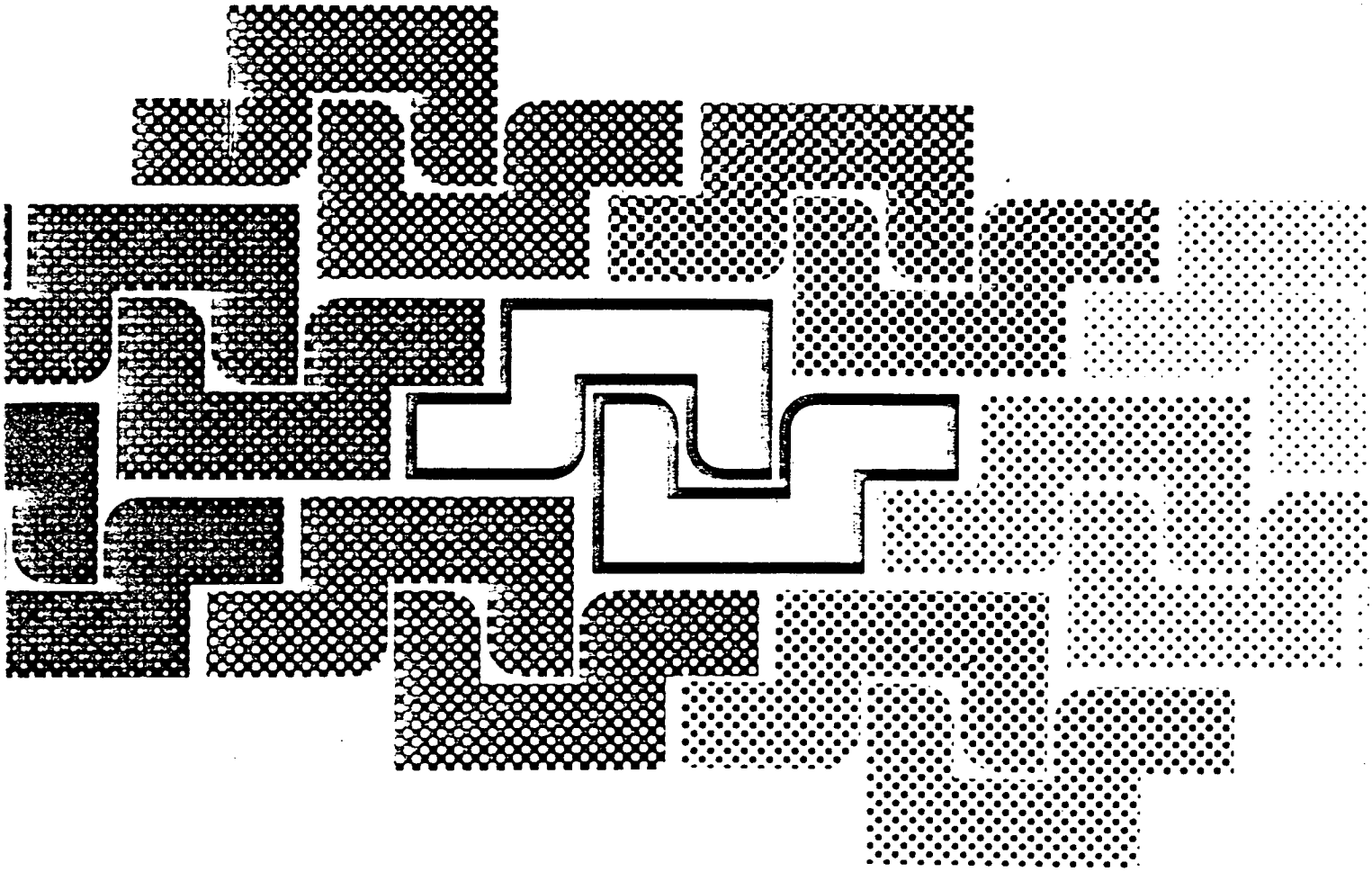

The Guanine-Nucleotide Binding Proteins

Common Structural and Functional Properties

Edited by

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STRUCTURAL AND FUNCTIONAL ANALYSIS OF *ypt* PROTEINS, A FAMILY OF *ras*-RELATED NUCLEOTIDE-BINDING PROTEINS IN EUKARYOTIC CELLS

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INTRODUCTION

The advance in cloning and sequencing of DNA has led in recent years to the discovery of a large number of genes encoding guanine nucleotide-binding proteins that are related to the potentially oncogenic *ras* proteins. *Ras* proteins (for review: Barbacid, 1987) and various *ras*-related proteins are, as far as this has been studied, evolutionary highly conserved. These proteins share similar biochemical properties, they are membrane-bound and likely to be involved in different regulatory pathways in all eukaryotic cells.

The first of the *ras*-like proteins discovered was the *Ypt1* protein of the budding yeast *Saccharomyces cerevisiae* (Gallwitz et al., 1983). Besides the genes of very diverse eukaryotic species coding for proteins that exhibit extensive homology to the mammalian H-, K- and N-*ras* proteins, genes encoding related proteins were subsequently identified in the mollusc *Aplysia* and in human cells, *rho* proteins (Madaule and Axel, 1985), and in mammalian cells, *ral* (Chardin and Tavitian, 1986) and R-*ras* proteins (Lowe et al., 1987). Mammals were also shown to express proteins highly homologous to the yeast *YPT1* gene product (Haubruck et al., 1987; Touchot et al., 1987), and a yeast gene known to be involved in protein secretion, *SEC4*, was found to encode a GTP-binding protein related to the *ras* gene products (Salminen and Novick, 1987).

All the sequence data presently available, together with the still very limited functional studies that have been performed, allow to distinguish between three families of *ras*-related proteins, *ras*, *rho* and *ypt* proteins (Haubruck et al., 1987).

This paper summarizes the present knowledge of the biochemical properties and the cellular functions of *ypt* proteins with special emphasis of the *S. cerevisiae YPT1* gene product.

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Biochemical Properties of ypt Proteins

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and mutant proteins (for review: Barbacid, 1987; Wagner et al., 1987) and by a recent crystallographic investigation (De Vos et al., 1988) to be of importance for GTP binding and hydrolysis. These domains, residues 5-17, 57-62, 116-119 and 143-147 (with respect to mammalian *ras* proteins) are similarly spaced with respect to the primary structures suggesting rather similar tertiary structures of all of these proteins build of roughly 200 amino acid residues.

GTP and GDP binding of *ypt* proteins from different eukaryotic species has been shown by protein blotting procedures and with the purified yeast *Ypt1* protein and the mouse *ypt1* protein in solution. The binding affinity for GDP is high (2.2×10^9 M^{-1} measured for the wild-type yeast *Ypt1* protein at 30°C), and different mutant forms of the protein exhibiting altered GTPase activity (substitutions Ser-17 Gly, in short S17G, or A65T) had only slightly impaired affinities for the nucleotide (P.W., C.V. and D.G., unpublished). As is the case for mammalian *ras* proteins, the A65T substitution in the yeast *Ypt1* protein, corresponding to the same change in position 59 of *ras* proteins, led to a significant decrease in GTPase activity and to an autophosphorylation of the protein (Wagner et al., 1987). The intrinsic GTPase activity of the yeast *Ypt1* protein was found to be lower than that of *ras* proteins and the S17G substitution resulted in an expected increase of the GTP hydrolyzing activity (Wagner et al., 1987). Whereas the two mutations mentioned did not affect the biological activity of the yeast protein, investigated in haploid cells after exchanging the wild-type *YPT1* gene with the respective mutant genes, the substitutions K21M of the first and N121I of the third nucleotide binding domain led to a significant impairment of GTP binding and a loss of cellular function of the *Ypt1* protein (Schmitt et al., 1986; Wagner et al., 1987). It is clear, therefore, that *ypt* and *ras* proteins are biochemically very similar.

Like the *ras* proteins that are bound to the inner surface of the plasma membrane via palmitic acid covalently attached to a cysteine four residues from the carboxyl terminal end (Willumsen et al., 1984; Deschenes and Broach, 1987), the yeast *Ypt1* protein requires one of its two C-terminal cysteine residues for fatty acid binding and membrane attachment in order to exert its cellular function (Molenaar et al., 1988).

A summary of amino acid substitutions and their effects on the biological function of the *S. cerevisiae Ypt1* protein is given in Figure 1.

Towards an Understanding of ypt Function

The very efficient system of homologous recombination in the yeast *S. cerevisiae* allows to easily exchange a wild-type gene with a mutant gene or to precisely inactivate a gene on the chromosome. These techniques have been successfully employed to get insight into the possible functioning of the *YPT1* gene. Gene disruption and the replacement of the wild-type gene with mutant genes encoding proteins with significantly impaired guanine nucleotide-binding capacity proved to be lethal (Schmitt et al., 1986; Wagner et al., 1987). The essential role of the *Ypt1* protein was further investigated by placing the gene under an inducible yeast promoter, *GAL10*, allowing to switch on and off its expression. Growth arrest following *Ypt1* protein depletion was accompanied by a conspicious disorganization of microtubules (Schmitt et al., 1986) and by an accumulation of intracellular membranes and vesicles (see Figure 2), by an increase of intracellular calcium and by a severe inhibition of protein secretion (Schmitt et al., 1988). Similar observations were made with a temperature-sensitive (Schmitt et al., 1988) and a cold-sensitive mutant (Segev et al., 1988) of the *Ypt1* protein at the nonpermissive tempera-

ture. It was shown in addition that with high concentrations of extracellular calcium the phenotypic alterations of the ts mutant at the restrictive temperature could be prevented partially (Schmitt et al., 1988).

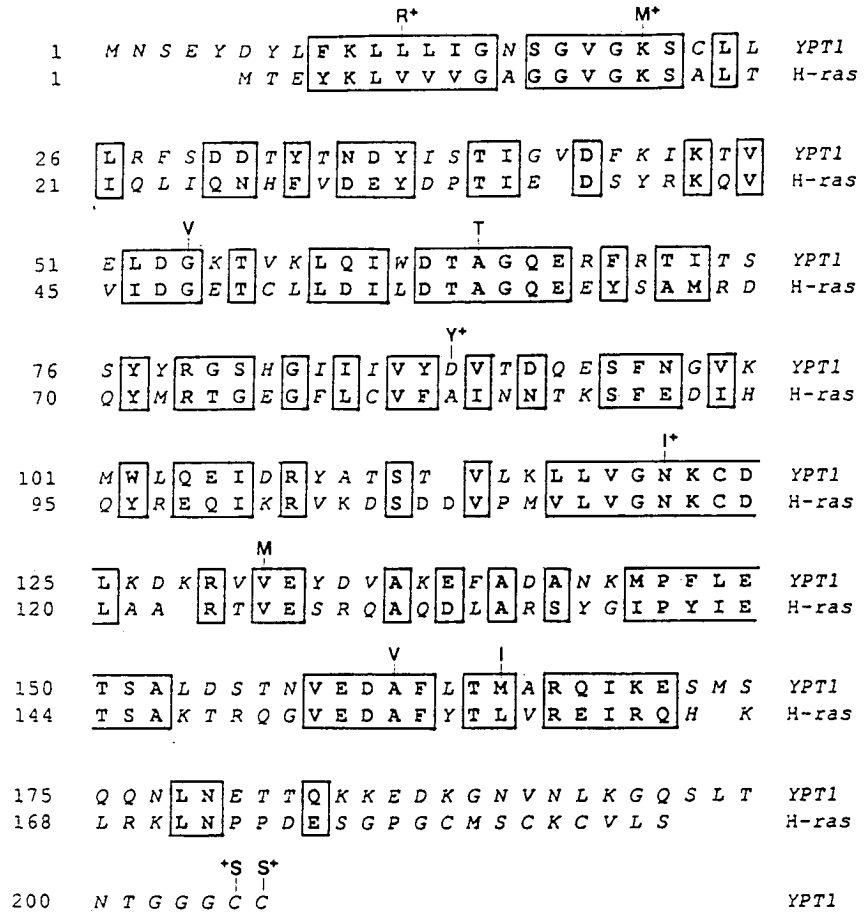

Fig. 1. Comparison of primary sequences of the *S. cerevisiae Ypt1* protein and the human H-*ras* protein. Identical residues and preferred substitutions are boxed. Amino acid substitutions of the *Ypt1* protein generated by in vitro mutagenesis of the gene (Wagner et al., 1987; Molenaar et al., 1988) or found by screening for second-site mutations suppressing the dominant-lethal N121I mutation (Schmitt et al., 1988) are shown above the *Ypt1* sequence. Substitions marked by asterisks resulted in a loss of biological activity and caused lethality, other mutations were neutral with respect to the protein's cellular function.

These observations have led to somewhat different conclusions as to the primary action of the *Ypt1* protein. Whereas Segev et al. (1988) suggest that the protein functions as a membrane label in vesicular transport, we consider the possibility that the regulation of intracellular calcium, known to affect different cellular activities including the cytoskeletal network, mitosis and protein secretion, might be the principle function exerted by the *Ypt1* protein (Schmitt et al., 1988).

In favor for a role of the *Ypt1* protein in the secretory pathway could be the finding by Segev et al. (1988) that an antibody directed against the yeast protein stained Golgi structures in mouse fibroblasts. Although we have recently identified a gene in mouse, called *ypt1*, encoding a protein with more than 70 % amino acid identities compared to

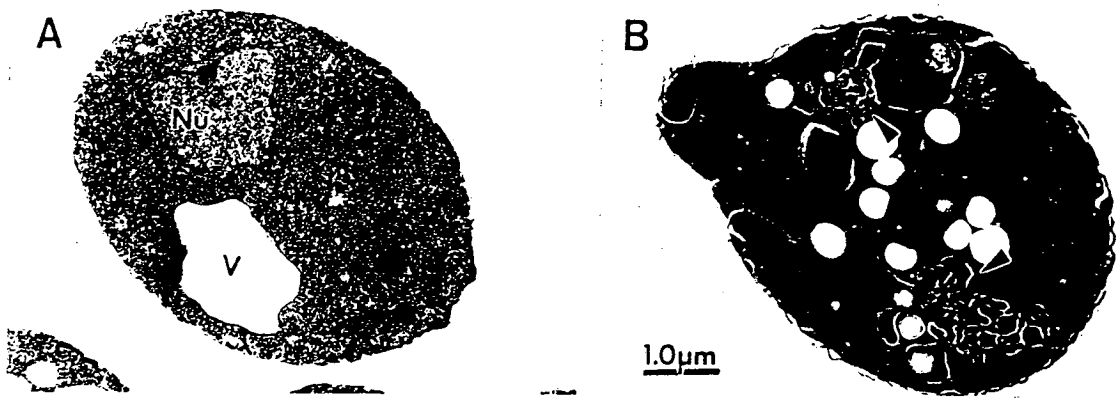

Fig. 2. Accumulation of intracellular membranes resulting from *Ypt1* protein depletion in a haploid yeast strain. Wild-type cells (A) and cells 15 h after the switch-off of the *GAL10*-controlled *YPT1* gene expression (B) are compared by thin-secretion electron microscopy.

the yeast *Ypt1* protein (Haubruck et al., 1987), the antibody against the yeast protein used by Segev et al. (1988) has neither been shown to crossreact with the mouse protein nor has it been demonstrated to not crossreact with any other of the *Ypt1*-related proteins recently identified in mammalian cells (Touchot et al., 1985). The conclusion that a *ypt* homologue in mammalian cells is associated with the Golgi apparatus is therefore premature at present and should be investigated with monospecific antibodies against the mammalian protein.

The involvement of *ypt* proteins in vesicle transport and protein secretion is nevertheless an attractive possibility since a conditional lethal mutant of the *Ypt1*-related *Sec4* protein in yeast leads to the accumulation of small vesicles, presumably caused by the failure of vesicles to fuse with the plasma membrane (Salminen and Novick, 1987; Goud et al., 1988). This led to the speculation that *ras*-related, GTP-binding proteins, like the *Sec4* and the *Ypt1* protein, might recognize specific proteins of different membrane compartments and thereby direct vesicle transport for fusion with different acceptor membranes (Bourne, 1988). The identification of proteins with which the *Ypt1* protein interacts within the yeast cell is now being tackled with the help of molecular genetic and biochemical methods.

Proteins with extensive homology to the *S. cerevisiae Ypt1* protein are likely to serve a very basic function in all eukaryotic cells. This is suggested by the essential function of a protein from the fission yeast *Schizosaccharomyces prombe*, called *ypt1* (M. Yamamoto, personal communication), and by the expression of the mouse *ypt1* gene in all tissues examined and in differentiating and non-differentiating F9 cells (Haubruck et al., 1987). Both the fission yeast and the mouse *ypt1* protein share more than 70 % amino acid identitites with the *S. cerevisiae Ypt1* protein.

Conserved Structural Features of ypt Proteins

As already pointed out above, four domains involved in guanosine nucleotide binding of *ras* and their related proteins are highly conserved and similarly contained in other GTP-binding proteins, like elongation factor EF-Tu (Jurnak, 1985) and the α subunits of G-proteins (Tanabe et al., 1985).

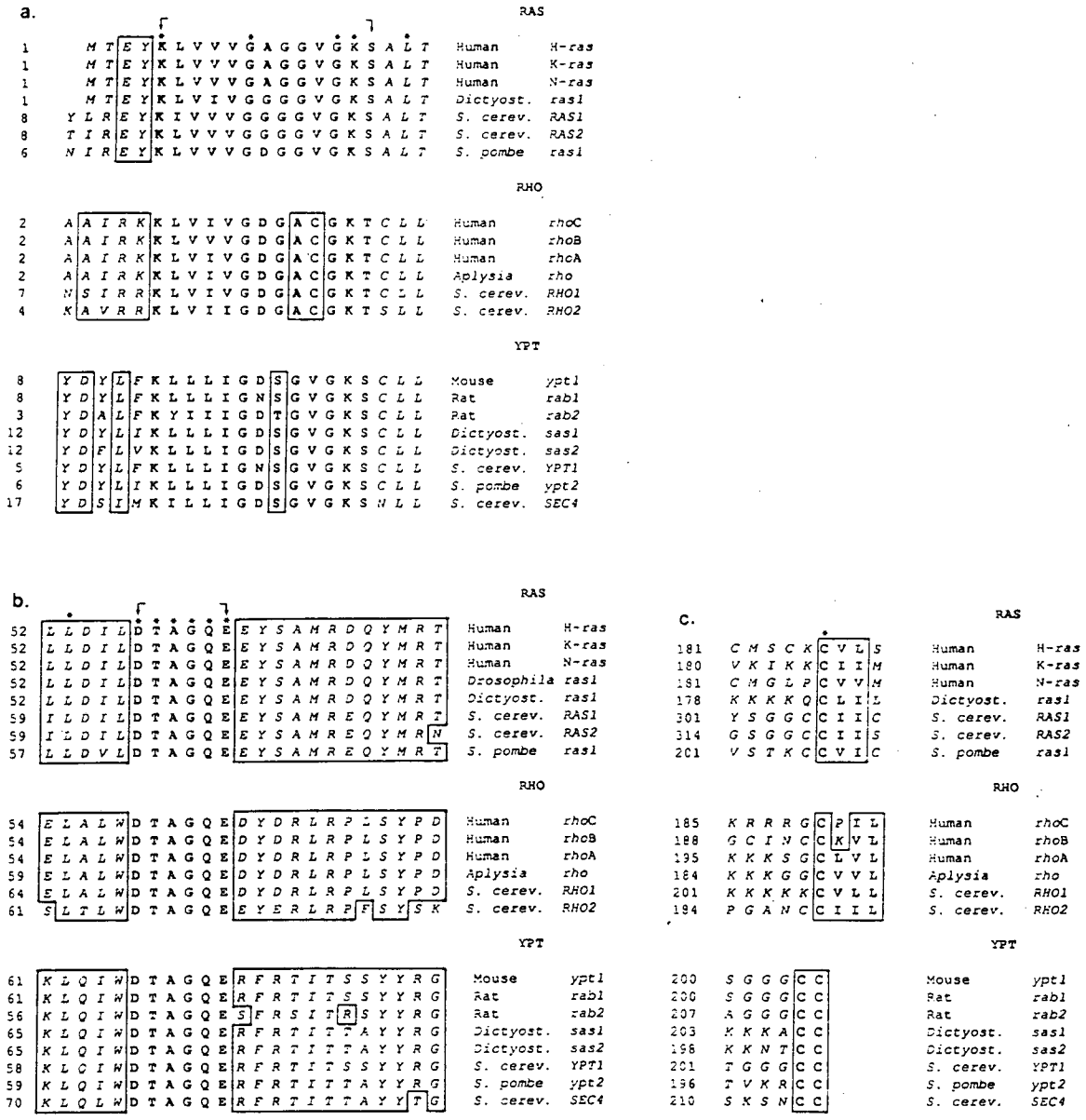

Fig. 3. Sequence comparison of segments of *ras*, *rho* and *ypt* proteins from different species. Sequences diagnostic for the three families of *ras*-related proteins that reside within or adjacent to the GTP-binding domains I (a) and II (b) or at the C-terminal end are boxed. Residues identical in all proteins are indicated by an asterisk above the H-*ras* sequence. Sequence data are from Barbacid (1987), *ras* proteins; Madaule et al. (1987), *Aplysia* rho and yeast *RHO* proteins; Chardin et al. (1988) and Yeramian et al. (1987), human *rho* proteins; Haubruck et al. (1987), mouse *ypt1* protein; Touchot et al. (1987), rat *rab* proteins; Saxe and Kimmel (personal communication), *Dictyostelium sas* proteins; Gallwitz et al. (1983), *S. cerevisiae Ypt1* protein; Salminen and Novick (1987), *S. cerevisiae Sec4* protein; and our unpublished results, *S. pombe ypt2* protein.

A prominent structural feature of *ypt* proteins is a serine instead of glycine-12 found in *ras* proteins, a residue critical for GTPase activity. The corresponding position in *rho* proteins is also occupied by a glycine, but a number of residues within the region forming loop 1 in *ras* proteins (De Vos et al., 1988) are either typical for *ras*, *rho* or *ypt*

proteins and so are residues adjacent to this region (Figure 3a). The distinction between these three groups of GTP-binding proteins can also be made by comparing the sequences flanking the absolutely conserved region comprising residues -Asp-Thr-Ala-Gly-Gln-Glu- in position 57 to 62 (with respect to mammalian *ras* proteins) (Figure 3b). In addition, *ras* and *rho* proteins terminate with a cysteine followed by three other residues whereas *ypt* proteins typically end with two consecutive cysteines (Figure 3c).

As the cysteine residues of the C-terminal region in *ras* (Willumsen et al., 1984; Deschenes and Broach, 1987) and *ypt* proteins (Molenaar et al., 1988) are the site for palmitic acid binding which in turn is required for membrane attachment, we already speculated that the distinct structural differences of the C-terminal sequences of *ras* and *rho* proteins on the one hand and *ypt* proteins on the other might be a means to direct these proteins to different membrane compartments within the cell (Molenaar et al., 1988). However, the elongation of the C-terminus of the yeast *Ypt1* protein by three amino acids did not affect the protein's proper biological function (Molenaar et al., 1988) but there are indications from recent experiments that these additional residues are being cleaved off before membrane attachment of the protein (C.M. and D.G., unpublished).

That *ras* and *ypt* are functionally different groups of GTP-binding proteins is not only shown by their sequence differences but also by the finding that mammalian *ras* proteins are able to functionally replace the essential yeast *Ras* proteins (Kataoka et al., 1985; De Feo-Jones et al., 1985) and that, likewise, the mouse *ypt1* protein can perfectly substitute for the loss of the essential *YPT1* gene product in yeast (R.P., H.H. and D.G., in preparation).

The recent discovery of several *ras*-related proteins in different eukaryotes has led to some confusion regarding the nomenclature. For instance, a rat protein identical in primary structure to the mouse *ypt1* protein, the structural and functional homologue of the yeast *Ypt1* protein, has been named *rab1* (Touchot et al., 1987), and two proteins from *Dictyostelium discoideum* with more than 50 % amino acid identities compared to the yeast *Ypt1* protein have been designated *sas1* and *sas2* (A. Kimmel, personal communication). As outlined above, the different *ras*-related proteins are characterized by the highly conserved and identically spaced four nucleotide-binding domains, but other, rather specific sequence features allow the distinction of three families: *ras*, *rho* and *ypt*. Proteins not fitting into either of these groups could be named *ryh* (for ***ras/rho-ypt*** homologue). Members of either family in one species might be functional homologous, like the *Ras1* and the *Ras2* protein of *S. cerevisiae*, or they might serve different and essential functions, like three *ypt* proteins in the fission yeast *S. pombe* that share more than 50 % of identical amino acid residues. Nevertheless, as proteins belonging to either family distinguished have been found in all eukaryotic kingdoms, it seems desirable to us to adopt a simplified nomenclature to avoid confusion.

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