

Perspective

Cellular virology

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Viruses need cells

Viruses cannot multiply without infecting cells. At each round of infection they must fall apart, deliver their genome to the proper site of replication and transcription and then rebuild infectious particles from scratch. As they are relatively 'simple' parasites, they require many host cell functions for the various steps of their life cycle. These include the host cell translation machinery, cellular translocation and sorting machinery to target their glycoproteins to their proper cellular destination. As viruses also lack a lipid-synthesizing machinery, they need host membranes to acquire their lipid envelope. Finally, with the exception of poxviruses, DNA viruses require the cellular nucleus for replication.

Although viruses strictly depend on cells, they have evolved the ability to use their host in the most effective ways. They use cellular cues to go through 'programmed' disassembly steps, with the aim of delivering their genome precisely at the right site of replication (Smith and Helenius, 2004). They subsequently assemble into stable structures within these same cells, obviously avoiding the disassembly cues previously used to allow the particle to fall apart. Cells respond to virus infection by inducing signalling events that favour cellular defence, often leading to cell death. Viruses have not only evolved strategies to prevent cellular apoptosis, but also to use cellular defence reactions to their benefit (Wurzer *et al.*, 2003). To travel faster through the cellular cytoplasm, they manipulate the cytoskeleton and cellular signal transduction pathways (Suomalainen *et al.*, 2001). They induce the proliferation of specific cellular membranes to increase surface areas and provide platforms for replication/assembly (reviewed in Salonen *et al.*, 2005). Considering that most viruses consist of no more than 5–10 proteins, their

impact on cells is really quite dramatic and fascinating at the same time.

Because various steps of viral life cycles mimic cellular processes, viruses have been and are being used as tools to study cellular processes. The advantage of viruses is that they highlight or induce certain cellular processes that are hard to study otherwise. The GFP-tagged version of the G glycoprotein of vesicular stomatitis virus, in particular its temperature sensitive Ts045 variant, has been used extensively to study transport from the ER to the Golgi complex, also in real time (Presley *et al.*, 1997; Scales *et al.*, 1997). The initial finding that the HA protein of influenza virus was insoluble in triton-100 in the cold (Skibbens *et al.*, 1989) opened up a whole field of research on rafts (Simons and Ikonen, 1997). The G protein of VSV and the HA of influenza, that are targeted to the basolateral and apical plasma membrane, respectively (Rodriguez-Boulan and Pendergast, 1980), have been widely used to gain insights into sorting in polarized cells. The small DNA virus, simian virus 40, revealed new insights into the dynamics of caveolae and the delivery of their cargo to the (smooth) ER, apparently bypassing the Golgi complex (reviewed in Pelkmans and Helenius, 2002). Clearly, without studying viruses we would know far less about cells. Obviously, knowledge about cells also helps us to understand viruses. As viruses require many different cellular functions, understanding their life cycle requires understanding cells as a whole. Thus, among cell biologists, the 'cell-virologist' is necessarily an 'all-rounder'.

Cell biology of viruses

In 2003 an estimated 3 million people died of HIV, 2 million of tuberculosis and 1 million of malaria, emphasizing that pathogens have a major impact on the world population (source: WHO). For each of these three 'big-killers' no effective vaccine is available, despite extensive (and in the case of HIV, well-funded) research. The emergence of newly evolved viruses that are more virulent (and deadly) than those they derive from, such as the coronavirus SARS and the increasing concern about a new influenza pandemic, emphasize that 'developed' countries are only

an aeroplane flight away from any life-threatening virus that may develop in other regions of the world.

Taking HIV as an example, only limited information is available on the interactions of this virus with the cells it infects. Although the overall sequence of events are known, many details of the steps of entry, uncoating, transport to the nuclear pores, nuclear export and transport to the site of budding remain to be elucidated. Similarly poorly understood are how these different steps of the HIV life cycle interact with, use and/or manipulate cellular structures such as membranes and the cytoskeleton. HIV primarily infects cells of the immune system and was long thought to leave these cells by budding at the cell surface. Two recent studies, however, showed by EM that in macrophages, the primary site of budding occurs in multivesicular endosomes, rather than at the plasma membrane (Raposo *et al.*, 2002; Pelchen-Matthews *et al.*, 2003). The latter structures are known, upon certain stimuli, to release their content as exosomes into the extracellular medium. An attractive implication of these observations is that HIV exploits the regulated exosome release of antigen presenting cells to 'present' infectious virus to T cells, which are subsequently infected. While this deadly scenario still needs to be proven, these studies have led to a stream of confocal microscopy studies with the aim to explain why HIV buds at different sites in different cell types. While this may be an important question to address, other parts of the HIV life cycle remain under-focused and consequently poorly understood.

The above exemplifies the state of most viruses; we virtually lack detailed cell biology of the majority of viral life cycles. This also implies that for anybody interested in the cell biology of a particular virus, there are many new things to discover, often using relatively simple tools. Is it important to understand viruses at the cellular level? Although some virus infections can be effectively prevented with vaccines, there are very few drugs that effectively prevent infection. A reasonable prospect therefore is that new insights into the complex life cycle of viruses may identify targets of new antiviral drugs. Furthermore, it is simply fascinating to study how viruses have evolved to use cells in the most effective way to go through basic steps, common to all viral life cycles.

Opportunities for cell biology in virology/microbiology

Understanding cellular virology means that the researcher needs to be both a 'virologist' and a cell biologist. Unfortunately, classically these two domains have been separated to a large extent into different journals, meetings and institutions. A young researcher is likely to start in either of the two disciplines and it is still an exception that virology is done in an environment of cell biology. This young

researcher may have to 'jump' many spatial and psychological hurdles to cross over to the other field. I argue that there is an urgent need to 'marry' these two disciplines more tightly. Fortunately, for those interested in the cell biology of pathogens there are an increasing number of opportunities. In Europe (as well as in the USA, without doubt) EURESCO- and EMBO-sponsored meetings are organized on a regular basis whose aim is to bring cell biologists and microbiologists together (see for instance Izaurrealde *et al.*, 1999; Roy and van der Goot, 2003; Sodeik *et al.*, 2005). These meetings are on the basis of small groups that meet with plenty of time to discuss and exchange ideas and reagents. Along this same line, *Cellular Microbiology* represents yet another opportunity to create a platform for cell biology in virology. In the near future we will publish reviews by Beate Sodeik and Stephan Ludwig on how viruses manipulate the cytoskeleton and signalling cascades. I furthermore greatly encourage any person to get into the cell biology of any virus and to submit details of their life cycles and how these interact with cells to *Cellular Microbiology*.

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