

RESEARCH HIGHLIGHT

Membrane traffic in the post-genomic era

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Abstract

A multi-parametric genetic screening approach sheds light on integrated control of the endocytic pathway in mammalian cells.

A defining feature of eukaryotic cells is the presence of an elaborate network of internal membrane compartments that communicate between themselves and with the cell surface via specific membrane fission and fusion reactions [1,2]. Such 'membrane trafficking' processes can be viewed as a network of intracellular transport pathways, whose operation is critical to normal physiology and disturbed in disease. A major goal in the field of cell biology, therefore, is to elucidate the mechanistic basis of these fundamental membrane trafficking processes and how they are regulated. Historically, genetic approaches have been instrumental in this effort, particularly forward genetic screens in model eukaryotes, such as budding yeast, by the traditional route of mutagenesis, phenotype selection, and subsequent identification of the affected gene. Such screens have led to the identification of a variety of essential proteins mediating membrane traffic in the biosynthetic pathway of yeast, many of which have orthologs in mammals [3,4].

A long-standing barrier to more comprehensive analysis of membrane-trafficking processes in mammalian cells has been the relative intractability of these cells to forward genetic analysis. The main barrier is that mammalian cell culture lines, unlike yeast, cannot be maintained in a haploid state. Therefore, traditional genetic methods based on mutations in the genome, because they typically disrupt only a single copy of a particular gene, rarely produce a screenable phenotype. This barrier is beginning to break down, however, based on the development of alternative methods. The sequencing and annotation of animal genomes, combined with the use of

RNA interference (RNAi) to knock down specific gene expression, are ushering in a new era of forward genetic analysis that extends to mammalian cells [5]. A recent study published in *Nature* from Marino Zerial's group in Dresden (Collinet *et al.*) [6], illustrates how such approaches are beginning to be applied to study the integrated function of the endocytic pathway in human cells.

Major gaps exist in our understanding of membrane traffic in mammalian cells compared with that in yeast. Such gaps quickly become evident when one begins to consider how membrane trafficking is integrated with other essential cellular processes. Endocytic membrane traffic is essential not only for 'classical' functions such as nutrient uptake from the extracellular milieu, but also plays critical roles in a wide range of superficially unrelated processes. One of the best recognized of these relationships is with cellular signal transduction. Multicellular life is dependent on a diversity of receptor-mediated signaling mechanisms, and animals have greatly expanded the representation of signaling receptors in their genome compared with yeast. Membrane trafficking of many signaling receptors in the endocytic pathway is essential for the proper organization and regulation of downstream information transfer. Such effects are not only critical for organized cell-cell communication under normal physiological conditions, but disturbances in the endocytic trafficking of receptors play a causative or supporting role in disease states such as cancer. There is also compelling and accumulating evidence for regulation in the converse direction - of the membrane machinery by signaling - at multiple stages of both the membrane-biosynthetic and endocytic pathways [7,8].

Analysis of endocytic pathways in mammalian cells

The main new advance introduced in the study of Collinet *et al.* is automated phenotyping of the endocytic pathway, using quantitative fluorescence microscopy. The investigators applied this method to carry out unbiased analysis of the phenotypes produced by knocking down gene expression using RNAi. Using the HeLa human cell line, Collinet *et al.* monitored two receptor-mediated endocytic processes - the uptake of the iron-transport protein transferrin bound to its receptor and the uptake of epidermal growth factor (EGF) bound to its receptor (a receptor tyrosine kinase), which are important to cellular

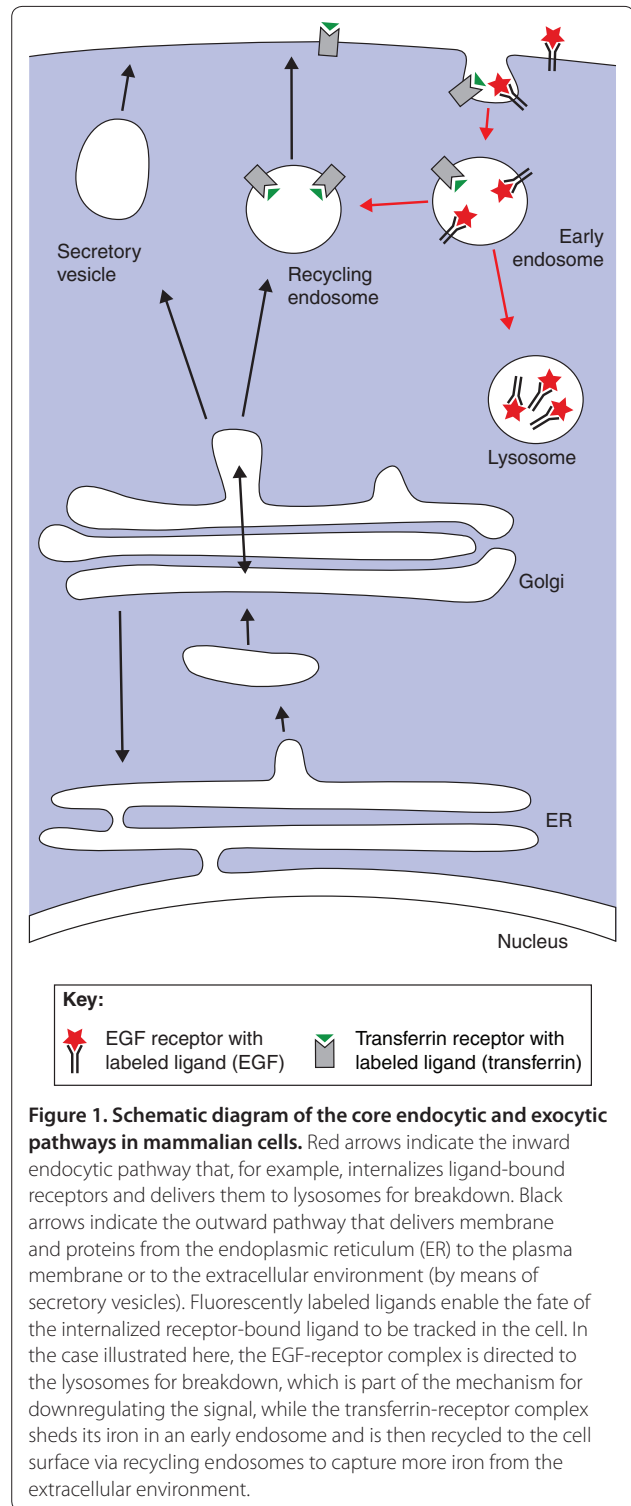
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nutrition and cellular signaling, respectively (Figure 1, which also illustrates the core membrane-trafficking pathways in the cell). Endocytosis of these two receptor-ligand complexes is thought to utilize much of the same 'core' endocytic machinery, yet each pathway differs significantly in its regulation, and in the specificity with which internalized ligands are trafficked to different internal membrane compartments (Figure 1). These two processes are also a good choice from the experimental perspective, because fluorochrome-conjugated ligands enable the visualization of these processes by fluorescence microscopy.

Fifty-eight different parameters describing, for example, vesicle amount, size and intracellular distribution, were extracted using a computer-controlled algorithm from automatic confocal images of HeLa cells. The investigators screened multiple libraries of synthetic small interfering RNAs (siRNAs) and an endoribonuclease-prepared siRNA (esiRNA) library, each covering every human gene several-fold. Cluster analysis of these 58 parameters led to 10 parameter groups describing distinct classes of endocytosis phenotypes. This approach resulted in 161,492 knockdowns and around 2.5×10^6 confocal microscope images, requiring 4.5×10^6 computing hours on a 2,584-core computer cluster to analyze. This is, first, an approach that excludes the subjective bias of a human observer. Second, the multi-parametric description of phenotypes potentially allows the detection of effects on endocytosis that would be missed by more conventional approaches, which are typically limited to relatively severe (or lethal) phenotypes. Taking advantage of their multi-parametric analysis, combined with deep coverage of the expressed genome, the authors have developed an impressively rich database of the effects of genetic disruption on the endocytic pathway in a human cell line.

What emerges from this analysis is both exciting and cautionary. On the exciting side, the authors identified a remarkably large number of genes - more than 4,000 - whose knockdown reliably affected some parameter of the endocytic analysis. On the cautionary side, this is a remarkably high hit rate - around 15% of the coding genome. The authors emphasize that their goal was not to identify particular genes that directly mediate a particular trafficking step or pathway but, instead, to develop a larger genetic profile that would enable appreciation of integrated 'design principles' of the endocytic pathway in mammalian cells. From this perspective, the list of implicated genes supports the existence of exquisitely close relationships, both direct and indirect, between the endocytic pathway and diverse cellular processes.

Returning to the question of how membrane trafficking is related to signal transduction, Collinet *et al.* identified



a particularly large number of genes that encode signaling receptors and mediator proteins. For example, the primary hit list includes a large number of seven-pass transmembrane receptors, including 'orphan' receptors whose physiological significance is currently not

established. The potential of this forward genetic screening approach to reveal new links in the signaling-endocytosis nexus is indeed very exciting. On the cautionary side, some of the identified hits (such as several neuropeptide receptors) are thought not to be expressed at significant levels in HeLa cells. Thus, despite the careful attention paid to verifying hits with multiple siRNA targets, the possibility that the current list still includes a number of false positives must be kept in mind. All in all, the recent work by Collinet *et al.* represents a bold and interesting effort, with great potential but also significant challenges.

In future studies we can anticipate integration of the strategy used by Collinet *et al.* with proteomics and protein biochemical methods, which will help distinguish direct from indirect genetic effects and provide insight into biochemical mechanisms. Further advances in automation and computational power may allow practical genetic analysis of endocytic effects produced by changes in the cellular environment or the activation of particular signaling pathways. We can also look forward to extension of the genetic approach to paired or combinatorial knockdowns, which may help organize the large number of hits identified into coherent genetic pathways. Such analysis could also provide crucial insight to the significance of hits representing the remarkably large number

of human disease-linked genes identified, most of which have not been implicated previously in endocytosis. The paper by Collinet *et al.* indeed makes a bold step into the future, and provides an intriguing preview of a new era in cell biological research.

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