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HIV’S VOYAGE INSIDE THE CELL

A few years ago, McDonald and Hope amazed virologists with impressive movies of HIV particles progressing along microtubules inside infected cells, on their way to the nucleus. In this issue, Charneau and colleagues offer another view of the intracellular whereabouts of HIV, with increased precision and scope. Whereas previous imaging studies used HIV particles labeled via a GFP tag on a viral accessory protein, Charneau’s group has found a way to label HIV Integrase protein without perturbing its function. Because the Integrase remains associated with the viral genome until its integration in the host-cell chromosomes, this labeling method permits to follow HIV complexes within the nucleus. Combined with sophisticated imaging software, this labeling strategy reveals details of the viral cytoplasmic movements never observed before.

Article p825

Investigating hESC culture heterogeneity

Before human embryonic stem cells (hESCs) can be implemented in their many promising therapeutic applications, a thorough understanding of how to maintain their pluripotency and self-renewal in cell culture is needed. hESC populations can display surface expression marker heterogeneity, which is often undesirable for practical applications. Rather than using drug-based or manual selection, Bhatia and coworkers offer an alternative flow cytometry–based method to isolate individual hESCs based on marker expression. These sorted cells can be regenerated into new hESC cultures by seeding onto a layer of fibroblast-like feeder cells derived from the parent hESC line. They found that these clonal colonies were self-renewing and retained pluripotency.

Article p807, News & Views p782

The new state of small-molecule discovery

The present small-molecule discovery paradigm is to screen large libraries of compounds for activity against a selected protein target. The systems-wide effects of small molecules, for example on an entire signaling network, is a concept just beginning to be appreciated. MacBeath and coworkers present a new strategy they term ‘state-based discovery,’ which identifies active small molecules based on the quantitative ‘state’ they induce in a signaling network. This strategy involves incubating cells with small molecules from a library, stimulating the target network with an appropriate ligand, arraying the cellular contents, and probing the lysate microarrays with different antibodies to assess protein levels. They demonstrate the implementation of this strategy by assessing the quantitative phosphorylation state of the ErbB signaling network in response to kinase and phosphatase inhibitors.

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Off-target effects in RNAi screens

Off-target effects are a well known limitation of RNA interference (RNAi) in mammalian cells—a limitation that requires investigators to include extensive controls in RNAi experiments. The ideal controls, however, may constitute a logistical challenge in large-scale RNAi screens. In a Commentary, a multi-institution group of investigators involved in genome-scale screens discusses what the best controls should be in such experimental settings, and they open the floor for a community debate on the question. An Article by Kulkarni and colleagues in this issue also reinforces the need for such debate by showing that, in contrast to a common assumption, off-target effects are a prevalent problem in screens performed in Drosophila melanogaster cells, where dsRNAs are the RNAi triggers of choice.

Commentary p777, Article p833

Spheres of influence

The so-called ‘neurosphere assay’ is widely used when cultivating neural stem cells. In some instances, the formation of spheres in clonal culture conditions has been used to ascertain and even quantify the presence of stem cells in a neural cell population. Now a study by Snyder’s group calls this practice into question by showing that under culture conditions conventionally used for the assay, neurospheres are not necessarily clonal entities. In contrast, they are dynamic and routinely merge with each other. These observations point to the risk of relying solely on the neurosphere assay to demonstrate ‘stemness.’

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