Baculovirus Bounty

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Autographa californica nuclear polyhedrosis virus (AcMNPV), the Rosetta stone of baculoviruses, is being used to decipher biological mysteries in organisms irrelevant to its own existence, such as human beings. This service to humanity is occurring even though we know little about AcMNPV's interactions with its own hosts, certain species of larval lepidopteran insects (moths and butterflies).

AcMNPV can survive outside its hosts: It becomes sequestered within proteinic crystals that protect it from desiccation. The AcMNPV-encoded crystal protein, polyhedrin, is produced late during infection in considerable quantities by virtue of the very strong transcriptional promoter of the polyhedrin gene. Because polyhedrin is not essential for the production of progeny virus (only for survival outside the host), and because insect cells can add eukaryotic post-translational modifications to proteins, enterprising baculovirologists have used the polyhedrin promoter for expression of foreign gene products (*1*). These baculovirus expression vectors (BEVs) are now used routinely in many basic research laboratories throughout the world (*2*). They also form the core of many commercial biotechnology enterprises aimed at the production of vaccines, therapeutics, and diagnostic reagents. More recently, BEVs have become the systems of choice for evaluating the effects of amino acid sequence alterations on protein function in drug discovery research. This wide use of BEVs for medical research is ironic considering that baculoviruses do not even infect vertebrates, and demonstrates the inherent difficulty in making accurate predictions regarding areas of research and dividends in application.

During the last several years, industrial development of BEV technology has been impressive, but only in areas most germane to the rapid development of commercially viable products. Industry has employed chemical engineers to develop scaled-up production of AcMNPV-permissive cells in culture, and molecular virologists to improve the existing repertoire of BEVs. However, the power of basic lepidopteran insect cell biology and physiology has not yet been tapped. For example, at a recent conference on baculovirus gene and insect cell expression a series of speakers lamented that the secretion of their BEV-expressed products declined during late stages of infection. The wild-type protein, polyhedrin, is not secreted, but BEV users often employ vectors with engineered secretory signals that facilitate purification of their products. The fact that the host insect cells have a compromised secretory system is thus not a problem for the virus, only for BEV users (*3*). The depolymerization of microtubules is also characteristic of AcMNPV-infected cells late in infection (*4*), but it is not known whether this depolymerization causes the secretory deficit—even though microtubules clearly are essential for secretion in mammalian cells.

The initial justification for studying AcMNPV and other baculoviruses was to develop their potential as biopesticides. This activity is still supported by industry because of the public's demand for effective, more selective, safer pesticides consistent with sustainable agriculture. Baculoviruses are superior to other viruses as pest control agents because their host range is limited to arthropods and because many infect larval lepidopteran insect pests. Thus, the use of baculoviruses for insect pest control is practically devoid of the risk of infecting humans and other vertebrates.

In spite of this advantage, progress on baculovirus pesticides has been slow. This is because wild-type viruses typically are less efficacious than classical insecticides due to the fact that these viruses kill their hosts too slowly: Insects die several days to weeks after initial infection. By standard infectious disease criteria, death within days or weeks would not be considered slow. Infected caterpillars, however, can consume a great deal of plant matter and inflict significant crop damage within a few days. When a well-fed host finally does succumb to infection, the virus wins the jackpot because the caterpillar becomes a blob of viscous tissue containing 200 million or so viral occlusions, sufficient (in the case of AcMNPV) to infect 100 million more *Trichoplusia ni* larvae (*5*). A quicker death almost certainly would lead to fewer viral progeny; thus, a quick-killing virus would be at a disadvantage outside of the dominion of pest managers. BEV-based technology has been used to "improve" AcMNPV killing times by the addition of genes encoding various insect-specific toxins, hormones, and hormone-regulating enzymes (*6*). Further improvement might be achieved with increased understanding of how this virus interacts with its host at the organismal and cellular levels. Very recently, results from studies with reporter genes have revealed that after primary infection of the midgut, the insect's respiratory (tracheal) system is the major conduit for viral movement from tissue to tissue (*7*). Indeed, insects can be infected through their spiracles, "valves" bridging the tracheal system and the external environment (*8*). Viral pesticides administered through spiracles could bypass the midgut and get a head start on systemic infection.

One fascinating aspect of the baculovirus-host interaction was discovered in a study of the AcMNPV ecdysteroid UDP-glucosyltransferase (EGT) gene (*9*). Ecdysone is an important caterpillar hormone that initiates molting both from one larval instar to the next and from the last larval instar to the pupa. The product of the viral EGT gene inactivates ecdysone and, at sufficient concentrations, blocks pupation. This is important because adult insects and pupae are not permissive for baculovirus infections. Hence, *egt* expression enables the virus to prevent its host from pupating into a resistant animal.

Resistance to viral infection also can be achieved by apoptosis. AcMNPV encodes a protein (p35) that prevents apoptosis in some of its hosts (*10*) and also in *Caenorhabditis elegans*, mammalian neural cells in culture, and in the developing eye of *Drosophila* (*11*). p35 acts by inhibiting the proteolytic activity of human interleukin-1β converting enzyme (*12*). Thus, p35 represents another AcMNPV-derived tool valuable for research in numerous organisms.

The good news is, the bountiful harvest of baculovirus-based treasures has just begun.

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