Capturing Genes

SIGHTINGS

Walk into any lab today and you can usually find a project involving an experiment on protein-protein interactions. One common theme is to search for a protein's binding partners in the hopes that they will shed light on its function. The methodology used to discover protein-binding targets has, to date, involved rather similar schemes. The basic idea is to isolate the gene encoding a binding protein by trapping it and its protein product together in an organism or virus. The two-hybrid system in yeast and the phage display system in bacteria are currently the most useful. They depend on the fact that a complex library of sequences can be hosted and expressed in an organism.

Phage display, for instance, works because each phage particle contains not only the protein target but also the gene to make additional copies of that target. You can then select for and isolate the gene indirectly with an affinity probe for the protein target that is exposed on the phage's surface. Unfortunately, these in vivo systems have their drawbacks. For instance, some peptides cannot be produced because of toxicity in vivo. Also, the complexity of the libraries cannot exceed about 10^9. What if the technology of the phage or two-hybrid systems could be reduced so as to remove all organisms from the picture—creating a totally in vitro selection system? Put another way, is there a method to covalently link a gene to its protein product in a test tube?

It is known that when a ribosome complex reaches the end of an mRNA transcript, the complex detects a termination codon. An active process then serves to terminate translation and release the ribosome from the message and nascent protein. If there is no stop codon in the message, the complex of mRNA–nascent protein–ribosome can remain intact (at least in vitro) for some time. Small molecules exist that can poison the ribosome translation machinery. One of these, puromycin, acts somewhat like a charged tRNA molecule, in that it enters the growing peptide chain. However, when puromycin is incorporated into the peptide, translation is terminated and a covalent puromycin-peptide species is released along with a free mRNA molecule.

The Szostak lab (*1*) has applied their significant expertise in RNA chemistry and in vitro evolution to create a powerful system to capture genes in vitro with probes specific to the gene's protein product. Using chemical synthesis, they first created an activated derivative of puromycin and attached this to a short DNA linker, which was then ligated to the 3′ end of a pool of RNA molecules in vitro. They correctly assumed that the ribosome would stall when it reached the RNA-DNA junction. Then, the puromycin moiety would loop back onto the ribosome's amino acid acceptor site and stop translation. In the end, a covalently joined species containing mRNA-DNA-puromycin-peptide was released into solution.

They then tested to see if they could capture the mRNA-DNA-puromycin-peptide species with an antibody targeting a protein epitope. They transcribed mRNA in vitro that encoded a portion of the *myc* gene to which a highly specific antibody has been made. In one experiment, they mixed the *myc* RNA with a pool of random messages and translated them in bulk. After selection steps which involved affinity capture with an antibody to *myc*, they amplified the selected mRNA through reverse transcription and polymerase chain reaction. Analysis of the resulting "cDNA" showed significant enrichment of the *myc* sequence over background levels.

Should this new technique be reproduced efficiently in other laboratories, it could well become a major new method of gene discovery. If past experience with technologies like phage display or the yeast two-hybrid system are any indication, the protein-binding targets discovered by the in vitro selection method is poised to yield a wealth of useful data. The technique lends itself to large-scale "fishing expeditions" and sequencing of many potential leads. In the end, the real problem may be how to handle the volume of data generated.

–Robert Sikorski and Richard Peters

References

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Cell Shocked

SIGHTINGS

Without central dogmas in immunology, it seems that the complex literature of this field would be impenetrable for the rest of us, mere mortal scientists. For example, the general mechanism by which antibodies are rearranged is well understood and has stood the test of time. Another example of a solid immunology principle is the mechanism by which antigens produce an immune response. Stop any student learning about how T cells, specifically the CD8 subset, recognize a foreign antigen and he or she will quickly recite the following: "CD8 cytotoxic T cells recognize antigens when associated with MHC class I proteins on the surface of cells. Antigens presented with MHC class I proteins are peptides that have been cleaved from endogenous proteins by intracellular proteases, which then become associated with class I MHC, and are transported to the cell surface."

The class I pathway is of particular interest because this is the pathway used by all our cells to alert the immune system to viral infection or to abnormal gene expression, as is the case with cancer. The main feature of antigen processing via the class I pathway, the dogma, is that the processed antigen is derived from a polypeptide synthesized within the presenting cell itself. In the common model, there is no cellular door through which external antigens can enter.

However, astute scientists have noticed recently that a class of polypeptides known as heat shock proteins (hsp) do not follow the above rules. Recent work has shown that one such hsp isolated from tumor cells, gp96, will produce a CTL (CD8) response even if given to cells exogenously (*1*). This surprising result provided the intellectual springboard for the development of a simple, but powerful, technique for generating a CTL-based immune response to other proteins.

The laboratory of Richard Young at MIT has taken this discovery and honed it into a general method that in theory could be used to produce a cellular immune response against any soluble antigen (*2*). To test their method, they devised a model system that consisted of a mycobacterial hsp70 protein as the "delivery vehicle" and the ovalbumin protein as a mock antigen. It was known that ovalbumin injection by itself could not elicit a CD8 T cell response. The question was: could the hsp protein fool the immune system into generating such a response?

They first created a fusion protein that linked hsp70 to the coding sequence of a large fragment of the ovalbumin gene. Next, they purified the fusion as expressed in *Escherichia coli*. This preparation was then used for injections, without the addition of an adjuvant.

In one experiment, they immunized cohorts of mice with either the hsp70-ova fusion or each of the individual components, hsp70 and ovalbumin, separately. The fusion protein generated a robust CD8 T cell response against the ovalbumin moiety, proving that the hsp component fosters the uptake of the protein and corrects internal processing. How this occurs exactly is under investigation. It could involve hsp70's ability to augment protein folding, hsp70's ability to facilitate the breakdown of intracellular proteins, or the immunogenicity of hsp70 itself.

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