Synthetic Protease Switch

SIGHTINGS

Every graduate student has been quizzed at least once on the life cycle and genetics of the bacterial phage lambda. This virus is perhaps the most well-characterized piece of genetic information in all of biology. Over the years, lambda has served mainly as a dependable laboratory workhorse for everyday molecular biology experiments. This bacterial virus is well suited for cloning purposes and can propagate complex mixtures of exogenous DNA of a convenient size. For genetic experimentation, sophisticated assays of gene and protein function have been designed based on the molecular components of the virion. The protein components of lambda gene regulation and their interactions are known at the most detailed level. In fact, many of these proteins have been examined at the atomic level through x-ray crystallography. Investigators can use their molecular knowledge of lambda to design experiments that manipulate the regulatory circuits in ways that produce defined results.

The lambda life cycle consists of two distinct phases, lytic and lysogenic. The lytic phase is characterized by infection of a bacterial host followed by intense phage replication and the production of a large number of progeny. Released virions infect adjacent cells and amplify quickly. The lysogenic phase is characterized by infection and integration into the host genome. No progeny are produced, and the phage is effectively dormant. A molecular switch composed of transcription factors and proteases controls which direction a phage will go. Sices and Kristie at the National Institutes of Health (NIH) took advantage of their knowledge of the molecular switch to alter it so that it could now be used to detect the presence of a specific protease within a bacterium (*1*).

The lambda repressor protein is a DNA-binding protein that binds to a sequence of DNA adjacent to the lytic genes. When the repressor is functional, lytic gene products are silenced and the phage enters a lysogenic phase. The repressor also binds upstream of a negative regulator of its own transcription, a gene called *cro*. Together, the concentrations of lambda repressor and *cro* protein determine whether a phage will lyse a bacterium or just remain dormant. An endogenous bacterial protease, RecA, cleaves the repressor at a specific molecular hinge region. When cleaved, the repressor cannot complex efficiently with DNA. Lytic genes are then turned on, and the phage produces infectious progeny and kills the bacterium.

How does one modify this system to study other proteases? First, the NIH group altered the repressor gene sequence to insert a restriction site at the natural RecA protease–sensitive site in the protein. Testing in vivo, they showed that the mutant form was now resistant to RecA cleavage. Next, they used the inserted restriction site to create a new protease target site in the repressor that was patterned after one of the target sites of the human immunodeficiency virus–1 (HIV-1) protease (repressor-HIV). The basic idea was to make a synthetic molecular switch in lambda that could be toggled by introduction of active HIV-1 protease.

They next introduced the HIV-1 protease (in a phage with an intact *cro* circuit) along with a plasmid that contained repressor-HIV. Gel electrophoresis showed the cleavage of the repressor-HIV polypeptide in vivo. In addition, examination of the growth rates of the HIV protease–containing phage showed that they replicated 7000 times more efficiently than controls. To see if they could use this growth advantage to select for a protease-containing phage in a complex mixture of phage in vivo, the researchers designed a mock experiment. They infected bacteria with a 10^5 mixture of control to HIV-1 protease phage and examined plaques for the presence of the protease. Amazingly, after only three rounds of selection, 40% of all phage contained the HIV-1 repressor.

They also went on to show that drugs that act as inhibitors of the HIV-1 protease would also alter the synthetic molecular switch in vivo. In fact, the inhibition by drugs such as crixivan seen in the phage assay paralleled the effective concentrations of the drug determined in humans.

The system described should be useful for selecting a protease in a mixed population of clones, as would be done in a cloning experiment. It can be used to test inhibitors of the isolated proteases or of known proteases. Because proteases are involved in a wide range of biological processes, from apoptosis to Alzheimer's disease, the new assay could have broad utility. This work also shows that the old lambda system still has more uses up its molecular sleeve.

–Robert Sikorski and Richard Peters

References

1. H. Sices and T. Kristie, *Proc. Natl. Acad. Sci.* **95**, 2828 (1998).

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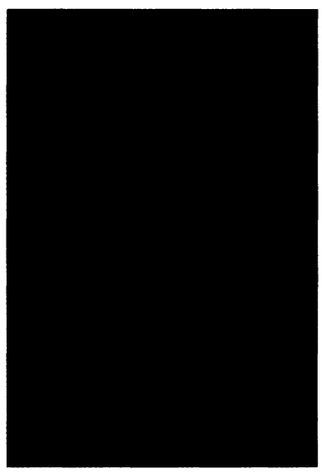
Beauty in Simplicity

SIGHTINGS

Each year, cardiovascular diseases cause approximately 12 million deaths worldwide. In developed countries, heart attack is the number one killer. The problem is simple on paper: Clots or plaques block the arteries that supply the heart itself. To open these blockages, hundreds of thousands of patients each year undergo some sort of revascularization procedure such as percutaneous coronary angioplasty or coronary artery bypass surgery. Researchers have been busy trying to come up with new methods to improve revascularization of damaged heart tissue.



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One experimental approach involves angiogenic factors that stimulate the growth of new blood vessels. Several methods are being investigated to achieve this. They include delivery of growth factors systemically, via gene therapy, or locally with microspheres. Intravenous administration of growth factors [such as fibroblast growth factor (FGF) or vascular endothelial growth factor (VEGF)] is the simplest method, but, because of intravascular dilution, large amounts of proteins have to be delivered, which may result in potentially dangerous side effects (such as hypotension). The promise of gene therapy has been that growth factors could be expressed locally in high concentration by targeting genes to damaged areas only. However, the technology behind gene therapy is still quite complicated, and it continues to face significant challenges in progressing to a clinical setting.

A more straightforward approach has been to use drug-containing microspheres to deliver growth factors. Several groups have attempted this by delivering the beads systemically or directly implanting them in the heart during open heart surgery. Intracoronary delivery of microspheres coated with basic FGF (bFGF) has been done in infarcted swine myocardium, and has resulted in increased angiogenesis (*1*).

Unfortunately, the beads also caused extensive myocardial necrosis, probably because of their large size (75 to 150 mm). Could smaller beads that would lodge more distally in the terminal capillaries provide a safer delivery system? A report by Arras *et al.* seems to show that this is indeed the case (*2*). The approach they used is elegant in