It's HIP to be a hub: new trends for old-fashioned proteins

Manuela Vecchi[1] and Pier Paolo Di Fiore[1,2,3]

[1]Fondazione Istituto FIRC (Fondazione Italiana per la Ricerca sul Cancro) di Oncologia Molecolare, 20139 Milan, Italy
[2]Dipartimento di Medicina, Chirurgia ed Odontoiatria, Universita' degli Studi di Milano, 20122 Milan, Italy
[3]Istituto Europeo di Oncologia, 20141 Milan, Italy

Many endocytic proteins shuttle between the nucleus and the cytoplasm; however, their putative function in the nucleus is unclear. Now, new data demonstrate that huntingtin interacting protein 1 (HIP1), an endocytic protein, modulates the transcriptional activity of nuclear hormone receptors. In network theory, therefore, HIP1 can be regarded as a hub connecting heterogeneous functional "territories:" a possibility with important physiological and pathological implications.

By textbooks' definition, endocytosis is a process through which cells internalize plasma membrane, surface receptors and ligands, bacterial toxins, immunoglobulins, viruses, and various soluble molecules. Yet, evidence is accumulating for a wider role of the endocytic machinery. For instance, in the case of signaling receptors, endocytosis is not only required for attenuation (removal of receptors from the cell surface) but also for optimal coupling of receptors with intracellular signaling effectors (Sorkin and Von Zastrow, 2002).

A more mysterious connection derives from observations that various endocytic proteins shuttle between the nucleus and the cytoplasm, with a possible involvement in transcription (Benmerah et al., 2003). However, there is no knowledge of the putative transcriptional targets and of their biological relevance. A better defined mechanistic case was reported for two endosomal proteins, APPL1 and 2, which, in response to signaling stimuli, translocate to the nucleus and interact with the nucleosome remodeling–histone deacetylase complex NuRD–MeCP1 (Miaczynska et al., 2004). However, it is not known whether APPLs are true endocytic proteins. Finally, Numb, an endocytic protein (Jafar-Nejad et al., 2002) probably involved in receptor recycling (Smith et al., 2004), is translocated to the nucleus by Mdm2, a p53 regulator. Numb is an antagonist of Notch, a receptor involved in cell fate determination and in differentiation/survival (Roegiers and Jan, 2004). Thus, Numb might integrate a complex circuitry involving receptor recycling, Notch function, and p53 stability—a scenario awaiting experimental corroboration.

Correspondence to Pier Paolo Di Fiore: pierpaolo.difiore@ifom-ieo-campus.it

The described findings point to some endocytic–nuclear connection. The picture, however, is still blurry, particularly in the following: there is no evidence so far for a well-defined biochemical/biological nuclear function of an authentic endocytic protein; by-and-large, endocytic proteins are not concentrated in the nucleus at steady state, and their shuttling can be unmasked only by inhibiting nuclear export with Leptomycin B; in the majority of cases, no signaling stimuli were identified that could induce or modulate the process; and shuttling endocytic proteins display nuclear export sequences, but no clear nuclear localization sequences (NLS). True enough, they can be translocated to the nucleus through association with other NLS-containing proteins. And yet, if they exert any function in the nucleus, why was there selective pressure for efficient extrusion and not for import? Are endocytic proteins actually "not desired" in the nucleus?

On page 191 of this issue, Mills et al. (2005), report observations that tie up several of the loose ends. They focus on huntingtin interacting protein 1 (HIP1), a player in the early step of endocytosis that, together with the related protein HIP1R, establishes connections between the forming pit and the actin meshwork (McPherson, 2002). Mills et al. (2005) show that, in prostate cells, HIP1 translocates to the nucleus in an androgen-dependent fashion. HIP1 associates with the androgen receptor (AR) and enhances the effects of the AR on the transcriptional activity of a known AR-inducible promoter. The effect is blocked by an antiandrogen drug, indicating that HIP1 directly modulates AR transcriptional activity. HIP1 also coactivates the transcriptional ability of other nuclear hormone receptors, including the estrogen and glucocorticoid receptors. Importantly, the authors established a direct, androgen-dependent association of HIP1 to androgen response elements. HIP1 also displays a putative NLS. More work will be needed to establish the relevance of this NLS on the nuclear translocation of HIP1 or of the HIP1–AR complex. Reassuringly, however, a mutation in the NLS abrogated the coactivator function of HIP1. Thus, HIP1 constitutes the first clear-cut example of a bona fide endocytic protein with a molecularly defined tie to a nuclear event (Fig. 1).

It is evidently too early in the game to draw general conclusions. However, data from Mills et al. (2005) tell us that, among all possible nuclear functions, endocytic proteins might be involved in regulation of gene expression. This begs the next "finalistic" question: why? Some speculative frameworks can be put forward.

The Journal of Cell Biology, Vol. 170, No. 2, July 18, 2005 169–171
http://www.jcb.org/cgi/doi/10.1083/jcb.200506039

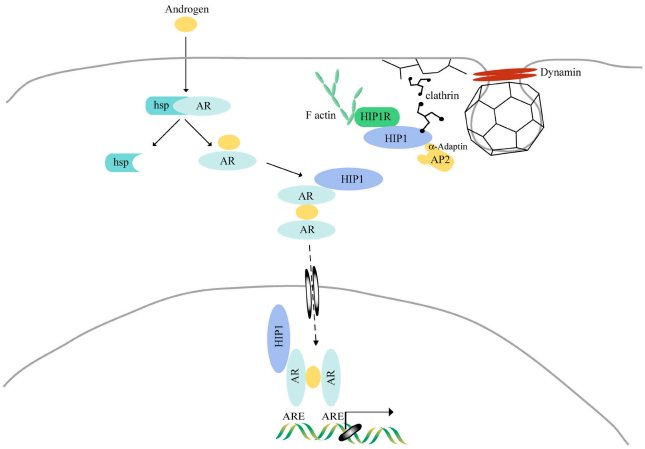

Figure 1. **HIP1 as a hub.** The molecular interactions of HIP1 in endocytosis (right) and regulation of androgen-dependent transcription (left) are depicted.

First, it is still endocytosis after all. Endocytic proteins might control the transcription of a limited set of genes involved in endocytosis and traffic. Prompted by the work of Mills et al. (2005), and without any claim to comprehensiveness, we looked at the presence of androgen response elements in the first 1,000 nucleotides preceding the ATGs of all human genes. We identified 227 genes, with no particular enrichment in genes encoding known endocytic/traffic proteins (unpublished data).

Second, coordination of endocytosis with other cellular processes. At some levels this possibility must be obviously true, if nothing else because two cellular processes (endocytosis and transcription) would be competing for common effectors. The issue, however, is whether the two processes are mechanistically interdependent; i.e., whether some "higher" cellular program needs their simultaneous execution. Initial evidence, although not denying this possibility, does not directly support it.

Mills et al. (2005) report that ~50% of HIP1 undergoes AR-dependent nuclear relocalization. This is in line with what was shown for other endocytic proteins upon Leptomycin B treatment (Vecchi et al., 2001). Although these results do not negate the possibility of "coordination," they suggest that there is functional segregation between the pools of endocytic proteins that work in the cytoplasm and in the nucleus.

The two functions of endocytic proteins can be apparently executed independently of each other. Mills et al. (2005) mutated two conserved lysine residues in the ANTH phosphoinositide-binding domain of HIP1. As expected, this mutant displayed reduced association to clathrin-coated vesicles. However, its transactivation ability was unperturbed and actually modestly increased. Similarly, it was previously shown that endocytosis and nucleocytoplasmic shuttling of endocytic proteins are independent processes (Vecchi et al., 2001).

Third, networks, hubs, and moonlighting. The definition of protein interaction maps is revealing interesting features. These maps are structured into areas of local networks connected by hubs. These hubs, represented by single proteins, are nodes displaying a large number of interactions. When a hub connects proteins in the same functional "territory," or belonging to two "affiliated" territories (e.g., coated pit formation and actin cytoskeleton), it is straightforward to infer its significance. In the present case, however, the two processes (endocytosis and transcription) are not immediately affiliated. It should be noted, however, that hubs are multifunctional connectors, frequently displaying many protein interaction modules. If hubs are scaffold organizers, then the cell might have found it convenient to use the same scaffold to organize different processes. Thus, the transcriptional function of HIP1 (and possibly of other endocytic proteins) might represent a true moonlighting job. This does not deny that the convergence of two heterogeneous processes on the same hub might have deeper physiological meanings. However, it means that, for many practical purposes (and if the hub is not rate limiting), the two functions can be studied separately.

Regardless of the physiological scenario (or combination of them), there are important implications for pathology. HIP1 is altered in human cancers and thought to contribute to the malignant phenotype through perturbation of receptor trafficking (Hyun and Ross, 2004). It is of note that other endocytic proteins are altered in cancer (Hyun and Ross, 2004), frequently as partners of fusion proteins in leukemia, which is a disease clearly associated to perturbations in transcriptional regulation. Furthermore, HIP1 was originally identified as an interactor of huntingtin (htt), a protein mutated in Huntington's disease. Mutated htt has a decreased affinity for HIP1, suggesting that this diminished interaction might play a role in the pathogenesis of the disease. Again, this has been connected to alterations in membrane traffic, leading to synaptic dysfunction, and also to a role of HIP1 in apoptosis (another cellular process enters the scene!), leading to neurodegeneration (Gervais et al., 2002). Mills et al. (2005) now open the possibility that transcriptional deregulation might be part of the pathogenetic involvement of HIP1 in cancer and neurodegenerative diseases. There might be a lesson here: hubs connecting heterogeneous cellular processes might constitute weak links in the cellular master plan in that alterations of a single protein might contribute more than one step to the pathogenesis of complex diseases.

Work in the authors' lab is supported by Associazione Italiana Ricerca sul Cancro, Human Science Frontier Program, European Community (VI Framework), Fondazione Monzino, and Italian Ministries of Health and of Education and Scientific Research.

Submitted: 6 June 2005
Accepted: 16 June 2005

References

Benmerah, A., M. Scott, V. Poupon, and S. Marullo. 2003. Nuclear functions for plasma membrane-associated proteins? *Traffic.* 4:503–511.

Gervais, F.G., R. Singaraja, S. Xanthoudakis, C.A. Gutekunst, B.R. Leavitt, M. Metzler, A.S. Hackam, J. Tam, J.P. Vaillancourt, V. Houtzager, et al. 2002. Recruitment and activation of caspase-8 by the Huntingtin-interacting protein Hip-1 and a novel partner Hippi. *Nat. Cell Biol.* 4:95–105.

Hyun, T.S., and T.S. Ross. 2004. HIP1: trafficking roles and regulation of tumorigenesis. *Trends Mol. Med.* 10:194–199.

Jafar-Nejad, H., K. Norga, and H. Bellen. 2002. Numb: "Adapting" notch for endocytosis. *Dev. Cell.* 3:155–156.

McPherson, P.S. 2002. The endocytic machinery at an interface with the actin cytoskeleton: a dynamic, hip intersection. *Trends Cell Biol.* 12:312–315.

Miaczynska, M., S. Christoforidis, A. Giner, A. Shevchenko, S. Uttenweiler-Joseph, B. Habermann, M. Wilm, R.G. Parton, and M. Zerial. 2004. APPL proteins link Rab5 to nuclear signal transduction via an endosomal compartment. *Cell.* 116:445–456.

Mills, I.G., L. Gaughan, C. Robson, T. Ross, S. McCracken, J. Kelly, and D.E. Neal. 2005. Huntingtin interacting protein 1 modulates the transcriptional activity of nuclear hormone receptors. *J. Cell Biol.* 170:191–200.

Roegiers, F., and Y.N. Jan. 2004. Asymmetric cell division. *Curr. Opin. Cell Biol.* 16:195–205.

Smith, C.A., S.E. Dho, J. Donaldson, U. Tepass, and C.J. McGlade. 2004. The cell fate determinant numb interacts with EHD/Rme-1 family proteins and has a role in endocytic recycling. *Mol. Biol. Cell.* 15:3698–3708.

Sorkin, A., and M. Von Zastrow. 2002. Signal transduction and endocytosis: close encounters of many kinds. *Nat. Rev. Mol. Cell Biol.* 3:600–614.

Vecchi, M., S. Polo, V. Poupon, J.W. van de Loo, A. Benmerah, and P.P. Di Fiore. 2001. Nucleocytoplasmic shuttling of endocytic proteins. *J. Cell Biol.* 153:1511–1517.