Genomics

Distribution and functional diversification of the ras superfamily in *Saccharomyces cerevisiae*

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Abstract The recent availability of the full *Saccharomyces cerevisiae* genome sequence offers a first opportunity to analyze the composition, function and evolution of GTPases in the ras-p21 superfamily. This superfamily in yeast is composed of 29 proteins divided into five families: ras with four sequences implicated in cell signalling; rho, six genes related to the cell shape machinery; ypt-rab, ten proteins with different roles in intracellular trafficking; arf-sar, seven proteins related to vesicular trafficking in secretory pathways; and ran, two proteins acting as components of the nuclear transport system. The superfamily covers a wide range of cellular functions from signalling to intracellular trafficking, while conserving the structural framework and a common mechanism of GTP hydrolysis.

Key words: Ras; p21; Yeast; Genome; *Saccharomyces cerevisiae*

1. Introduction

The genomic information on yeast now available allows, for the first time, the cataloguing of all the members of broad protein families. One of the largest and most interesting protein families is constituted by the ras-related sequences, the so-called ras superfamily [1]. This superfamily is currently composed of more than 700 sequences from different species. This information will certainly contribute to the understanding of the origin, evolution and functional potential of this important protein superfamily.

It therefore seems to be the moment for revising the sequence information available for the yeast genome in the context of the large body of information available on the specific function of each protein. This review illustrates how, on the one hand, the ras superfamily has achieved a high degree of functional divergence, with striking examples of adaptation to very different cellular functions, and on the other hand, it has retained a high degree of redundancy, with many pairs of proteins of overlapping function.

The importance of this dichotomy between functional diversity and gene redundancy is discussed in the context of the comparison of the yeast ras superfamily with the available information about *Schizosaccharomyces pombe* and human ras sequences.

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Abbreviations: ORF, open reading frame; SW, SwissProt data base; mYr, million years

2. Results and discussion

2.1. Composition of the superfamily

The ras superfamily is characterized by the presence of a set of highly conserved GTP binding motifs and a characteristic C-terminal Cys motif subject to post-translational modifications [2–5].

There are 29 open reading frames (ORFs) in yeast that fulfil these characteristics and clearly belong to the superfamily, classified in five families: arf-sar, ran, ytp-rab, rho and ras (Fig. 1) [1,3,6].

A number of other proteins can be considered relatives of the ras superfamily, since they present most of the characteristic motifs, including Yhi2 (SW:yhi2_yeast), an ORF of unknown function in chromosome VIII [7], Mss1 (SW:mss1_yeast), a mitochondrial GTPase that belongs to

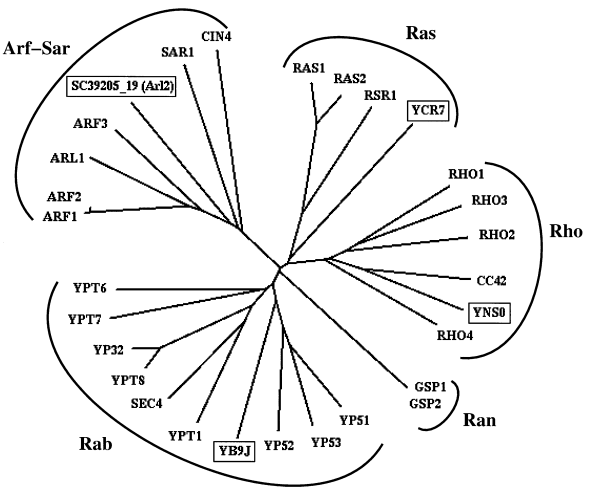

Fig. 1. Dendrogram of the ras superfamily in yeast. The different proteins are named using the SwissProt or EMBL identifiers. The names in boxes indicate new putative genes discovered by the total sequencing of *S. cerevisiae*. So far, there is no experimental evidence on their expression or function. Multiple-sequence alignments and trees of all available ras sequences or all ras sequences in yeast are available at http://www.cnb.uam.es/~cnbprot/ras_yeast. The identification of the ras sequences in the yeast genome ([75] and http://www.mips.biochem.mpg.de/mips/yeast/) was carried out with the GeneQuiz system [76,77]. GeneQuiz is an automated system for large scale sequence analysis that includes a careful selection of updated databases, a composition bias mask procedure, well-known algorithms such as BLAST [78], and FASTA [79] and other searching methods [80–83]. The system has been implemented experimentally in the form of a server for the analysis of single sequences in the context of a collaborative research project. Multiple-sequence alignments and trees were obtained with ClustalW [84] that implements a neighbor-joining algorithm [85].

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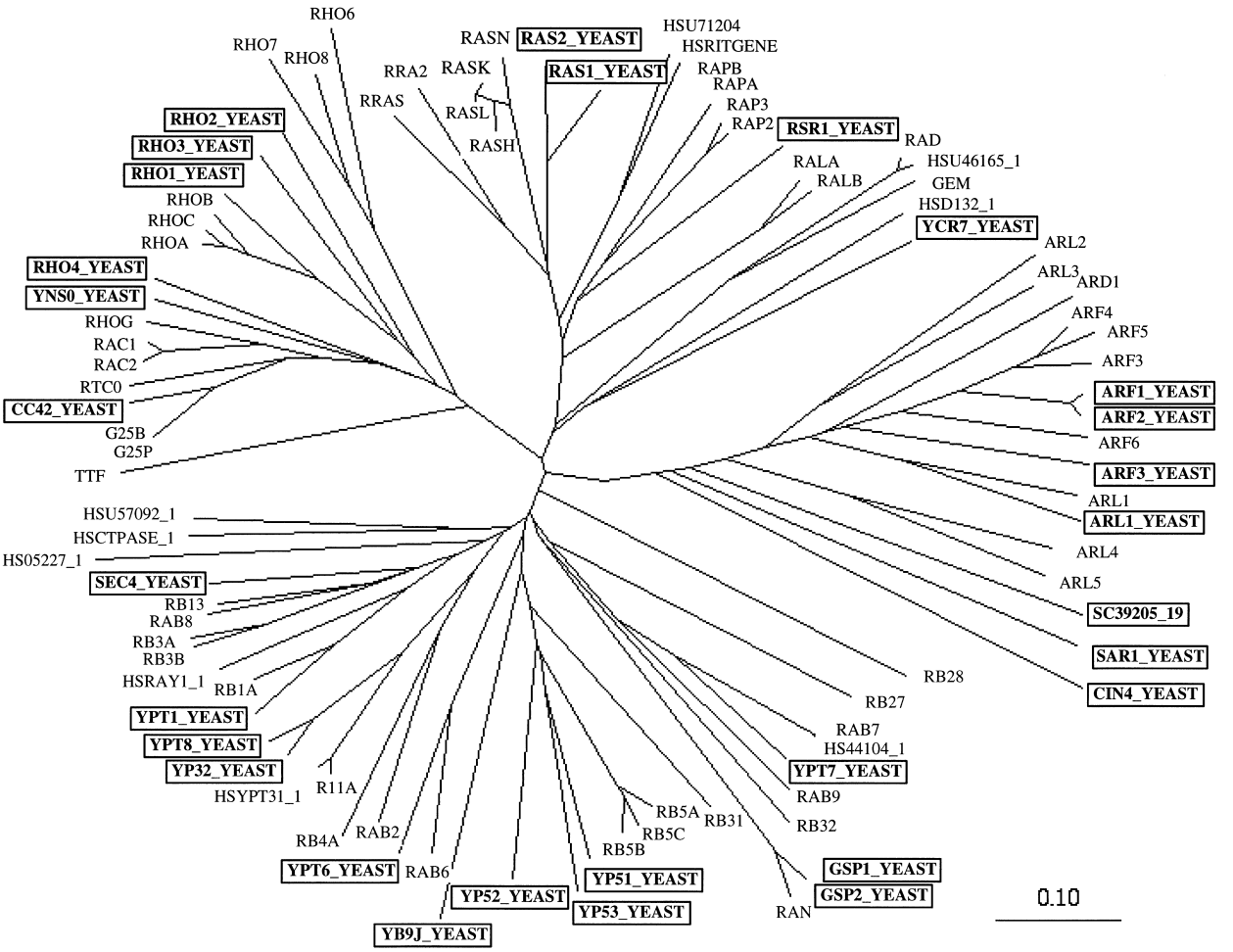


Fig. 2. Dendrogram of all known yeast (boxes) and human sequences of the ras superfamily. Sequences are indentified by their SwissProt or EMBL data base names.

the ERA-THDF family of prokaryotic proteins [8], TEM1 (SW:tem1_yeast), related to the termination of meiosis [9] and Yn44 (SW:yn44_yeast), an ORF in chromosome XIV. Yn44 is the only protein that presents not only the main GTP binding motifs, but also the C-terminal Cys motif; it has already been classified in the rab family by overall sequence similarity [10]. The presence of a large insertion and the impossibility of finding a clear match for the (F/Y)xExSA(K/L) GTP-binding motif [5] makes this classification tentative until some functional information becomes available.

2.2. The arf-sar family

Arf-sar sequences are implicated in vesicular transport in both exocytic and endocytic pathways [11,12]. This family covers a broad range of sequence divergence while retaining a good degree of functional complementation. For example, human Arf1, Arf4, Arf5 and Arf6, and *Giardia* Arf are able to complement and rescue the Arf1-Arf2 lethal yeast double mutant (see also Fig. 2) [13,14].

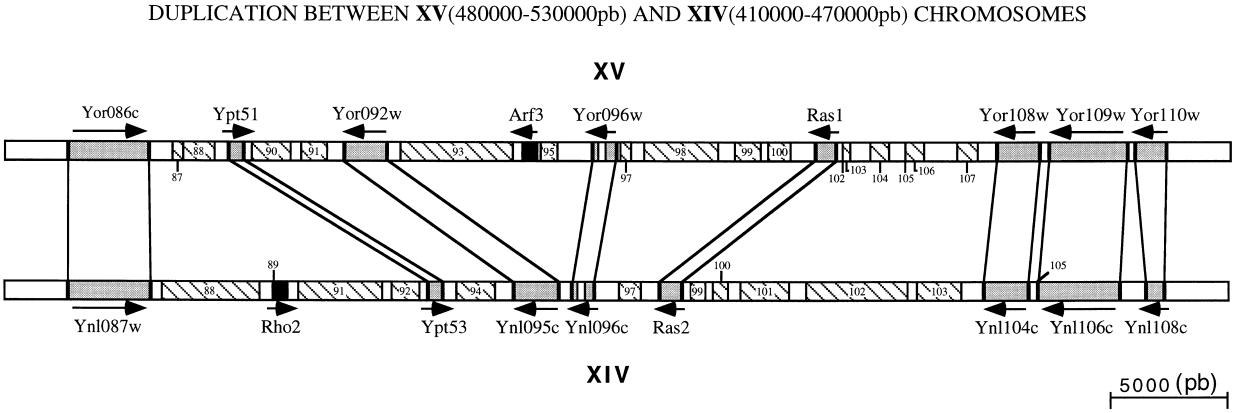
Arf1 and **Arf2** are very similar (96% identity), conforming a well-differentiated branch of the family. Both genes are apparently the product of an ancient duplication of chromosome IV [15]. The deletion of both genes is lethal [16], but their individual deletion produces viable cells. While the deletion of Arf2 is indistinguishable from the wild-type, deletion of Arf1 is slightly defective, giving rise to slow growth, cold sensitivity, and supersensitivity to fluoride. They also differ in their expression: Arf1 is constitutively expressed, whereas Arf2 is repressed by glucose [14]. The differences between the Arf1 and Arf2 genes suggest specific functional adaptations after their duplication.

Arf3 is a distant member of this family, and its function in yeast is unclear. It is not essential for cell viability and is not required for endoplasmic reticulum-to-Golgi protein transport [17]. A certain relation to Arf6 sequences has previously been proposed, based on detailed sequence analysis and molecular modelling [18].

For **Arl2**, detailed analysis shows a close relationship to *Drosophila* and human Arl2. There is no significant evidence about the possible role of Arl2 [19].

Cin4 is not an essential gene in yeast [20,21]. Its function has been partially characterized as a benomyl-hypersensitive mutant implicated in microtubule function [16]. It is the most distant sequence of the arf family and seems to be a gene specific to yeast, since no similar sequences have been found in other organisms.

Sar1 is the only arf family gene known to be essential in yeast [22]. Sar1 is located in the endoplasmic reticulum membrane [23], is involved in the secretory pathway and regulates trafficking between the endoplasmic reticulum and Golgi [24].



Chr. XV	Chr. XIV	Protein Classification
Yor086c	Ynl087w	Unknown proteins
--------	Ynl090w	Rho2
Yor089c	Ynl093w	Ypt51/Ypt53
Yor092w	Ynl095c	Unknown proteins
Yor094w	--------	Arf3
Yor096w	Ynl096c	Ribosomal proteins (S7)
Yor101w	Ynl098c	Ras1/Ras2
Yor108w	Ynl104c	2isopropylmalalate synthases (Leu4)
Yor109w	Ynl106c	Phosphatidylinositol phosphate phosphatases
Yor110w	Ynl108c	Unknown proteins

Fig. 3. Representation of the Ras1/Ras2 duplicated cluster. ORFs are represented by boxes. Dark boxes point to clearly duplicated ORFs. Black boxes represent Arf3 and Rho2 proteins. Dashed boxes represent other ORFs in this region. ORFs are labelled by their sequential order. The names and functions of the duplicated ORFs plus the two ras proteins Arf3 and Rho2 are given.

Arl1 is clearly similar to Arl1 sequences in other organims. In *Drosophila*, it has been shown to be an essential gene [25]. The specific function of Arl1 is still unclear, but its location in the cell cytosol and Golgi apparatus raises the possibility of a role in secretion [26].

2.3. The ran family

Ran proteins are involved in nucleo-cytoplasmic transport, including protein import into the nucleus and RNA export [27,28]. All sequences in this family are very similar, including the yeast sequences **Gsp1** and **Gsp2**. Interestingly, there are two copies of this gene in plants and yeast, and apparently only one in animals [29].

Both genes in yeast appear to be the result of a duplication of chromosomes XII and XV [15]. The sequence of these genes seems to be highly constrained, with only five amino acid differences in non-core regions, and 92% of the nucleotide changes restricted to non-coding positions (synonymous changes). The disruption of Gsp1 is lethal for the cell, whereas the disruption of Gsp2 produces a viable phenotype [30] and its overexpression is able to rescue the disruption of Gsp1 [31].

The remarkable protein conservation after a large DNA divergence is accompanied by very different expression levels of these two genes [31]. It is possible that the presence of these proteins is justified by their adaptation to different cellular conditions, achieved by regulating expression rather than by differences in their biochemistry. Unfortunately, little is known about the evolution of the regulation of gene expression.

2.4. The rab family

Rab proteins play a role in the regulation of vesicular trafficking [32]. The specific function of all rab proteins is nevertheless not yet fully understood. They seem to be required in assembling or proofreading the general docking/fusion machinery of the cell [33].

In yeast as in other organisms, rab constitutes the largest ras family, with ten members. The full sequencing of yeast has brought to light a new sequence of this family, Yb9j.

Ypt6 is clearly similar to the Rab6 subfamily. Indeed, the Rab6 gene from *Arabidopsis thaliana* complements yeast Ypt6 mutants [34]. Ypt6 is implicated in transport regulation between late Golgi and prevacuolar endosome-like compartments [35].

The Rab5 cluster includes three sequences, **Ypt51**, **Ypt52** and **Ypt53**. These proteins appear to be implicated in the endocytic pathway. Disruption analysis of Ypt51 affects endocytic transport and vacuolar protein sorting. Their functions overlap partially, since Ypt51 disruption is viable, but cell viability is progressively affected by double (Ypt51/Ypt52) and triple mutants (Ypt51/Ypt52/Ypt53) [36,37].

Sequence analysis suggests two subsequent duplications, first including the Ypt52 and Ypt51/Ypt53 ancestors, and second, the duplication of Ypt51 and Ypt53 (Fig. 1). Ypt51 and Ypt53 are part of a region duplicated between chromosomes XV and XIV that also includes the Ras1 and Ras2 genes (Fig. 3).

Yb9j can be considered a very distant member of the Rab5 cluster. It is not yet known if it is really expressed or what function it has [38]. Deletion of Yb9j does not disable the cell

in any observable manner [10]. No similar sequences have been found so far in other species.

Ypt7 belongs to the Rab7 cluster, sharing similarities in its effector domain and C-terminal region. Ypt7 seems to be implicated in protein transport between endosome-like compartments [39]. Disruption of Ypt7 is viable, although it produces disorders in vacuolar transport and maturation [40]. Analogously, Rab7 is localized in the late endosomal compartment in animal cells, and its mutation leads to accumulation of small vesicles [41].

Ypt31 (also called Ypt8) and **Ypt32** belong to the Rab2–Rab4 cluster, both of them are derived from a duplicated region of chromosomes VII and V [15]. Only the double disruption of Ypt31 and Ypt32 is lethal [42]. Ypt31 and Ypt32 are implicated in the budding of vesicles from the trans-Golgi compartment [43].

Ypt1 is an essential gene of the Rab1 cluster [44], necessary for vesicular transport from the endoplasmic reticulum to the Golgi apparatus [45]. The similarity of function between Rab1 and Ypt1 is supported by the complementation of the Ypt1 mutant by mouse Rab1 [46].

Sec4 is an essential gene for cell growth [47]. Its mutation gives rise to cells which accumulate secretory vesicles [48]. Sec4 is required at the post-Golgi stage of the secretory pathway for protein transport to the cell surface [49]. Sec4 belongs to the Rab8–Rab10 cluster and has a clear homologue in *S. pombe*, denoted Ypt2.

2.5. The rho family

The rho family controls actin cytoskeleton dynamics in response to extracellular signals, and has an important function in the budding process [50]. The rho family is divided in two sub-families, rho and rac, which apparently regulate partially overlapping pathways. Yeast has a complete representation of these proteins.

Rho1 is an essential gene that localizes at the growth site and it is required for bud formation [50,51].

Rho2 is not essential and its disruption produces no detectable phenotype; it may, however, regulate aspects of the budding process [52]. Rho2 has no clearly related sequence besides the *S. pombe* Rho2.

Rho3 and **Rho4** are relatively different (57% sequence similarity), although they are functionally related. Rho3 and Rho4 seem to be required after initiation of bud formation to maintain cell polarity during the maturation of the daughter cells [53,54]. No clearly related sequences have been found

Table 1
Functional information about the ras proteins in yeast

Protein name	Type of mutation	Functional relation with other genes	Phenotype		
			Normal	Some (viable)	Essential gene
Ras1 (ras) (acc: p01119)	Disrupted	Double mutant with **ras2** is lethal	X		
Ras2 (p01120)	Disrupted	Double mutant with **ras1** is lethal	X		
Rsr1 (p13856)	Deletion	Overlapped function with Rap1A		X	
Ycr7 (p25378)	–	Hypothetical protein	–	–	–
Rho1 (rho) (p06780)	Disruption	With human RhoA			X
Rho2 (p06781)	Disruption	–	X		
cc42 (Cdc42) (p19073)	Deletion	Overexpression of **cdc42** rescues Rho3			X
Rho3 (q00245)	Deletion	Double deletion mutant (Rho3, Rho4) is lethal at 30°C		X	
Rho4 (q00246)	Deletion	Double deletion Rho4/Rho3 is lethal. Overexpression **Rho4** rescues Rho3	X		
Yns0 (p53879)	–	Hypothetical protein	–	–	–
Ypt51 (rab) (p36017)	Deletion	With mammals Rab5. And triple mutant (Ypt51, Ypt52 and Ypt53) is lethal		X	
Ypt52 (p36018)	Deletion	Triple mutant (Ypt51, Ypt52 and Ypt53) is lethal	X		
Ypt53 (p36019)	Deletion	Triple mutant (Ypt51, Ypt52 and Ypt53) is lethal	X		
Ypt31 (ypt8) (p38555)	Disruption	Double mutant (Ypt31, Ypt32) is lethal	X		
Ypt32 (p51996)	Disruption	Double mutant (Ypt31, Ypt32) is lethal	X		
Ypt6 (q99260)	Disruption	With *Arabidopsis thaliana* Rab6		X	
Yb9j (p38146)	Deletion	–	X		
Ypt7 (p32939)	Disruption	With mammals Rab7		X	
Ypt1 (p01123)	Disruption	With mouse Rab1			X
Sec4 (p07560)	Disruption	–			X
Gsp1 (ran) (p32835)	Disruption	Overexpression of Gsp2 rescues Gsp1 mutant			X
Gsp2 (p32836)	Disruption	Overexpression of Gsp2 rescues Gsp1 mutant	X		
Cin4 (arf) (p39910)	Disruption	No homologous		X	
Arf1 (p11076)	Deletion	**Arf2** (96%), double mutant (Arf1/Arf2) is lethal		X	
Arf2 (p19146)	Deletion	**Arf1** (96%), double mutant (Arf1/Arf2) is lethal	X		
Arf3 (p40994)	Disruption	–	X		
Sc39205_19 (u39205)	–	Probable *Drosophila* Arl2 homologous (no publications)	–	–	–
Sar1 (p20606)	Disruption	Sar1 of *S. pombe* rescues Sar1 mutant of *S. cerevisiae*			X
Arl1 (p38116)	–	Hypothetical protein (no publications)	–	–	–

in other organisms. Disruption of the Rho3 gene produces viable cells with very poor growth, whereas Rho4-disrupted cells are normal, and the double mutant has enhanced growth defects. Overexpression of Rho4 is able to rescue Rho3 mutants [55].

Cdc42 belongs to the Rac subfamily, and has a close homologue in *S. pombe*. The Cdc42 sequences are highly conserved in different species and involved in controlling cell polarity and organizing the actin cytoskeleton [56]. Yeast Cdc42 is an essential gene.

Yns0 is a newly characterized ORF in the Cdc42 group (62% similarity to Cdc42), whose function is unknown. The comparison of Yns0 protein and DNA sequences with those of Cdc42 suggest that Yns0 may be expressed as a functional protein. The relatively larger proportion of differences at DNA level suggests a rather large divergence time, but the fact that these differences accumulate mostly in the third codon positions (data not shown) suggests a selective pressure to retain function.

2.6. The ras subfamily

This family has a major role in signal transduction (Table 1).

Ras1 and **Ras2** are derived from a duplication of chromosome XIV and XV [15]. In yeast, they have different patterns of gene expression that allows a certain redundancy of function [57]. The double disrupted mutant is lethal, whereas neither Ras1 nor Ras2 are essential genes by themselves [58]. They appear to perform different tasks in the cellular senescence process [59,60].

Rsr1 is related to the Rap subfamily, with a function similar to human Rap1A [61]. Deletion of Rsr1 does not affect growth, but its presence is required for the selection of bud site [62,63], probably by recruitment of one or more cell polarity proteins to the bud sites [64].

Ycr7 is the most divergent sequence of the ras family, distantly related to the Rad and Gem subfamilies. Its function remains unclear.

Ral is the only subfamily not present in yeast. Ral proteins seem to be important in early steps of endocytosis in specialized superior cell types [65,66].

2.7. Ras, a large protein superfamily with a high degree of functional redundancy

Duplications, deletions, translocations, mutational drift and functional adaptations have shaped the current composition of the Ras family. Despite its long evolutionary history, a large ancient duplication (approx. 100 mYr-old) took place of which the involvement of at least five pairs of ras sequences is still visible, involving 35% of the superfamily (Ras1/Ras2, Ypt31/Ypt32, Ypt51/Ypt53, Gsp1/Gsp2, and Arf1/Arf2) [15]. Remarkably, the duplicated genes still retain similar functions in all these cases and only the double mutants are lethal.

Apart from these duplications, a general overview of the genomic distribution of ras sequences reveals no particular trend (not shown). A case that deserves special attention is that of the Ras1 and Ras2 pair, which is part of a duplicated region including other ras-related genes, i.e. Ypt51, Arf3 and Ras1 in chromosome XV, and Ypt53, Rho3 and Ras2 in chromosome XIV (Fig. 3). Most of the genes in this region are found in the same order and orientation. The original duplication has suffered a number of alterations, for example; the distance between the different duplicated ORFs is not conserved and two of the genes may have been derived from other deletion and/or insertion processes (Arf3 and Rho2), since they are not directly related. It would be interesting to know whether there is a functional reason for the survival of the gene order in these regions after such a long time.

Indeed, the functional redundancy of the system is even more general, with at least 14 sequences (almost 50% of the superfamily) showing some type of overlapping functional relation (Ras1-Ras2, Cdc42-Rho3, Rho3-Rho4, Ypt51-Ypt52-Ypt53, Ypt31-Ypt32, Gsp1-Gsp2, and Arf1-Arf2). This redundancy probably guarantees the stability of the organism, while the progressive functional divergence acquired over time

Table 2
Comparision of known functions of ras proteins in and *Schizosaccharomyces pombe* and their homologues in *Saccharomyces cerevisiae*

Schizosaccharomyces pombe	Type of mutation	Phenotype			*Saccharomyces cerevisiae*	Type of mutation	Phenotype		
		No effects	Some effects (viable)	Essential gene			No effects	Some effects (viable)	Essential gene
Ras[a] (acc.: p08647)	Disruption			X	**Ras1**	Disruption	X		
					Ras2	Disruption	X		
Cdc42 (rho) (q01112)	Disruption			X	**cc42** (rho)	Deletion			X
Rho1 (rho) (q09914)	Disruption			X	**Rho1** (rho)	Disruption			X
Rho2 (rho) (q10133)	No data	–	–	–	**Rho2** (rho)	Disruption	X		
Spi1 (ran) (p28748)	Disruption			X	**Gsp1** (ran)	Disruption			X
					Gsp2 (ran)	Disruption	X		
Ypt1 (rab) (p11620)	Disruption			X	**Ypt1** (rab)	Disruption			X
Ypt2 (rab) (p17609)	Deletion			X	**Sec4** (rab)	Disruption			X
Ypt3[a] (rab) (p17610)	Disruption				**Ypt31** (rab)	Disruption	X		
				X	**Ypt32** (rab)	Disruption	X		
Ypt5 (rab) (p36586)	Disruption		X		**Ypt52** (rab)	Disruption	X		
Ryh1 (rab) (p17608)	Disruption	X			**Ypt6** (rab)	Disruption		X	
Arf1 (arf) (p36579)	No data	–	–	–	**Arf1** (arf)	Deletion		X	
					Arf2 (arf)	Deletion	X		
Arl (arf) (q09767)	No data	–	–	–	**SC39205_19**	No data	–	–	–
Sar1 (arf) (q01475)	Rescues *S. cerevisiae* Sar1	–	–	–	**Sar1** (arf)	Disruption			X

[a]Genes related to duplications in *S. cerevisiae*, apparently not duplicated in *S. pombe*.

provides broader functional plasticity and improved adaptation to the environment.

One interesting aspect of the duplication of the ras proteins is the different rate of divergence between corresponding pairs (Fig. 1, see for example the difference in branch length between the pairs Gsp1/Gsp2 vs. Ypt51/Ypt53). The apparent coexistence of different molecular clocks in the same protein family is striking.

2.8. Comparison of the ras superfamily between Saccharomyces cerevisiae and Schizosaccharomyces pombe

The species most closely related to *S. cerevisiae*, for which a large number of sequences is known, is *S. pombe*. The information available on *S. cerevisiae*, a budding yeast, and the partial information available on *S. pombe*, a fission yeast, is compared in Table 2.

In *S. cerevisiae*, at least six ras genes are essential for cell viability, seven produce some phenotypic disorders, and 11 show no significant differences from the wild type. For five genes, there is no functional information.

In *S. pombe*, there are 13 known ras-related proteins. Nine of them have been mutated and only two are not essential: Ypt5, the homologue of yeast Ypt52 [67], and Ryh1 [68], the yeast Ypt6 homologue. In *S. pombe*, as in *S. cerevisiae*, Ras [69], Cdc42 [70], Spi1, the Gsp1 homologue, [71], Rho1 [72], Ypt1 [73] and Ypt2, the Sec4 homologue, [74] are essential genes. With this partial data, the two systems appear to have a similar number of essential genes.

At the same time, some of the proteins that perform fundamental functions show different copy numbers. For example, the disruption of ras is lethal in *S. pombe*, whereas in *S. cerevisiae* only the double Ras1/Ras2 mutant is lethal. Ypt3 is essential for *S. pombe*, although for *S. cerevisiae* only the double Ypt31/Ypt32 deletion is lethal. These two related organisms have apparently been subject to different duplication and gene rearrangement processes while retaining a similar core of essential functions.

2.9. Comparison of the ras superfamily in humans and yeast

The full sequencing of the human genome will complete the puzzle of all possible ras functions, but the information already available and the possibility of manipulation in yeast already facilitates the direct functional study of human proteins in yeast.

The available sequence relationships between the ras proteins in yeast and humans are displayed in Fig. 2. It is striking to note the existence of highly specialized protein subfamilies not present in yeast (e.g. ral) or specialized proteins such as human rap. In the case of yeast, it would be interesting to assess the role of the distantly related members of the superfamily such as Ycr7. There are other clear examples of specialization after duplication in the ras and rac subfamilies that are more extended in humans, while the arf subfamily contains examples of the opposite behavior, with apparently more duplications in yeast than in humans. In the near future, with the sequencing of other eukaryotic genomes, it should become possible to assess the existence of different duplication processes, including the yeast genome duplication [15], and the proposed concurrent duplications in the vertebrate lineage 500 mYr old [86].

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