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An integral process for the production of virus-like particles by insect cells

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Virus-like particles (VLP) are produced when the structural proteins of a non-enveloped virus are expressed in a recombinant system. As a result, particles identical to the native virus, but devoid of the viral genetic material, are obtained. VLP are useful as vaccines, as carriers, and for basic research. Their production represents a challenge, as several proteins need to be simultaneously expressed and assembled into a complex structure. Other aspects that complicate process development include the difficulty of accurately quantifying complete VLP, as well as possible intermediates, and the difficulty of designing effective purification procedures.

The versatility of the insect cell-baculovirus expression vector system has made it one of the most frequently used systems for the production of VLP. In this work, our recent advances on the production of double-layered rotavirus-like particles (dlRLP) by insect cells will be presented. The use of a viral vector, such as the baculovirus, allows the manipulation of the concentration of the recombinant proteins by manipulating the multiplicity of infection, allowing the production of rotavirus proteins at a stoichiometry that maximizes protein assembly [3]. *In vivo* analysis of the accumulation of rotavirus proteins in insect cells showed that assembly occurs intracellularly. However, differences in the intracellular distribution of rotavirus proteins when they were individually expressed suggest that the formation of RLP involve more than only the contact between two proteins [2]. We will present *in vivo* studies of the assembly or rotaviral proteins, where we have identified and characterized the limiting steps and intermediaries in the assembly process. Finally, tools for the characterization and quantification of dlRLP and assembly intermediates will be presented [1], along with the development of a purification scheme for the 'preparative characterization of dlRLP. The results obtained and the several strategies presented are an example of the characteristics, limitations and specific requirements of animal cells in culture.

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