PROTEIN BIOCHEMISTRY

Putting suicide genes to work

Overexpression of an enzyme that degrades *Eschericia coli* mRNA leaves lots of room for the protected expression of exogenous proteins, and makes worries about background a thing of the past.

E. coli is firmly established as a workhorse in every laboratory. It willingly takes up foreign DNA and expresses it at high levels. When it comes to giving up the fruit of its labor, however, the workhorse becomes less cooperative, and purification of exogenous proteins from endogenous *E. coli* proteins can be tedious. Masayori Inouye and his colleagues at Robert Wood Johnson Medical School, found a way to change that by enzymatically persuading *E. coli* to get rid of all its endogeous mRNA and to focus entirely on the production of the foreign protein (Suzuki *et al.*, 2005).

In this method, Inouye is exploiting a system *E. coli* developed to help it through times of stress. The bacterium continuously expresses the suicide operon, encoding the toxin mazF and the antitoxin mazE; in times of abundance these two proteins form a complex and the toxin is neutralized. If, however, *E. coli* encounters stress, protein production is halted and mazE, the less stable of the two, is degraded. This leaves the toxin to recognize the specific sequence ACA on any cellular mRNA and destroy it, pushing the cell into a dormant state during which it does not grow but can wait out the crisis.

Inouye's team, less interested in helping *E. coli* through the tough times, but more intrigued by the implications of destroying all cellular mRNA, overexpressed mazF together with a gene of interest in which all ACA sequences had been replaced by silent mutations. This ACA-less mRNA was translated very efficiently, while all endogenous mRNA had disappeared, resulting in *E. coli* cells filled almost exclusively with the protein of interest.

Inouye emphasized two applications of his system. The first is the study of protein structure in their natural environment by NMR. He explains, "We don't even want to break open the cell, but [instead] try to discover the protein structure inside the cell." The second involves finding new jobs for *E. coli*. "We think this system can be used to convert a living cell into a bioreactor," says Inouye. "It won't be restricted to proteins, it could be producing small biochemical molecules." Expression of mazF would ensure that all the endogeous mRNA is destroyed and the cell could channel all its energy to making, for example, a specific amino acid.

Despite all these intriguing possibilities, *E. coli*, being a bacterium, has difficulties correctly folding and modifying eukaryotic proteins. Therefore, Inouye is now working on adapting his method of single protein production also to yeast and mammalian cells. It remains to be seen whether these will be the same willing workhorses that *E.coli* has been.

Nicole Rusk

RESEARCH PAPERS

Suzuki M *et al.* Single protein production in living cells facilitated by an mRNA interferase. *Mol. Cell* **18**, 253–261 (2005).

VIROLOGY

How do your prions grow?

A system for the rapid amplification of misfolded prion oligomers provides new evidence supporting the prion hypothesis and may offer a powerful diagnostic tool.

The prion hypothesis remains controversial even now, almost ten years after earning discoverer Stanley Prusiner the Nobel Prize in Medicine. The idea of an infectious protein that is capable of propagating its own misfolding to trigger the formation of proteinaceous plaques and neurological disease runs athwart much of the conventional wisdom of infectious disease research, and although much evidence has accrued to support this hypothesis, scientists on both sides continue to search for data that could conclusively prove or disprove the prion hypothesis.

One supporter of the hypothesis, Claudio Soto, has dedicated much effort to the study of scrapie, an animal disease linked to misfolded prion protein (PrP). In 2001, Soto's group described the development of the 'protein misfolding cyclical amplification' (PMCA) assay, in which brain tissue from scrapie-infected hamsters is incubated with uninfected brain tissue, triggering the misfolding of native PrP, after which the sample is disrupted by sonication and rediluted into normal brain tissue for further amplification of PrP misfolding (Saborio *et al.*, 2001).

Now, Soto and his colleagues at the University of Texas Medical Branch describe the optimization of PMCA (Castilla *et al.*, 2005), rapidly performing numerous rounds of scrapie PrP amplification to an extent that they are eventually left with purely *in vitro*–generated misfolded protein. This amplified protein not only has the same biochemical properties as misfolded PrP in samples from scrapie-infected hamsters, but is also infectious and capable of efficiently inducing scrapie in hamsters that is indistinguishable from the natural disease.

Soto sees potential for PMCA as a sensitive tool for clinical detection of the presence of misfolded prion protein. "This is a cyclical process," explains Soto, "and you can do as many cycles as you want, depending on how much you need to amplify the signal in order to see it. In a way, it's very similar to PCR."

He also feels that his group's findings lend strong support for the prion hypothesis. He acknowledges that not all prion skeptics will be converted: "Some people... I don't believe they will be convinced by anything." But, he adds, "if you want to blame something to be the infectious agent behind [a] disease," says Soto, "the best way to do that is to isolate the infectious agent, cultivate it *in vitro*, and show that this... will maintain and produce infectivity in a live animal—and this is exactly what we have done here."

Michael Eisenstein

RESEARCH PAPERS

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Castilla, J. *et al. In vitro* generation of infectious scrapie prions. *Cell* **121**, 195–206 (2005).