The Sla2p/HIP1/HIP1R family: similar structure, similar function in endocytosis?

Irit Gottfried*, Marcelo Ehrlich† and Uri Ashery*†

*Department of Neurobiology, Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69978, Israel and †Department of Cell Research and Immunology, Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69978, Israel

Abstract

HIP1 (huntingtin interacting protein 1) has two close relatives: HIP1R (HIP1-related) and yeast Sla2p. All three members of the family have a conserved domain structure, suggesting a common function. Over the past decade, a number of studies have characterized these proteins using a combination of biochemical, imaging, structural and genetic techniques. These studies provide valuable information on binding partners, structure and dynamics of HIP1/HIP1R/Sla2p. In general, all suggest a role in CME (clathrin-mediated endocytosis) for the three proteins, though some differences have emerged. In this mini-review we summarize the current views on the roles of these proteins, while emphasizing the unique attributes of each family member.

Introduction

CME (clathrin-mediated endocytosis) is a well-studied mechanism involved in many important cell functions, such as the continuous uptake of essential nutrients [1,2], recycling of synaptic vesicles [3], modulation of signal transduction by controlling the levels of surface receptors and by serving as a mechanism for signal compartmentalization [4,5]. The coordination of coated-pit assembly, cargo inclusion and vesicle scission requires the dynamic interaction of many proteins, one of which is HIP1 (huntingtin-interacting protein 1). The mammalian HIP1 was identified in 1999 as an interactor of huntingtin, a protein that when mutated is involved in the genetic neurodegenerative disorder Huntington’s disease [6,7]. The mutated huntingtin has reduced affinity for HIP1, suggesting that the impaired interaction could be part of the disease mechanism [7]. HIP1’s yeast homologue, Sla2p, had been described a few years earlier as a regulator of membrane cytoskeleton assembly [8], and a third member of this family, HIP1R (HIP1-related), was identified on the basis of structural homology [9,10].

Sla2p/HIP1/HIP1R structure and function

Common to the structure of all three Sla2p/HIP1/HIP1R proteins is an N-terminally localized ANTH (AP180 N-terminal homology) domain, a central coiled-coil domain, and a talin-like domain at the C-terminus [6,7,9] (Figure 1). However, despite their structural similarity, experimental evidence suggests functional diversity among these three related proteins.

The classical E/ANTH domains of epsin 1 and AP180 strongly bind PtdIns(4,5)P2 [11,12]. Moreover, this protein–lipid interaction plays a determining role in their recruitment to the membrane (the presumed site of action for endocytic proteins). Similarly, the ANTH domain of Sla2p was found to preferentially bind PtdIns(4,5)P2 [13]. However, deletion of the ANTH domain did not prevent Sla2p’s localization to cortical endocytic patches, suggesting its recruitment to be dependent on additional protein–protein interactions [13]. Though, despite being dispensable for recruitment, the interaction of Sla2p’s ANTH domain with PtdIns(4,5)P2 was found to be required for the organization of actin at the endocytic site [13]. Interestingly, the ANTH domains of mammalian HIP1 and HIP1R do not preferentially bind PtdIns(4,5)P2, but PtdIns(3,4)P2 and PtdIns(3,5)P2 [14], suggesting interactions with membranes that have different lipid contents. This is puzzling as PtdIns(4,5)P2 is considered to be an interaction hub in the clathrin interactome [15]. Furthermore, our unpublished results and others’ suggest a high degree of co-localization of HIP1 and HIPR with additional structural components of the clathrin-coated pit. Thus, either there exists a diversity in lipid composition in the immediate proximity of the coated pit or additional molecular elements play a determining role in the recruitment of HIP1 and HIP1R to the coated pit (discussed below).

The talin-like domain, also called the I/LWEQ module or the THATCH domain [16], is an actin-binding domain found in proteins that serve as linkers between the actin cytoskeleton and cellular compartments. The talin-like domains of Sla2p and HIP1R have been shown to bind F-actin (filamentous actin) [16–20], and indeed, several mutations in these proteins caused disruption in actin binding or organization [16,17,21,22]. In contrast, controversy exists as to the actin-binding capabilities of the HIP1 talin-like domain. While Legendre-Guillemin et al. [19] did not find significant actin binding by HIP1, Wilbur et al. [18] reported actin binding that was approx. 7.5 times weaker than that of
HIP1R, and Senetar et al. [20] found HIP1’s affinity to actin to be very close to that of HIP1R. Notably, no disruption of actin organization has so far been described on alteration in the expression levels of HIP1. It is important to note that the actin-binding capacity of HIP1 and HIP1R is regulated by both intramolecular and intermolecular interactions. The USH (upstream helix) domain, which is present in all three proteins, inhibits actin binding by an intracellular inhibitory mechanism [20]. Moreover, the proline-rich domain, which is present in HIP1R but not HIP1, mediates its binding to the SH3 (Src homology 3) domain of cortactin, an interaction which potentially determines its actin-modifying potential and its function in CME [23].

The central domain of the Sla2p/HIP1/HIP1R proteins contains a coiled-coil domain and several consensus sequences enabling protein–protein interactions. Binding experiments have shown that all three proteins interact with the clathrin light chain [19,24–26]. This binding is of functional importance as it may modulate not only recruitment to the clathrin-coated pit/vesicle, but also the ability of HIP1 and HIP1R to bind to actin [18]. Furthermore, even though reduced affinity to actin on clathrin binding is common to both HIP1 and HIP1R, the extent of this phenomenon is substantially larger in the case of HIP1R [18], a fact that may be of importance in the context of a coated pit, where all three factors (clathrin light chain, HIP1 and HIP1R) are thought to be present. HIP1’s central domain has been shown to have several additional partners, such as clathrin heavy chain, AP2 (adaptor protein 2), and huntingtin [6,19,27–29] (Figure 1). Interactions of HIP1 have also been detected with androgen receptor, EGF (epidermal growth factor) receptor and NMDA (N-methyl-D-aspartate) receptor [30–32]. In this context, HIP1 may function as a cargo-specific endocytic adaptor. Sla2p’s central domain was found to additionally bind Sla1p, another regulator of actin organization [33]. Another interaction attributed to the central domain is the homo/heterodimerization of the proteins. While there seems to be agreement on their homodimerization [18,22], the heterodimerization reported by Legendre-Guillemin et al. [19] was recently apparently contradicted by the findings of Wilbur [18] that a mixture of the coiled-coil domains of HIP1 and HIP1R results in strong homodimerization of each protein, with no significant heterodimerization.

The effects of Sla2p/HIP1/HIP1R silencing

The roles of the endogenous proteins were investigated using deletion mutants and silencing techniques. However, these studies yielded conflicting results: several HIP1-deficient mice created over the years unexpectedly exhibited different phenotypes. Rao et al. [34] were the first to create mice with targeted deletion of murine Hip1. These mice were viable and developed normally, except for testicular degeneration with increased apoptosis of post-meiotic spermatids found in the adult male mice. Another mouse, created by Metzler et al. [35], displayed a more severe phenotype: from the age of 12 weeks the mice developed growth retardation, tremor, thoracolumbar kyphosis and a reproductive defect. The phenotype was progressive and resulted in premature death several months after development of the kyphosis. Additionally, those authors showed that cultured neurons from these mice are less susceptible to NMDA-induced excitotoxicity, and that long-term depression measurements from hippocampal slices show a slight reduction for Hip1-deficient mice compared with wild-type mice [30]. It is worth noting that brain morphology, cortical, hippocampal and striatal volumes, and densities of hippocampal and striatal neurons were indistinguishable from those of wild-type mice [35]. Additional mice were created by Oravecz-Wilson et al. [36]. Aside from the previously described spinal defect, these mice displayed haematopoietic abnormalities, micro-ophthalmia and cataracts [36]. Possible explanations for the phenotype discrepancies are the possible creation of hypomorphic alleles, differences in mouse strains, and neighbouring gene effects [36].

Symptoms specific to the endocytic pathway were described in the mice created by Metzler et al. [35]. At the cellular level, internalization of AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptors following stimulation was impaired in cortical neurons derived from Hip1-knockout mice, while surprisingly, transferrin-receptor internalization remained intact in these cells [35]. These results suggest a role for HIP1 in only specific forms of endocytosis, i.e. AMPA-receptor internalization. However, other studies showed that transferrin uptake is inhibited in cells expressing a fragment of HIP1 [27], and thus the question of HIP1’s functional specificity in endocytosis remains open.

In neurons, HIP1 was shown to be localized to dendritic structures and to affect AMPA receptor trafficking [35]. A morphological study using immunogold labelling confirmed that HIP1 is localized predominantly post synaptically but also demonstrated that some HIP1 labelling is found at presynaptic terminals [37]. Further examination of synaptic transmission in Hip1–/– mice reported a small but significant increase in paired-pulse facilitation and reduced recovery from synaptic depression [38]. In addition, the Caenorhabditis elegans HIP1 homologue (hipr-1) was suggested to be involved in presynaptic activity [38]. These findings suggest that HIP1 affects presynaptic function, but further studies are needed to examine if these effects relate to alterations in endocytosis or to other processes.
Figure 2 | Suggested roles for Sla2p, HIP1 and HIP1R in the initial steps of endocytosis

(A) Sla2p arrives at the pre-existing coated pit in the cell cortex, recruits actin-remodelling proteins and dissociates in parallel to clathrin. (B) HIP1R arrives at the pit in parallel to clathrin, and probably participates in clathrin assembly; it interacts sequentially with actin-remodelling proteins and then dissociates in parallel to clathrin. HIP1 dynamics have not yet been characterized. Similar to HIP1R, it is capable of clathrin assembly (in vitro), so it probably arrives in parallel to clathrin; its actin-binding capabilities are controversial and it has not yet been shown to have an effect on actin structure, so it may dissociate earlier than has been proposed for HIP1R and Sla2p, or in parallel to clathrin as shown for the other two proteins. Questions remain as to HIP1’s time of arrival, its interaction with actin and the actin-remodelling proteins, and the timing of its dissociation (indicated by question marks).

Deletion of Hip1r in mice caused no noticeable phenotype. Hip1r homozygous mutant mice were viable and fertile without obvious morphological abnormalities [39]. However, double-knockout of both HIP1 and HIP1R aggravated the phenotype of the Hip1-deficient mice [39]. Surprisingly, no abnormalities were found in endocytosis, PtdIns 3-kinase signalling or actin dynamics in any of the examined cell types [MEFs (mouse embryonic fibroblasts), spleen cells] [40]. In contrast, reducing HIP1R levels using siRNA (small interfering RNA) altered endocytosis and caused abnormal cytoskeletal actin organization [41]. In particular, clathrin-coated structures and their endocytic cargo became stably associated with dynamin, actin, and the Arp2/3 (actin-related protein 2/3) actin-remodelling complex [41].

In yeast, cells lacking Sla2p have severe defects in actin organization, cell morphology, and endocytosis. Interestingly, the early endocytic factors are recruited and form cortical patches, but these do not internalize. In addition, actin comet tails are formed at these sites and grow into the cytosol [8,22,42]. Therefore, although these experiments suggest a role in endocytosis, it is not clear why in some cases the different deletion methods do not affect endocytosis, thus a clear classification of the proteins’ functions is prevented. This apparent discrepancy may stem from the timeline of the imbalance of expression levels (between HIP1/HIP1R and its interactors), which differs between the two methods, and may allow for a different compensatory response.

Suggested modes of action

Based on their domain structure, binding partners, knockdowns and other experimental evidence, several models for Sla2p/HIP1/HIP1R function have been proposed.

The most conclusive results were found for yeast Sla2p: dynamic characterization of Sla2p shows that it arrives at existing endocytic patches with a ~25-s delay relative to clathrin and dissociates simultaneously with clathrin upon recruitment of actin-related proteins [26,42]; in addition, Sla2pΔ cells show normal recruitment of the early endocytic machinery but no internalization, and creation of abnormal actin structures [8,22,42]. These results strongly support the notion that Sla2p serves as a bridge, connecting the endocytic patch to the cortical actin cytoskeleton [25] (Figure 2A).

For HIP1 and HIP1R, the picture is not as clear. It has been suggested that these proteins act as heterodimers and, similar to Sla2p, can connect the coated pit to actin structures [43–45]. This was further supported by the effects of Hip1r siRNA, which were quite similar to those of Sla2p knockouts [41]. However, in light of the new evidence for the unlikelihood of these proteins’ heterodimerization [18], this model of their function becomes less feasible. In addition, the different phenotypes of the knockout mice also imply functional differences for the two mammalian proteins. A more likely notion is that HIP1 and HIP1R perform partially overlapping functions, as their combined knockout produces a more severe phenotype [39], and re-expression of
HIP1 alone is enough to ameliorate many of the symptoms [40]. It is very possible that HIP1R, like Sla2p, has a role in actin regulation during endocytosis, as inferred from its silencing experiments [23,41]. Yet, due to the potentially different roles played by actin in endocytosis in yeast and mammals, HIP1R might have an additional, earlier role. In support of this notion is the arrival of Sla2p to pre-existing clathrin patches, which contrasts with the parallel dynamics of HIP1R and clathrin [24] (Figure 2B). Furthermore, both HIP1 and HIP1R are endowed with the capacity to promote clathrin assembly in vitro [19,24]. Moreover, actin polymerization may also play distinct roles at different steps of the endocytic event in mammalian cells [46,47]. Apart from its endocytic role, recent results associate HIP1R to the apoptotic pathway, through interaction with a member of the Bcl-2 pro-apoptotic family [48]. The function of HIP1 is even less clear. Unlike the other two members of this family, no effect on actin organization has so far been reported for HIP1. In addition, the time frame of HIP1 recruitment and dissociation from coated pits/vesicles is currently unknown (Figure 2B, question marks). Nonetheless, different reports have suggested that HIP1 functions in different stages of endocytosis: from the very early recruitment of clathrin coat components [29], through the internalization of the coated pit/vesicle [35] and later, in the transition from early to late endosomes [14]. Apart from endocytosis, HIP1 has also been associated with other cellular processes such as tumorigenesis [49], transcription regulation [32] and cell death [50,51]. The association of HIP1 with the EGF receptor and its proposed role in tumorigenesis [31,49] is of particular interest: as internalization is considered to be a mechanism of receptor down-regulation; this is in apparent contrast to a proposed role for HIP1 as a cargo-specific endocytic adaptor. Importantly, HIP1 has been described to interact with a broad spectrum of factors in different intracellular compartments [6,19,27–32,51]. This and the divergent consequences of alterations in HIP1 expression levels support the notion that HIP1 functions at several points along the clathrin–endocytic pathway, in addition to clathrin-independent regulation of cellular processes. Of particular interest is the nuclear localization of HIP1, due to the presence of a C-terminally localized nuclear localization signal [32]. In this sense, HIP1 is an additional member of the group of endocytic proteins that perform nucleocytoplasmic shuttling [52]. Taken together, these considerations raise the need for further quantitative measurements of the dynamic parameters of HIP1 localization, under different cellular conditions, to unravel the relevant stages of endocytosis in which HIP1 participates, and the full extent of its functional capacities.

**Concluding remarks**

Despite their structural similarity, the members of the Sla2p/HIP1/HIP1R family probably perform unique functions. In light of this potential functional diversity, the tendency to minimize their uniqueness and accentuate a presumed redundancy should be avoided. Despite extensive research over the years there is a need for further experimentation, especially using high-resolution techniques, to unravel the steps of these proteins’ functions. Better characterization of both their common and unique functions will provide us with a better understanding of key processes in the cell.

Since submission of the present paper, we have published our findings that HIP1 shares similar dynamics to those of clathrin in coated pits and functions in the last steps of coated pit maturation and the formation of coated vesicles [53].

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**References**

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