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Systems biology of virus infection in mammalian cells

Editorial overview

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Lucas Pelkmans obtained a master's degree in Medical Biology in 1999 from the University of Utrecht, The Netherlands, and received his PhD in Biochemistry from the ETH Zürich in 2002. During that period he used time-lapse imaging and single-particle tracking of fluorescent virus particles to study the infectious entry route of Simian Virus 40, and studied basic mechanisms and properties of caveolar membrane domains. From 2003 to 2005 he was a postdoctoral fellow at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden, Germany, where he continued to study caveolae assembly and dynamics at the cell surface using total internal reflection microscopy. There, he also performed the first comparative RNAi screens of virus infection in mammalian cells, namely Simian Virus 40 and Vesicular Stomatitis Virus, and on the properties of the endocytic entry routes that these viruses use. In 2005, he became an assistant professor at the ETH Zürich in Molecular Systems Biology (www.imsb.ethz.ch/researchgroup/plucas). In 2007 he cofounded the company 3-V Biosciences (www.3vbio.com), which uses RNAi screening to find targets in the host instead of the pathogen to fight viral infections. Currently, his laboratory studies heterogeneity patterns of infection in populations of mammalian cells, how this is determined, and which molecular mechanisms are involved.

The host cell in virus infection: in the past a major focus for some — now for many

In the 1960s, Samuel Dales did groundbreaking work when he started to examine cells exposed to mammalian viruses with electron microscopy [1]. He was able to visualize a wide range of different mammalian viruses interacting with their host cells, from which he concluded that all these viruses were taken up into the cell by endocytosis, and that this was necessary for the viruses to infect their host cell. Even more, in his models they already indicated that enveloped viruses would fuse their membrane with the limiting membrane of endocytic organelles to pass the membrane barrier. Look for instance at Dales' drawings in a review in 1973 [1].

Similarly, the work by Ira Herskowitz and colleagues in the 1970s on phage infection in *E. coli*, can be regarded equally groundbreaking and ahead of time. Although using a system much simpler than that of virus infection in mammalian cells, it is remarkable to read his conclusions on how the environment of the host cell may influence processes in virus infection, and how that involves cellular signaling and host cell components [2]. Much of these topics are still highly relevant today and poorly understood.

Today, the host cell receives a lot of attention in the virology field. This may sound trivial to a molecular cell biologist, but it is still something not every virologist feels confident with. Viruses, because of their simplicity and ease to purify and to obtain in large quantities, are great objects of study, in particular for structural analysis, and for use in *in vitro* assays. This revealed many basic aspects of membrane fusion and pore formation, capsid assembly, DNA and RNA replication, transcription, and translation. As a consequence, we now understand a great deal of viruses, often with structural models that very accurately describe the molecular changes over time as they occur in these *in vitro* assays.

However, one can easily come up with a number of questions that remain unanswered, even at this basic level. For instance, does Influenza Hemagluttinin (HA) undergo the same structural rearrangements as in the model when it actually arrives in an endosomal compartment with the pH necessary to trigger the change? This is certainly expected, but we do not know it for certain. And, how many HA molecules on the envelope of an individual Influenza virus particle will actually undergo structural rearrangements when it is entering a living cell, and how fast, and how stereotypic are these steps for a population of virus particles?

To address such questions, one needs to apply methods to measure dynamic events over time, with single-molecule resolution and within living cells. Clearly, fluorescent molecular sensors and single-particle and single-molecule

tracking in living cells are needed here. With the current development of super-resolution light microscopy [3], and the possibility to measure dynamic molecular events in tissues and large populations of cells [4], virologists can indeed now address these questions.

Such work is a logical continuation of the earlier work done on virus particles *in vitro*, and will confirm, expand or reject our molecular models of viruses during entry, replication, and egress. But, those experiments demonstrate a multitude of phenomena that current models would never have predicted. As we observe virus particles on the plasma membrane, inside endocytic organelles, moving along microtubules, and undergoing membrane fusion, we see a daunting complexity of very heterogeneous behavior. While some of that heterogeneity might be caused by the fact that a population of virus particles demonstrates a certain variation in genetic information, structural assembly, shape, size, and number of functional molecules on their outer surface, the host cell determines most of it.

The host cell in virus infection and the systems approach

We now know that most viruses indeed require endocytosis for infectious entry [5], and, ironically, even the paradigm virus thought to fuse with the plasma membrane, HIV, has now in several publications been reported to depend on endocytosis for infectious entry [6]. However, our textbook models of virus entry are today, from a host cell point of view, not that different from the drawings done by Samuel Dales 40 years ago. Yes, we know a number of cellular molecules involved, and we understand the importance of certain cues that the host cells give to a virus particle, such as low pH, to trigger particular steps in the stepwise entry program of a virus. But we can still not predict which route a virus will take into the cell, and on which host factors it will rely. This stands in contrast to the molecular biology of virus replication itself, which, once the genetic information is known, can be better predicted.

To fill in that gap, many virologists are currently implementing discoveries from molecular cell biology labs in experiments with their favorite virus. This is an important development, which will generate a wealth of new information, especially when combined with super-resolution imaging methods. Indeed, the recent literature contains ample examples of such studies. But how are we going to integrate all this information? And more importantly, how far should we go in trying to measure every detail of the virus infection process? Should we try to find principles that explain behaviors at a more global level, and that reveal properties of the infection process from a 'systems-level' point of view? Perhaps, such principles are more universal and more predictive, will focus us on general rules, and will make it unnecessary to scrutinize all aspects of virus infection behavior.

From –omics, RNAi screens and interactomes to predictive models of virus infection and evolution

In this special issue of *Current Opinion in Microbiology*, I gathered opinions from researchers who take very different angles at this problem. Perhaps the first impression after reading all contributions is that we are only at the beginning of a paradigm shift. We need to learn each other's languages, and to think about how we should integrate all the large datasets being generated right now. Michael Katze and colleagues nicely describe the challenges in systems biology to map out the relevant information, to integrate it, and to apply data-driven approaches to obtain predictive models of virus infection. Even more challenging will be to come up with strategies by which a complex physics approach to explain and model virus particle dynamics in living cells, as outlined by David Holcman and his colleagues, could be combined with large-scale perturbation and interaction screens as outlined by Sara Cherry, and Jürgen Haas and colleagues.

Clearly, the experimental and methodological limitations are substantial. The (to some shocking) realization that RNAi screens on the same virus infection process performed in different labs reveal very different sets of host cell factors makes that very clear. Is it only the technology that has problems, or should we change the way we interpret and compare genetic perturbations in cell populations? Similarly, to which extent must we expect variation in a virus–host cell interactome, and how can we know which interactions do actually take place in a particular cell when virus particles are infecting it? In addition, what do changes in the host cell transcriptome averaged over millions of individual cells mean for the virus infection process in an individual cell? What are responses of the cell, and what are purposely virus-induced changes in the cell?

All those questions bear the fundamental deeper question of whether variation in a set of measurements is of a stochastic or of a deterministic nature. That this is a relevant question is clear from both David Holcman's article and Leor Weinberger's contribution. Brownian motion needs to be included in models to describe and predict the collective behavior of virus particles inside cells, but (ironically) implies that the exact behavior of an individual virus particle can never be fully predicted. Similarly, the transcription of genes is to a certain extent stochastic, making it fundamentally impossible to fully predict this activity. Uncertainties also exist in protein–protein interactions, phosphorylation reactions, and the behavior of organelles, to name a few.

In *in vitro* biochemical experiments, or in experiments involving millions of cells that were lysed before analysis, we were blind to this variation, which undoubtedly has led to many wrong interpretations. Part of the discrepancy

between results from large-scale experiments of today, most of which still generate averaged measurements *albeit* in a high-throughput scale, can probably be related to this problem. Thus, one challenge for the future of large-scale experiments is to develop methods to quantify variation of activities and molecular interactions in single cells, and to reveal rules that can be used to predict this variation. Analogous, since we are now able to follow individual virus particles, individual host cell molecules and organelles, and we can monitor the transcription of individual mRNA molecules and the translation of individual proteins inside individual living cells, we will also need to understand the causes of variation in these processes.

What is purely given by chance, and what is determined by factors in the host cell. Ultimately, we will need to understand how virus infection, when considered as the activity of a very complex cellular process, can behave deterministic even though each individual component of the process displays a certain degree of intrinsic noise. These systems properties of virus infection will, once understood, greatly contribute to a more complete understanding of virus infection in mammalian cells, including our capacity to predict the dynamics, heterogeneity, and the reactions of virus infection to molecular perturbations.

In trying to elucidate these mechanisms it will eventually be helpful to consider virus infection in the context of evolution. The contribution by Luis Villareal nicely outlines the concept of how viruses may have actually driven evolution and survival of their host. Although it is clear that we are far away from understanding this at a molecular and mechanistic level, we should keep in mind that the complexity of interactions between host cells and viruses is the product of coevolutionary processes in the past, and also contains the properties that will allow further coevolution in the future. At the end of the day, 'nothing in biology makes sense except in the light of evolution' [7].

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