

Research Roundup

A view of antibody maturation

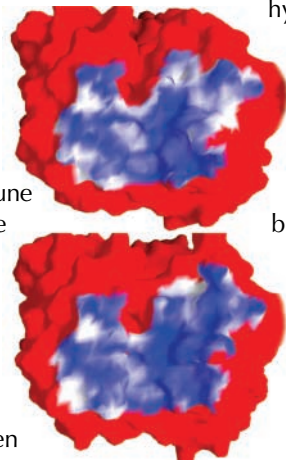
Using X-ray crystallographic snapshots of antibodies with increasing affinities for a protein antigen, Yili Li, Roy Mariuzza (University of Maryland Biotechnology Institute, Rockville, MD), and colleagues suggest that protein–protein interactions are optimized by increasing hydrophobic stickiness and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



Mariuzza/Macmillan

Antibody regions that fit snugly against the antigen, shown in blue, are more abundant in the antibody with higher affinity (bottom).

Reference: Li, Y., et al. 2003. *Nat. Struct. Biol.* 10.1038/nsb930.

GPR-1/2 support unequal division

Polarity 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.

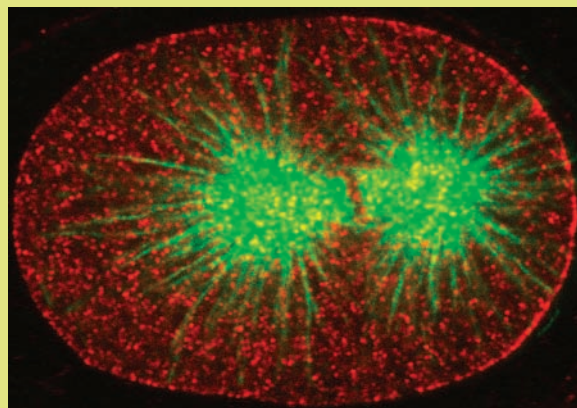
Polarity in worm embryos, which is set by the PAR proteins, produces an unequal first mitotic division, and thus a small posterior and large anterior blastomere. A previous screen by Gönczy identified two proteins, GPR-1 and GPR-2, necessary for this unequal division. Although direct interactions between 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 G α subunits. The group used RNAi and spindle severing experiments to show that these two G α '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 check-



Gönczy/AAAS

More GPR-1/2 (red) at the posterior end (right) of the embryo leads to asymmetric cell division.

point 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. ■

References: Colombo, K., et al. 2003. *Science*. 10.1126/science.1084146. Brauchle, M., et al. 2003. *Curr. Biol.* 13:819–827.