Gene expression platforms for lepidopteran cells as tools for functional annotation of genome sequences, basic research and added-value applications

L. Swevers, R. Efrose, P. Tsitoura, K. Kousis, C. Magrioti, V. Labropoulou, K. Ioannidis and K. IATROU
Insect Molecular Genetics and Biotechnology Group, Institute of Biosciences & Applications, National Centre for Scientific Research "Demokritos" 153 10 Aghia Paraskevi, Athens, Greece
iatrou@bio.demokritos.gr

With the successful completion of the silkworm genome sequence determination, the great challenges for the future are the annotation of the available sequences and their exploitation for research and value-added applications. While *in silico* analysis is a major tool for sequence annotation, robust functional testing tools are required in order to validate postulated functional properties. This talk will summarize how available gene expression platforms developed from genetic elements of lepidopteran insects and viruses infecting these organisms may be used as effective tools for the functional annotation of genes of hypothesized or unknown function mined from the available genome sequences of the domesticated silkworm and other lepidopteran insects.

Two types of broad expression platforms will be described. The first system is a plasmid-based expression platform that employs various combinations of genetic elements of the silkmoth and its baculovirus (BmNPV) in modular configurations. This system is used for transient or stable transformation of cell lines derived from multiple lepidopteran species. Because of the modular nature of this system, the levels of expression of selected genes may be modulated according to need over a range spanning at least three orders of magnitude. Examples of the usage of this system for expressing genes encoding various secreted, intracellular and membrane anchored proteins of insect origin including "difficult to express" transmembrane and nuclear receptors will be presented. These examples will also illustrate how the coupling of such an efficient system for recombinant protein expression with various reporter platforms that can report reliably on the activity of different classes of proteins allows the validation of functions predicted by the *in silico* annotations.

The second type of expression platform, which is under continuous development, is based on the use of recombinant baculovirus-based (BmNPV and AcNPV) transducing nanoparticles, which incorporate expression cassettes similar to some of the plasmid-based expression platforms. The distinctive feature of these vectors is that they incorporate in their genome elements capable of reconstituting a transposition system directing the excision of the expression constructs from the recombinant baculovirus genome and their stable integration into the genome of the cells of the transduced host insects. These vectors have the major advantage of allowing expression of recombinant proteins for prolonged periods of time in a variety of tissues of the transduced insects. Since an essential requirement for this group of transducing vectors is the non-lethality of the baculovirus nanoparticles for the transduced insects, the infections are carried out in a species non-reciprocal manner. Thus, given the extremely narrow host range of BmNPV, recombinant BmNPV nanoparticles are used as transducing vectors for essentially all lepidopteran species except the domesticated silkworm. On the other hand, recombinant AcNPV nanoparticles are used for non-productive infection of silkworm larvae. Besides deducing biological functions for functional annotation purposes, the transducing baculovirus nanoparticles should also be ideal tools for long-term production of proteins of added value in lepidopteran larvae using simple larval injection procedures.