdk1–cyclin B promoted both DNA replication and mitosis.

“I’m a biochemist,” says Hunt. “I tend to think in terms of specificity between substrate and enzyme. So it was a shock to find the different [CDKs] might not discriminate their substrates.” Vertebrate cells apparently avoid the danger that cyclin B might initiate S phase via its cytoplasmic localization and low levels during G1. In yeast, a single cyclin can, under some circumstances, promote both S phase and mitosis. Yeast may not have as severe a need to restrict cyclin activities because the G2 to M transition is less clear than in higher eukaryotes. “Maybe it’s okay to start mitosis early in yeast because chromosomes are still able to replicate as they are set on the metaphase plate,” Hunt suggests.