

## Lactamase Live!

Technologies that can detect intracellular processes in intact, living organisms are invaluable. Visualizing and measuring one of these processes—messenger RNA transcription—is a particularly tricky undertaking, especially in real time. To yield reliable data about transcription levels, the probe must possess some critical properties. First and most obviously, the molecular probe should be nontoxic to the cells of interest. Second, the probe must be able to enter the target cell, either by direct injection, diffusion through membranes, or transport. And third, the signal generated by the probe should be strong enough such that only a few RNA copies per cell can be detected.

The only practical method for measuring RNA transcription today in real time is one that uses the green fluorescent protein (GFP) as a reporter molecule. In these studies, the GFP gene is usually placed downstream of the promoter sequence of a specific gene whose transcription is of interest. The amount of intracellular fluorescence can then be measured and used as a gauge of the level of transcriptional activity. Unfortunately, the GFP reporter system is not very sensitive, and it takes about  $10^5$  to  $10^6$  GFP molecules to allow reliable detection over background fluorescence in a single cell (1). This limits the use of GFP-based technology to studies of very strong promoters, such as those found in viruses.

Now, a group led by R. Y. Tsien has applied a few tricks of fluorescent dye chemistry to the problem of real-time transcription measurement. As reported in a recent issue of *Science* (2), they designed a new system capable of detecting as few as 100 molecules of a GFP reporter per cell.

The technique makes use of some unusual properties of dye molecules. In experiments that use fluorescent dyes, the dye molecule is typically excited at one wavelength of light and data is collected at a longer wavelength. An interesting thing can happen, though, when two different dye molecules are placed very close together. Light can be absorbed by one molecule (the donor, D), and its emission can then be immediately captured by the adjacent molecule (the acceptor, A). Light at a still longer wavelength is then emitted from the acceptor. This fluorescence resonance energy transfer phenomenon (FRET) provided the basis for the probe used in the experiments.

The Tsien group chose the enzyme  $\beta$ -lactamase as their reporter. It has a robust activity that cleaves the  $\beta$ -lactam ring of certain antibiotics, such as cephalosporins.

To produce a useful fluorescent probe that could measure  $\beta$ -lactamase activity in live cells, some new chemical synthesis was needed. They linked two fluorors, a donor D and an acceptor A, to parts of a cephalosporin molecule (where D is a coumarin and A is fluorescein). In addition, they created an esterified derivative that was nonpolar enough to cross cell membranes. By itself, D absorbs light most efficiently at a short wavelength, 409 nm, and emits at about 447 nm. One synthetic D-cephalosporin-A derivative absorbed at the low frequency of D but emitted at the fluorescence wavelength of A (520 nm), indicating efficient FRET. When the cephalosporin moiety of the molecule is cleaved by the enzymatic activity of  $\beta$ -lactamase, A and D are released free into solution. In this state, D then emits at a new frequency, 447 nm. By measuring the ratio of light emitted at 447 nm and 520 nm, the activity of the enzyme in a cell can be easily gauged. Also, positive cells can be visualized with the naked eye as blue at the shorter wavelength.

The researchers then carried out a series of experiments to test the sensitivity and specificity of their method. In one experiment, they created cell lines that differed from each other only in the amount of  $\beta$ -lactam protein they produced from a regulated promoter. They could show a 70-fold increase in activity in lines with elevated enzyme activity. They could also recover  $\beta$ -lactamase-positive cells by cell sorting. In a mock experiment, they created a mix at a ratio of one positive cell for  $10^6$  negative cells. The impressive result is that they could detect 50% of the positive cells and actually isolate 30% of them as viable clones.

As an application of the technology, they applied the system to the detection of small-molecule drugs that act to stimulate or block transcription from a promoter responsive to stimulation of the muscarinic receptor. In a test of more than 400 compounds that contained six known agonists of the receptor, they were able to detect the activity of all six of the compounds. In addition, they detected many new antagonists that are good candidates for further drug studies.

What's next for these new transcription probes? Clearly, the detection methods described by Zlokarnik *et al.* (2) can be used to screen for molecules (or genes) that effect important transcription pathways. Given the fact that the assay allows for cells to be sorted in a live form, complementary DNA selection schemes should be possible. Also, if small-molecule probes can be synthesized for other enzymes, a combinatorial approach may be possible in which multiple transcription readouts are measured simultaneously.

—Robert Sikorski and Richard Peters

## References

1. K. Niswender, S. Blackman, L. Rohde, M. Magnuson, D. Piston, *J. Microsc.* **180**, 109 (1995).
2. G. Zlokarnik *et al.*, *Science* **279**, 84 (1997).

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## Droplet Discovery

In just a few years, the concept of "genomic science" has truly transformed many fields.

The basic idea is to apply the explosion of genome-based information to the process of making new discoveries. In drug discovery, for instance, genomic-minded scientists are always looking for new methodologies to turn gene sequence information into drug targets. The buzzwords these days are "array," "massive," and "combinatorial." Researchers seek to screen in arrays, on a massive scale, using molecules made in a combinatorial manner.

To bring the power of selection to the screening process, one can use a biologic readout system, such as yeast. Here, the extensive tools afforded by genetics are a great bonus. Complex genotypes can be created that allow positive and negative selection schemes to be used to screen small molecules. One specific screening scheme in yeast is actually a variant of the widely employed two-hybrid system, where the interaction of two protein products turns on transcription. In theory, small molecules that block transcription in the two-hybrid system can be selected if the protein-protein interaction that is formed is lethal to a cell. In effect, this reverse two-hybrid system is a way of selecting for molecules that block protein-protein interactions.

Turning this simple logic into a real experimental, scalable system is not trivial for several reasons. First, the small-molecule inhibitor must be delivered to the target cells before the lethal proteins are expressed. Second, the amino acid selection schemes often used to select for plasmids in two-hybrid positive selections do not work as well in the reverse method, because amino acids made by neighboring cells can cross-feed in culture. Third, only small amounts of compounds are generated during combinatorial synthesis, so that screening schemes must be done on a very small scale to be at all practical.

An ingenious solution to all of these problems may have just come from the laboratory of S. Schreiber. In a recently published report (1), Huang and Schreiber describe a method that fuses the scale of combinatorial chemistry and genomics with tools of yeast molecular biology.



They first created plasmids that could be used in their own version of the reverse two-hybrid system. Basically, the plasmids were designed to create yeast that could synthesize fusion proteins containing the transcriptional activator B42 and the DNA-binding protein LexA in the same cell. When the two proteins dimerize via the fusion protein portion, they turn on transcription of a gene that is toxic and kills the cell. This lethal interaction was made inducible by using the Gal promoter, which can be rapidly switched on with a simple change of media.

Next, they set out to scale the system such that it would work in nanoliter cultures. To rapidly deliver small quantities of a test small molecule, they took advantage of the fact that combinatorial synthesis can be performed on small beads. By incorporating an ultraviolet (UV)-sensitive linkage into the process, they were able to make a molecule of the form drug-linker-bead. In their test case, the drug was FK506. With the photolytic effect of a short UV pulse, FK506 could be rapidly released.

To make cultures in which the small amount of released drug (about 100 pmol/bead) would have a reasonable concentration, they used a new technique that can deliver droplets of defined numbers of yeast, media, and a drug bead in 100- to 200-nl increments (2). In these nanodroplets, growth can be easily scored visually in large arrayed formats. They tested the whole system with a mock experiment by showing that FK506, a known inhibitor of the interaction between the proteins FKBP12 and TGF- $\beta$  receptor, could actually block the lethal action of this toxic two-hybrid construct.

The new nanodroplet yeast technique is significant in that it serves as a method that can bridge two fairly evolved fields, combinatorial chemistry and yeast molecular genetics, with the young field of genomics. The ever increasing number of proteins found to interact in the two-hybrid screen can now be tested in bulk for small inhibitors. Tests can even be performed on mixed populations of two-hybrid-positive proteins to sort out those interactions that can be blocked by a selected inhibitor.

—Robert Sikorski and Richard Peters

## References

1. J. Huang and S. L. Schreiber, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 13396 (1997).
2. A. B. Borchardt et al., *Chem. Biol.* **4**, 961 (1997); A. You et al., *ibid.*, p. 969.

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## Jamming Spam

No, we're not talking about some odd new cuisine here, but rather a plague: the problem of junk e-mail.

### NET TIPS

Known in the Internet culture as "spam," junk e-mail can be mass mailings to bulletin boards, newsgroups, or lists of people. While mailings from listserves that you subscribe to can be helpful, spam mailings can be annoying. Spam mail can also use up considerable resources on the Net. At some Internet service providers, as much as 10% of their resources is devoted to handling junk e-mail.

How do the spammers—the companies that distribute e-mail ads—get your e-mail address in the first place? One way is simply from online directories. These databases, such as Bigfoot (<http://www.bigfoot.com/>) and Four11 (<http://www.four11.com/>) house extensive collections of addresses. Another way is by using software robots that traverse the Net and look for addresses, focusing specifically on the @ sign in World Wide Web pages or newsgroups. This means that if you have put your e-mail address on your lab, university, or company Web page, it is fair game for the "spambots." Newsgroup fishing for e-mail addresses in particular is very easy and efficient. For example, if you post to a newsgroup discussing computer software, the spammer already has a clue to target you with junk e-mail advertising software.

How to avoid the spam? There are three different strategies: protect your e-mail address, use several e-mail addresses, or filter your e-mail.

**Strategy 1:** To protect your e-mail address, you can send your outgoing messages through an intermediate program called a remailer. This software (usually free) strips the header fields from the message so that the recipient never gets your original address. If you want the recipient to have the ability to send you a reply, you can use a remailer service that tags on its own special address, such as [msg1233@remailer.com](mailto:msg1233@remailer.com). During the return trip, the remailer then acts as a switchboard to direct the message back to you. In fact, a remailer can strip the addresses on the reverse path as well so that the entire communication is anonymous. To find out more about how to use remailers, check out <http://www.well.com/user/abacard/remail.html>.

**Strategy 2:** Use multiple e-mail addresses. If you intend to post to newsgroups or participate in mailing lists, this is an appealing option. The idea is to have a set of public e-mail addresses that are used exclusively for public purposes. That way, when the inevitable spamming comes, the

damage is localized to just these e-mail boxes. To make it even easier to switch e-mail accounts, you can take advantage of the fact that you can now get free addresses that can be accessed through a Web browser. One popular free service is Hotmail (<http://www.hotmail.com/>). The e-mail service acts like a post office box for regular mail.

**Strategy 3:** Block incoming spam with software filters. This can be done in two ways: by using the filtering capabilities of your e-mail program or by acquiring a separate anti-spam filter. In the Pro version of the e-mail program Eudora, for example, you can set any number of filters that examine the headers or body of incoming mail. If you get spam from one particular address or it has phrases such as "make money quick" in the subject, it is easy to configure a blocking filter. However, the spammers usually change their address, so you have to change the filter as well. That is where anti-spamming software is useful. This software looks at your e-mail before your mail program does. The better software is designed such that it can learn about new spamming operations and adapt. For the Mac, there is really only one anti-spammer program, called Spam Blaster (<http://www.cnet.com/Resources/Swcentral/Mac/Result/Download/0,162,39634,00.html>). For the PC, there are several. You might try SpammerSlammer (<http://www.cnet.com/Resources/Swcentral/PC/Result/TitleDetail/0,160,0-29003-g,0,0.html>).

Use one or all of the above tips, and you should be one step ahead of the spammers. Unfortunately, technology solutions work both ways, and spam operations evolve rapidly. Also, the fact that e-mail advertisement costs about one-thousandth that of printed ads ensures that spamming will continue. To find out more about spamming and anti-spam tactics, we've put together a list of useful Web sites at [www.medsitenavigator.com/tips](http://www.medsitenavigator.com/tips).

—Robert Sikorski and Richard Peters

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