Insights into biological functions across species: examining the role of Rab proteins in YIP1 family function

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Abstract

The YIP1 family comprises an evolutionarily conserved group of membrane proteins, which share the ability to bind di-prenylated Rab proteins. The biochemical capability of YIP1 family proteins suggests a possible role in the cycle of physical localization of Rab proteins between their cognate membranes and the cytosol. YIP1 is essential for viability in yeast and a deletion of YIP1 can be rescued with the human homologue YIP1A. We have made use of this evolutionary conservation of function to generate a series of mutant alleles of YIP1 to investigate the biological role of Yip1p. Our findings indicate evidence for the participation of Yip1p in both Rab and COPII protein function; at present, we are not able to distinguish between the models that these roles represent, i.e. independent or dependent activities of Yip1p.

Introduction

Proteins of the Ras GTPase superfamily share a common structure that operates as a molecular switch by cycling between GTP-bound and GDP-bound conformational states [1]. Each conformational state binds a distinct set of proteins to regulate diverse intracellular events. A common theme is that small GTPases also cycle between membrane-associated and cytosolic pool, membrane attachment being a prerequisite for the events following GTP activation. Reversible membrane-interaction offers cells additional flexibility in temporal and spatial regulation of the GTPase switch, and the mechanisms that facilitate reversible membrane attachment are of great interest in cell biology.

The Ras, Rho and Rab subfamilies are post-translationally modified by the covalent attachment of lipid moieties on the C-terminus. There are three major types of isoprenylation reactions, mediated by three prenyl transferases that are conserved from yeast to human [2]. Cysteine-containing motifs at the C-terminus dictate the isoprenylation type, either farnesylation (C15 isoprenoid) received by CAAX box (X = A, C, E, M, S or V) proteins, or a geranylgeranylation (C20 isoprenoid) moiety when X = leucine or a hydrophobic residue. The majority of Rab proteins contain two cysteine residues at the C-terminus (such as CXC, CC, CCX, CCXXX and CCXXXX). The two cysteine residues are subject to isoprenylation with two geranylgeranyl moieties in a reaction catalysed by geranylgeranyl transferase II together with an additional subunit, REP (Rab escort protein) [3]. Isoprenylation is a stable modification that persists for the lifetime of the protein.

Until recently, it was generally assumed that the type of prenyl modification has little functional influence, it merely served to confer sufficient hydrophobic character so that the protein could make stable attachments to lipid bilayers. We have recently demonstrated that the double prenylation motif, unique to Rab proteins, plays a specific functional role in the correct localization of Rab proteins to their subcellular compartment [4]. Correct membrane targeting and function of two essential yeast Rab GTPases, Ypt1p and Sec4p, require dual prenylation of two C-terminal cysteine residues. Swapping the dual prenylation motif for any other type of mono-prenylation motif resulted in mislocalization and cessation of growth. We extended this finding to three other Rab proteins in yeast, Ypt6p, Vps21p and Ypt7p, and similar results have also been reported for the mammalian Rab5 and Rab27 GTPases, demonstrating that the requirement for dual prenylation is evolutionarily conserved [5]. Thus the di-geranylgeranylation of Rab proteins is more than just a ‘greasy tail’ for Rab proteins but adds a unique functionality that is necessary for the restricted compartmentalization of Rab proteins.

Yip1p recognizes di- versus mono-geranylgeranylated Rab proteins

Our findings led us to propose the existence of a factor which is selective for di- versus mono-prenylated Rab proteins. A candidate gene approach, looking at known Rab-interacting proteins that were universally expressed and known to recognize Rab proteins in a prenylation-dependent manner, identified Yip1p [4]. We found that Yip1p recognized Rab proteins non-selectively in a manner dependent on double prenylation. Significantly, neither human YIP1 nor yeast Yip1p would interact with Rab8 or Rab13. These Rab proteins are members of a small minority of Rab proteins that contain...
‘Rho-like’ CAAX boxes in place of the usual double cysteine motif, and these results confirm the preference of Yip1p for di-prenylated Rab proteins.

The YIP1 protein family
Database searches identify Yip1p orthologues in all euca- ryotes for which sequence information is available. For the human counterpart of Yip1p, YIP1A, the sequences are of similar overall size with the homology extending through- out the entire length of the ORF (open reading frame) and a similarity value of approx. 30%. A graphical representation of the HMM (Hidden Markov Model) for the YIP1-related sequences is shown in Figure 1. Using Yip1p as a departure point we also identified Yip1p paralogues in Saccharomyces cerevisiae [6]. This analysis uncovered Yif1p, and two unknown ORFs, YGL198W and YGL161C, which we have termed Yip4p and Yip5p respectively (http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=yip4 and http:// genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=yip5). Because Yip1p is a small protein with stretches of hydrophobic residues, which greatly reduces the confidence level of homology assignments, we sought other criteria to demonstrate the relatedness of these sequences. We demonstrated that Yip1p-related proteins possess several features: (i) a common overall domain topology, (ii) are capable of biochemical interaction with a variety of Rab proteins in a manner dependent on C-terminal prenylation, and (iii) share an ability to physically associate with other Yip1p paralogues. As these sequences share common characteristics, we suggest that they constitute a family, and because Yip1p is the founder member or prototype for this family, we have termed it the YIP1 family [6].

Human YIP1A can functionally replace its yeast homologue
Two hybrid experiments revealed that human YIP1A and yeast Yip1p show striking cross-species conservation of interactions, with human YIP1A capable of interactions with yeast Rab proteins, and yeast Yip1p with mammalian Rab proteins. These results prompted us to test if the conservation of protein interactions had functional significance. We asked whether human YIP1A could functionally replace its yeast homologue and act as the only cellular source of the otherwise essential YIP1 gene. This experiment revealed that the human YIP1 sequence can fully replace a deletion of yeast YIP1. We made use of this result to dissect the molecular action of Yip1p [7] with a site-directed mutagenesis strategy. This study targeted residues that are conserved between human YIP1A and yeast YIP1. We additionally focused on charged residues with the rationale that such residues have the highest probability of protein surface exposure and intermolecular contact, and therefore their mutation is less likely to perturb the overall structure and more likely to disrupt interactions with binding partners. An overview of the mutagenesis data is shown in Figure 2. Of these mutants, three point mutants (yip1-41, yip1-9 and yip1-19) were found to be recessive null mutants, one (yip1-6) was a dominant negative null allele, four (yip1-4, yip1-40, yip1-14 and yip1-42) were thermosensitive and three alleles (yip1-43, yip1-12 and yip1-2) were sensitive to formamide at 37°C.

Functions of Yip1p and YIP1 family members
The hydrophobicity of the two C20 lipid moieties of Rab proteins provides a stable association with the lipid bilayer. This biophysical characteristic, however, presents a barrier to the existence of Rab proteins in the cytoplasm. Rab proteins have solved this problem with the aid of a chaperone protein, Rab-GDI (GDP dissociation inhibitor) which binds the hydrophobic prenyl moieties [8]. The cytosolic Rab protein pool, therefore, consists of Rab proteins in a dimeric complex with Rab-GDI. The high-affinity, nanomolar interaction between a Rab protein and Rab-GDI in turn creates a second problem. For cells to draw upon the cytosolic Rab reservoir, Rab-GDI must be induced to release its Rab protein at an appropriate membrane site. Proteins that promote GDI displacement and help recruit Rabs on to membranes are of great importance because such mechanisms will determine where and when the Rab proteins are activated. Certainly, the YIP1-family possesses features that suggest a capability for participation in Rab membrane recruitment; they are capable of biochemical interaction with a variety of Rab proteins in a manner dependent on C-terminal prenylation [6], and so might compete with Rab-GDI for Rab protein interactions in vivo in a simple ‘tug o’ war’ model (Figure 3). Furthermore,
in a milestone study, mammalian PRA1/YIP3 has been biochemically characterized as a GDF (GDI displacement factor) [9]. The yeast counterpart of PRA1/YIP3 is the membrane protein Yip3p. Yip3p does not fit the HMM definition of a YIP1 protein family member, and psi-blast searches with YIP1 family members do not identify Yip3p. In addition, YIP3 will recognize Rab proteins with a single prenyl modification [10], compared with YIP1 family members who require the di-geranylated protein for productive interaction. However, Yip3p does share all the functional characteristics of the YIP1 protein family: it has a cytoplasmically oriented N-terminus, it is capable of biochemical interaction with a variety of Rab proteins in a manner dependent on C-terminal prenylation, and it can physically associate with Yip1p [11].

Currently, it is of intense interest whether the isolated Yip1p polypeptide displays GDF activity in vitro, and studies to address this question are under way in our laboratory.

If YIP1 family members display in vitro GDF activity, does this make them an indispensable part of the Rab localization pathway in vivo? What are the physiological consequences of loss of YIP1 function on the cycle of Rab action? Is GDF activity the sole function of YIP1 family members or will they use this activity to co-ordinate other events of membrane traffic? Furthermore, if YIP1 family members show GDF activity but have pleiotropic Rab interacting capabilities, what accounts for the specificity of Rab membrane recruitment? One way to begin addressing these questions is to catalogue the number and location of YIP1 proteins. Simply put, are there enough Yips, or specific Yip complexes to cover every known individual stage of membrane transport? Such considerations have been made previously for SNARE (soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor) proteins and Rab proteins. However, both SNAREs and Rabs can act at
Summary of interaction data between \textit{yip1–4} and genes encoding Rab proteins

<table>
<thead>
<tr>
<th>Mutant category</th>
<th>Lethal when combined with \textit{yip1–4}</th>
<th>Viable when combined with \textit{yip1–4}</th>
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<tbody>
<tr>
<td>Rab genes</td>
<td>\textit{ypt6}\textsuperscript{Al36O}, \textit{ypt1–3}, \textit{ypt31\Delta}, \textit{ypt32}\textsuperscript{Al36O}, \textit{ypt6\Delta}, \textit{vps2}\textsuperscript{1\Delta} \textit{ypt52\Delta}</td>
<td>\textit{vps2}\textsuperscript{1\Delta}, \textit{ypt52\Delta}, \textit{ypt33\Delta}, \textit{ypt7\Delta}, \textit{ypt10\Delta}, \textit{ypt11\Delta}, \textit{sec4–8}</td>
</tr>
<tr>
<td>Rab regulators and modifiers</td>
<td>\textit{gd1–11}</td>
<td>\textit{sec2–41}, \textit{bet3–1}, \textit{gyp1\Delta}, \textit{bet2–1}</td>
</tr>
<tr>
<td>Other tested</td>
<td>None identified</td>
<td>\textit{api1\Delta}, \textit{sec7–1}, \textit{sec14}\textsuperscript{G}, \textit{dnm1\Delta}</td>
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*\textit{Lethality indicates that the double mutant is inviable at 25\degree C; viability indicates that examination of the double mutant showed no deleterious synthetic effects.}

more than one stage of membrane transport, which renders arguments based on this type of data inconclusive. We have also undertaken a genetic approach to gain insight into this question. If Yips act in association with Rab proteins, are Rabs genetically required for Yip function, i.e. how are \textit{yip1} mutations affected by mutations in genes encoding Rab proteins? To provide a complete analysis, we investigated genetic interactions between \textit{yip1–4} and each Rab ORF identified in the yeast genome. There are 11 Rab genes in the complete \textit{S. cerevisiae} genome. Table 1 summarizes our analysis of genetic interactions between Rab-encoding genes and \textit{yip1–4}. No synthetic lethality was observed with \textit{sec4–8}, \textit{ypt10\Delta}, \textit{ypt11\Delta}, \textit{vps2}\textsuperscript{1\Delta}, \textit{ypt32\Delta}, \textit{ypt33\Delta} or \textit{ypt7\Delta}. In contrast, synthetic lethality was observed with \textit{ypt1}\textsuperscript{Al36O}, \textit{ypt31\Delta}, \textit{ypt6\Delta}, \textit{ypt32}\textsuperscript{Al36O} and the combination mutant \textit{vps2}\textsuperscript{1\Delta} \textit{ypt52\Delta} \textsuperscript{[7]}. The shared feature of the Rab genes that genetically interact with \textit{yip1–4}, is that these genes influence Golgi functions at all levels from the cis-Golgi to the trans-Golgi network. These data suggest that Yip1p action \textit{in vivo} does indeed require Rab protein functionality, and that this requirement is confined to a subset of Rab proteins that act at the Golgi apparatus.

Are Rab proteins the major clients of YIP1 family members? One surprising initial discovery was that the conditional mutant \textit{yip1–4}, that displays genetic interactions with Rab proteins, showed an ER (endoplasmic reticulum) accumulation phenotype \textit{in vivo}. Together with \textit{in vitro} reconstitution, and cell biological and genetic studies, this demonstrated that Yip1p functions at an early stage in ER vesicle budding \textsuperscript{[12,13]}. This function of Yip1p is difficult to reconcile with its connection to Rab proteins. Although Rab proteins are required for vesicle budding and cargo selection in several systems, it is possible to form fusion competent transport vesicles from the ER in the absence of Ypt1p function, the Rab protein thought to regulate this particular transport step \textit{in vivo} \textsuperscript{[14]}, although Ypt6p may substitute for the absence of Ypt1p \textsuperscript{[15]}. Using the novel mutant alleles of \textit{yip1} generated by our targeted mutagenesis studies, we carried out a series of genetic experiments to directly address the issue of whether these proteins work in vesicle budding or Rab membrane attachment. Was the synthetic lethality observed between \textit{yip1–4} and \textit{sec12–4}, \textit{sec13–1} and \textit{sec23–1} (all mutants of the COPII ER budding machinery), specific to the E70K (Glu\textsuperscript{70} \textrightarrow{Lys}) mutant? Would any of the other alleles of \textit{yip1} fail to show synthetic lethality with the COPII mutants while still retaining synthetic lethality with Rab mutants or vice versa? Would any mutant show synthetic lethality with COPII and not with Rab GTPases? If either of these was found to be true, then this would be strong evidence to suggest that Yip1p functions independently in both pathways. If they could not be genetically distinguished, then one of these apparent functions may be a secondary effect of dysfunction in the other, or indicate that Yip1p acts in a common pathway. Our results showed that we were unable to generate any conditional allele that was selectively defective in either Golgi Rab or COPII gene interaction. In particular, every conditional \textit{yip1} allele tested showed synthetic lethality with a mutant in the nuclear pore and COPII subunit gene \textit{SEC13} \textsuperscript{[7]}. These data provide additional support for Yip1p acting in the COPII vesicle biogenesis pathway and also suggest that the action of Yip1p on Rab proteins cannot be distinguished from its role in COPII vesicle biogenesis.

In addition to Rab proteins and other YIP1 family members, two-hybrid interaction screens have identified a number of other potential binding partners for Yip1p \textsuperscript{[16,17]}, including PX-domain-containing proteins \textsuperscript{[18]}, and other proteins with known roles in membrane traffic. Another intriguing Yip1p-interactor, Yos1p was identified through high-copy suppression of \textit{yip1–4} \textsuperscript{[19]}. Yos1p is a small (87 residues) membrane protein that is essential for viability in yeast. Yos1p physically associates with Yip1p and Yip1p, and the interaction between Yip1p and Yos1p is diminished in strains bearing conditional mutations of either Yip1p or Yos1p. \textit{In vitro} assays of Yos1p function reveal that Yos1p is also required for transport between the ER and the Golgi.

Given our current level of knowledge, how can we rationalize roles for Yip1p on both the ER and Golgi? Perhaps the budding vesicle needs to incorporate proteins required for its downstream targeting and fusion. While the need for such proteins can be bypassed in a reconstituted \textit{in vitro} system, quality control \textit{in vivo} will block vesicle formation if the vesicle does not contain proteins such as Rab proteins that are needed for subsequent targeting and fusion. Even if the vesicle does not contain Rab proteins, it must have the capability to acquire them, hence perhaps the requirement for Yip1p. Another view could be that Yips act as some kind of intracellular equivalent of GPCRs (G-protein-coupled receptor) for small GTPases. In this manner, they may be useful for integrating information from a variety of sources, such as the lipid requirements for membrane traffic,
or the balance between anterograde and retrograde traffic. Recently, a mechanism has been identified that prevents ‘back-fusion’ of newly formed COPII vesicles with the ER [20]. Yips could conceivably play a role in the mechanism by which uncoated vesicles can prevent inappropriate fusion with their compartment of origin. This could explain the ER accumulation phenotype of yip1–4 mutants, if the vesicles are produced transiently, and then rapidly fuse back to the ER. We currently favour a model whereby YIP1 proteins act in a common pathway, via Rab proteins, to integrate the Golgi/endosomal system and the compartments with which the Golgi communicates via membrane traffic.

Clearly we have a considerable amount of information about the YIP1 protein family. We can list protein interaction partners, mutant phenotypes and the genetic pathways within which they participate. However, a molecular description of YIP1 action and its physiological role(s) remain elusive, and further experiments are necessary to address our current hypotheses. Certainly, the universal requirement for these proteins and their intractability makes their understanding a worthwhile and challenging goal.

References

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