Database search for yeast vacuolar nucleic acid hydrolases

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Master thesis

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Nov 2008 - Jan 2009

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Abstract

The yeast vacuole has an important role in the degradation of cellular material. The vacuole has a low pH that is regulated by H^+-ATPases at its limiting membrane that is necessary for the activation of hydrolases involved in the catabolic process. This organelle contains a variety of hydrolases, such as proteases, lipidases, glycosydases and phosphatases, which are delivered to the vacuole via biosynthetic routes. Degradative pathways deliver the cargoes that are targeted for destruction from different locations, including structures containing nucleic acids. It remains completely unclear, however, how the DNA and RNA is degraded in the vacuole. The most obvious possibility is that there are one or more nucleases in this organelle. Here, we present a computational database search for nucleic acid hydrolases in the yeast genome. This search identified a few potential genes that could potentially act as vacuolar nucleases and they are discussed in detail.

Abbreviations

V-ATPase: Vacuolar H^+-ATPase

TMD: Transmembrane domain

MVB: Multi vesicular body

Vps: Vacuolar protein sorting

Cvt: Cytoplasm-to-vacuole

Sec: Secretory

PAS: Phagophore assembly site/preautophagosomal structure

PM: Plasma membrane

ESCRT: Endosomal sorting complex required for transport

PMN: Piecemeal microautophagy to the nucleus

NV: Nucleus-vacuole

Vde1: Vma1-derived endonuclease

CAS: Conserved active-site segment

Introduction

The yeast vacuole has a role in multiple cellular processes such as maintaining the pH and osmotic homeostasis, and as a storage compartment for amino acids, ions and polyphosphates. The storage of components in the vacuole requires that this organelle has a direct role in the regulation of the availability of these metabolic compounds and ions. To perform this function, numerous specific membrane transmembrane transporters for the different stored components mediate their transport across the limiting membrane of the vacuole. The yeast vacuole is considered to be the equivalent of the mammalian lysosome as they both have an important role in the degradation of substrates containing the hydrolases needed for this function. The focus in this report will be on the yeast vacuole in its role as a catabolic organelle.

The vacuole receives a wide variety of material; the components targeted for degradation but also all the hydrolases necessary for this catabolic function. There are multiple routes that deliver compounds to the vacuole; these pathways can be biosynthetic or degradative transport routes. The degradative routes deliver their cargo that is targeted for destruction from different locations, for example plasma membrane and cytoplasm. The biosynthetic routes, in contrast, deliver the hydrolases. To regulate the vacuolar and cellular pH the vacuole limiting membrane contains H^+-ATPases (V-ATPases). The regulation of the vacuolar pH by V-ATPases is needed for its function as a degradative compartment because it is necessary for the activation of these hydrolases, including proteases, lipidases, glycosydases and phosphatases.

Importantly, material containing nucleic acids is delivered to the vacuole for destruction but it remains completely unclear how the DNA and RNA is degraded in the vacuole. The most obvious possibility is that there are one or more nucleases in this organelle. Here, we present a computational search for nucleic acid hydrolases in the yeast genome. First, a short overview is given about the important components of the vacuole and the main transport pathways to this organelle. Then, the genome search for nucleases in the *S. cerevisiae* genome database is illustrated. The presence of a transmembrane domain (TMD) and/or a signal peptide plus a nuclease domain are the important properties that should be present in the potential vacuolar nucleases. The search generated a few potential genes that contain one or more of these features and can potentially act as vacuolar nucleases. The most potential nuclease gene is *RNY1*, which codes for an RNase that has the best features to be a potential vacuolar hydrolase. Also, few other interesting genes came up, but they seem less likely vacuolar nucleases. All the hits of this search are discussed in detail in a final chapter.

1 The vacuolar H^+-ATPases (V-ATPase)

The yeast vacuole is, like the mammalian lysosome, the most acidic cellular organelle. In the vacuole the pH varies from 5 to 6,5 depending on the growing conditions. Compared to the lysosome, which generally has a pH of 4,5-5; the vacuole pH is higher and varies more than that of the lysosome. The low pH in the organelle is coupled to its function in a variety of cellular processes as for example the endocytic and biosynthetic transport sorting, the zymogen activation and the transport across the membrane of metabolites and ions. To establish the acidic environment, the vacuole limiting membrane contains an ATPase proton pump, the so-called vacuolar H^+-ATPase (V-ATPase). This pump hydrolyzes ATP to generate the energy necessary for the transport of protons from the cytosol into the lumen of the vacuole. (Review: Li 2008) The loss of V-ATPases (*vma* mutants) is lethal in eukaryotes except for fungi because these organisms can uptake protons from the extracellular environment by endocytosis. The yeast *vma* mutants do show phenotypes, but can function. Next to the pH regulation, the V-ATPase also generates a membrane potential, which is critical for the functioning of the transporters present in the vacuole membrane.

1.1 The V-ATPase structure and regulation

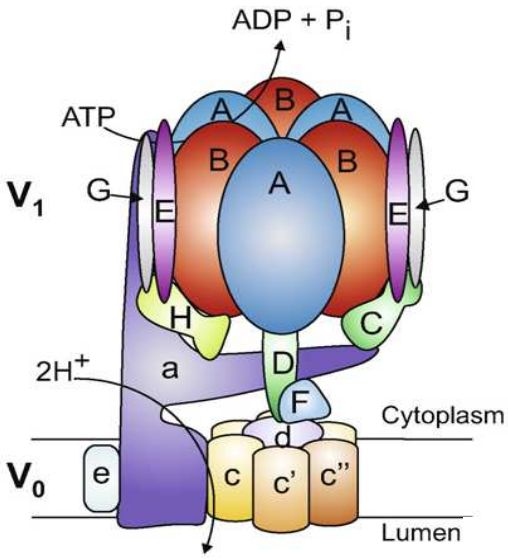

Fig. 1 **A model of the vacuolar H^+-ATPase proton pump -** The subunits of the vacuolar H^+-ATP (V-ATPase) are represented. The cytosolic domain (V1) is membrane bound and drives the ATP hydrolysis. The integral membrane domain (V0) translocates the protons to the vacuole lumen. (Figure: Jefferies 2008)

The V-ATPase proton pumps are conserved enzymes. They are protein complexes containing two functional multi-subunit domains. The complex of subunits termed V1, which contains three catalytic ATP binding sites, is located peripherically to membranes. Bound to V1 is a complex of integral membrane subunits, V0, which is the pore necessary for the proton translocation into the vacuole. Hydrolysis of ATP leads to conformational changes in the V1 complex. This makes the central stalk in the middle of V1 (subunits D, F and d) rotate. The V0 connected to V1 also rotate through the static large subunit a. This rotation drives the proton translocation across the V0 domain. (Fig. 1; review: Jefferies 2008)

In yeast, all the subunits are present in one isoform, except for the largest membrane subunit a that couples the cytosolic domain to the integral membrane domain, which has 2 isoforms. The two

isoforms of subunit a, are Vph1p and Stv1p (Manolson 1994) and are localized to the vacuole and Golgi, respectively. The vacuole located, Vph1-containing V-ATPase has been shown to have a more efficient coupling between ATP hydrolysis and proton transport than the Stv1p-containing one in the Golgi.

The regulation of the acidification of the vacuole occurs via different mechanisms. One of them occurs at the level of the V-ATPase and involves a rapid and reversible disassembly of V1 from the V0 domain, simultaneously blocking the ATP hydrolysis in V1 as well as the proton transport through V0. The assembly and disassembly of the V-ATPases is a way to regulate their use. For example, upon a decrease in glucose levels, the disassembly occurs. The mechanism how V-ATPases senses and respond to glucose is yet unclear. For the reassembly, however, a protein complex named RAVE is essential but is not directly involved in the sensing of glucose (Smardon 2002). (Review: Li 2008; Jefferies 2008)

2 The vacuolar hydrolases

One of the major functions of the vacuole is the degradation of biological components. The delivered cargo targeted for degradation can be cytosolic, organellar, extracellular or plasma membrane material. This material is transported to the vacuole via multiple degradation routes (see chapter 3). To catabolize this variety of material, the vacuole contains a subset of hydrolases. The hydrolases can be soluble or membrane bound and each one of them is able to degrade a specifc substrate. Most vacuole hydrolases are transcribed in an inactive precursor form (= zymogen) and are processed into a mature active form once in the vacuole. (Table 1)

2.1 The activation cascade

As mentioned, most of the hydrolases are generated as a zymogen and need to be processed to become active before they can function in the vacuole. Their activation requires the proteolytic removal of a propeptide. The activation of the zymogens in the vacuole dependents mostly on Pep4, but also Prb1 is important. An active form of Pep4 is required for the initiation of the activation cascade (Nebes 1991), to lead to the processing of all the inactive precursors (Table 1).

Maturation of proPep4 into a mature pseudo-Pep4 can occur autocatalytically, under the influence of the low pH in the vacuole. This self-processing of this protease is incomplete, but is able to cleave proPrb1 to Prb1, and partially proPrc1 and proCps1 into a pseudo-Prc1 and pseudo-Cps1. The complete removal of the propeptide from the pseudo-Pep4 to generate a fully mature Pep4 depends on active Prb1, formed by the pseudo-Pep4. The mature Prb1 is able to process proPep4,

Table 1. **The vacuolar hydrolases**

Proteinases	Name	Abbre-viation	Zymogen	Vacuolar localization	Gene	Pathway to vacuole	References
Endoproteinases	Proteinase A Proteinase B	Pep4 Prb1	Yes Yes	Soluble Soluble	*PEP4* *PRB1*	Prc1 Prc1	(Knop 1993; Van den Hazel, 1996; Parr 2007)
Carboxypeptidases	Carboxypeptidase Y Carboxypeptidase S	Prc1 Cps1	Yes Yes	Soluble Soluble	*PRC1* *CPS1*	Prc1 Prc1*	(Knop 1993; Van den Hazel, 1996)
Aminopeptidases	Aminopeptidase I Aminopeptidase Co	Ape1 ApCo	Yes	Soluble	*APE1*	Cvt	(Knop 1993; Van den Hazel, 1996)
Dipeptidylamino-peptidase	Dipeptidylamino-peptidase B	Dap2	No	Membrane bound	*DAP2*	Prc1	(Knop 1993; Van den Hazel, 1996; Roberts 1989)
Other hydrolases							
	Trehalase		Yes	Membrane bound			(Huang 2007)
	α-mannosidase		Yes	Membrane bound	*AMS1*	Cvt	(Klionsky 1990)
Phosphatase	Alkaline phosphatase	Pho8	Yes	Membrane bound	*PHO8*	Pho8	(Klionsky 1990)
Lipase	Cvt17			Membrane bound	*CVT17*	Prc1*	(Teter 2001)
Lipid phosphate phosphatases	Diacylglycerol pyrophosphate	Dpp1		Membrane bound**	*DPP1*		(Oshiro 2003)

* travels as a membrane associated enzyme
** active site is located at the cytosol

but also proPrc1, pseudo-Prc1, proApe1, proPrb1 and proPho8. The processing of pseudo-Pep4 by Prb1 is expected to occur, since the conversion of proPep4 into Pep4 can also occur upon the presence of Prb1 (Fig. 2). When mature Prb1 is present upon the autocatalytic activation of proPep4 to pseudo-Pep4, under the low pH conditions present in the vacuole, the activation of all the inactive hydrolases can occur and then the autocatalytic activation of Pep4 is not required anymore. (Van den Hazel, 1992)

3 Transport routes to the vacuole

Proteins at different cellular locations can be transported to the vacuole through different pathways (Fig.3). Depending on their nature, cargoes can have different fates. They can be degraded or as in the case of hydrolases, they are processed into their mature, active form and fulfill their function. There are multiple routes to deliver the newly synthesized hydrolases to the vacuole and they are called the biosynthetic routes. The delivery of cargo targeted for degradation can come from different locations in the cell as well. Thus the routes that these cargoes follow depend on where the material is located.

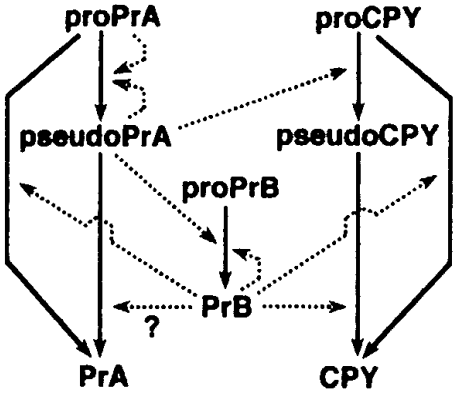

Fig. 2 **The maturation of proPep4, proPrb1 and proPrc1** - Part of the activation cascade is shown here. All the possible processing steps for proPep4, proPrb1 and proPrc1 are indicated with a solid arrow. The dashed arrows indicate the enzymes involved in the maturation step. The step from pseudo-Pep4 is indicated with a question mark, because this step has not yet been demonstrated, but is assumed to occur. (Figure: Van den Hazel 1992)

3.1 The biosynthetic routes

Via the biosynthetic routes, the newly translated hydrolases are transported to the vacuole and sometimes undergo post-translational modifications on their way to this destination. There are three major biosynthetic pathways to the vacuole. Numerous proteins are involved in the different biosynthetic pathways, but the focus here will be on the most important components and the main events that characterize each pathway.

3.1.1 The Prc1 pathway

Most of the soluble hydrolases, the membrane bound hydrolase Dap2 and the V-ATPase traffic via a part of the secretory pathway and reach the vacuole passing through endosomes. The most studied hydrolase following this route is Prc1 and consequently this pathway has been named the Prc1/CPY pathway. In this pathway, the precursor hydrolases contain signal peptides that lead them to their translocation into the ER, where the signal peptide is then cleaved off by a signal peptidase. The proPrc1 also undergoes N-glycosylation in the ER and then the protein is transported to the Golgi. In the Golgi, the oligosaccharides get further modified before that proPrc1 reaches the late Golgi compartments. At this location, the soluble vacuolar proteins are sorted out from the secretory pathway by receptors. The proPrc1 contains a signal sequence in its propeptide that is recognized by its specific sorting receptor, which is encoded by the *VPS10* gene, in this way the proPrc1 is directed to the vacuole (Marcusson 1994). Mutations in the propeptide signal sequence (Johnson 1987; Valls 1987) or overexpression of Prc1 (Stevens 1986) lead to the secretion of this protease in the medium. The Vps10-proPrc1 complex is transported to late endosomes, the convergence point with the endocytic pathway, where the receptor dissociates from proPrc1 and recycles back to the late Golgi to bind more proPrc1. The limiting membrane of late endosomes invaginates forming a multivesical body (MVB). Mature MVB then fuses with the vacuole and proPrc1 is delivered in the interior of this organelle (Review: Bryant 1998).

The proteinase Cps1 uses a variation of this route, as it is transported as a membrane protein to the late endosomes and is inserted into the invaginations in order to end up in the MVB luminal vesicles before being delivered into the vacuole, where the processing by Pep4 removes its transmembrane domain (Li 2008). To enter the MVB internal vesicles, the cytoplasmic tail of Cps1 has to be ubiquitinated. In contrast, the membrane bound lipase Cvt17, which has a topology similar to that of Cps1, travels in an ubiquitin-independent way into the internal vesicles of MVB (Epple 2003).

3.1.2 The Pho8 pathway

The vacuolar membrane hydrolase Pho8, but also the SNAREs Vam3 and Nyv1, uses a distinct route than Prc1 and Cps1. This was determined by making vacuolar protein sorting (*vps)* mutations that abolishes certain steps of trafficking between endosomes. In particular, both the *vps45Δ* and the *pep12Δ* mutants show defects in targeting vesicles derived from the Golgi to the endosome (Cowles 1994; Piper 1994). In a mutant strain, containing one or both these mutations, Pho8 transport to the vacuole is not affected. This revealed that Pho8 does not travel through endosomes and takes a distinct route direct from the late Golgi to the vacuole. This protein contains a signal sequence in its cytosolic amino-terminal tail, what actively targets it for this direct pathway known as the Pho8/ALP pathway. When there is overexpression of Pho8 or the Pho8 pathway is defective, part or all this protein can be transported to the vacuole via the same pathway as Cps1 and Prc1 (Cowles 1997).

3.1.3 The cytoplasm-to-vacuole (Cvt) pathway

The vacuolar hydrolases Ams1 and Ape1 are transported directly from the cytoplasm into the vacuole through the cytoplasm-to-vacuole (Cvt) pathway. They do not contain a signal sequence to target them into the ER and are not glycosylated, but they possess a signal that is required for targeting them into the vacuole. With the help of secretory (*sec)* mutants, the trafficking of these proteins through ER or Golgi has been excluded. First, Ams1 was identified as a protein that uses a direct route from the cytosol to the vacuole (Yoshihisa 1990) and successively this biosynthetic route was shown to be specific for both hydrolases (Hutchins 2001; Klionsky 1992). The specificity is guarantee by the cargo receptor Atg19/Cvt19 (Scott 2001). In this route, the proApe1 first forms dodecamers in the cytosol that further assemble in a more complex structure called the Ape1 complex through the self interaction of its propeptide. The Ape1 complex binds to the receptor Atg19 as well as proAms1, and together they form the Cvt complex. Then another protein, Atg11/Cvt9, associates to the receptor and targets the Cvt complex to the phagophore assembly site/preautophagosomal structure (PAS). The PAS generates a double-membrane vesicle, called a Cvt vesicle, which encloses these two hydrolases and Atg19. The Cvt vesicles successively fuse with the vacuole releasing the internal vesicle in the lumen of this organelle. The membrane of this vesicle is

degraded resulting in the liberation of prApe1 and prAms1 (Shintani 2002). This route uses almost the same molecular machinery as autophagy, which will be discussed later (chapter 3.2.2).

3.2 The degradative transport routes

The degradative transport routes deliver a wide range of material that has to be degraded to the vacuole. Extracellular or plasma membrane (PM) proteins are transported via endocytosis while cytosolic or large cellular components such as organelles are targeted to the vacuole via autophagy. Again, numerous proteins are involved in these two routes pathways, but the focus here will be on the most important components and the main events that characterize these two transport routes.

3.2.1 The endocytic pathway

The uptake of extracellular or PM material occurs via vesicle-mediated pathways. In this process the vesicles form at the cytosolic site of the PM through a mechanism of budding and pinching off in the cytoplasm. The targeting of PM proteins to the vacuole for degradation can be constitutive to renew them or signal-dependent to regulate signaling by internalization. There are multiple endocytic routes, like the well-studied receptor-mediated endocytosis via clathrin-coated pits, but also there are the non-clathrin mediated endocytic routes such as phagocytosis, micropinocytosis or caveolae-mediated endocytosis, but those appear not to exist in yeast (Review: Mayor 2007). Although the mechanism of cargo selection and vesicles budding can be different, most of the endocytic routes pass through the early and the late endosomes/MVB before reaching the vacuole.

The continuous signal-independent endocytosis of PM proteins depends on the recognition and binding of targeting signals in the cytoplasmic tail by adaptor proteins of the clathrin-dependent pathway, like AP-2 and clathrin itself. When a ligand binds a receptor at the PM, signaling cascades are activated but also the signal-dependent receptor-mediated endocytosis by phosphorylation and mono-ubiquitination of the receptors at their intracellular tails. These events trigger the recruitment of the adaptor proteins necessary for the budding of the membrane. At this site, numerous proteins are recruited but AP-2 and clathrin are the ones required to form a coated-pit, which invaginates and pinch off in a dynamin-dependent manner to form clathrin-coated pits (Review: Mousavi 2004). After endocytosis, the clathrin coat of the vesicle is dissembled and the vesicle can fuse with early endosomes. From there, the multiubiquitination of the receptors direct them to the late endosome and then into the MVB. Finally, the fusion between MVBs and vacuole lead to the degradation of the receptors.

3.2.2 Autophagy

The term autophagy is generally used to define the process of degradation of cytosolic components by the vacuole/lysosome. The degradation of cytosolic contents occurs constitutively, but also under stress-related conditions such as starvation. Multiple types of autophagy are known. For example, there can be direct uptake of cytoplasm by invagination and pinching off of the vacuole limiting membrane through microautophagy or intermediate vesicles can be formed that sequester cargo and deliver it to the vacuole through macroautophagy. Here we will discuss the process of macroautophagy and a particular type of microautophagy, the piecemeal microautophagy to the nucleus (PMN).

3.2.2.1. Macroautophagy

Macroautophagy is a process used for the sequestration of cytosolic content into large double-membrane vesicles known as autophagosomes and its transport into the vacuole for degradation. It allows the elimination of cytoplasmic proteins but also entire organelles. The Cvt pathway resembles to this route, as they share most of the molecular machinery. The autophagy-related (*ATG*) genes are involved in autophagy and to date, 30 *ATG* genes involved in either starvation-induced autophagy and/or the Cvt pathway have been identified.

The process of autophagy starts with generation of a phagophore, which occurs at the PAS. At this site, the core Atg machinery, which are involved in the formation of the autophagosome, mediate the formation of the phagophore, an intial cisterna that is the precursor of the sutophagosome. The events that occur at the PAS and the origin of the membranes are still not exactly known. Expansion and sealing of the phagophore lead to the enclosure of the cargo into the autophagosome. The core machinery that is responsible for the formation of the vesicle does not localize at the completed autophagosome, but is retrieved back via an Atg1-Atg13 signaling complex (Reggiori 2004). Once completed, autophagosomes are transported to the vacuole, where their outer membrane fuses with the vacuole limiting membrane while the internal vesicles is liberated inside vacuole and called an autophagic body. Finally, the vacuole hydrolases Pep4, Prb1 and Cvt17 degrade the autophagic body and its content.

Under stress conditions autophagy is non-specific. The Cvt pathway, in contrast, is a form of selective type of autophagy because it is involved in the exclusive transports of a specific cargo (chapter 3.1.3). The two pathways share the core machinery, but other specific factors dictate the specificity and the vesicle size, e.g. autophagosomes are 300-900 nm in diameter and Cvt vesicles are ± 150 nm in diameter (Shintani 2002; reviewed by Xie 2007).

3.2.2.2 Piecemeal microautophagy to the nucleus (PMN)

During microautophagy cargo is directly sequestered into a vesicle generated through invagination of the vacuole limiting membrane and its pinching off into the vacuole lumen. In *S.*

cerevisiae there can be degradation and recycling of part of the nucleus via a form of microautophagy, PMN. In yeast, there is a connection between the nucleus and the vacuole at nucleus-vacuole (NV) junctions. These junctions are formed by the interaction of Vac8 at the vacuole membrane and Nvj1 at the nuclear membrane (Pan 2000). This connection is important for the selectivity and induction of PMN. During PMN, the nuclear membrane protein of the NV junction recruits at least two other proteins in process to the PMN, Tsc13 (Kvam 2005) and Osh1 (Kvam 2004). The molecular mechanism of PMN can be divided in the following steps: 1) the NV junction is formed by interaction between Vac8 and Nvj1, 2) the nuclear envelope bulges, 3) the bulge becomes a thin-necked bleb, 4) the bleb pinches off into the vacuole lumen and finally 5) the vesicle gets degraded. During starvation the amount of NV junctions becomes higher and at these locations, a portion of the nucleus can be targeted for degradation. When the bleb is in the vacuole, the proteins involved in the formation of this structure are also degraded along with their nucleoplasmic content. (Roberts 2003). In contrast to earlier reports, a recent work of Krick and co-workers has shown that the core autophagic machinery is also required for the first stages of PMN. Pep4 and Cvt17 are also required for the completion of PMN, but they are implicated in the last stages of this pathway (Krick 2008).

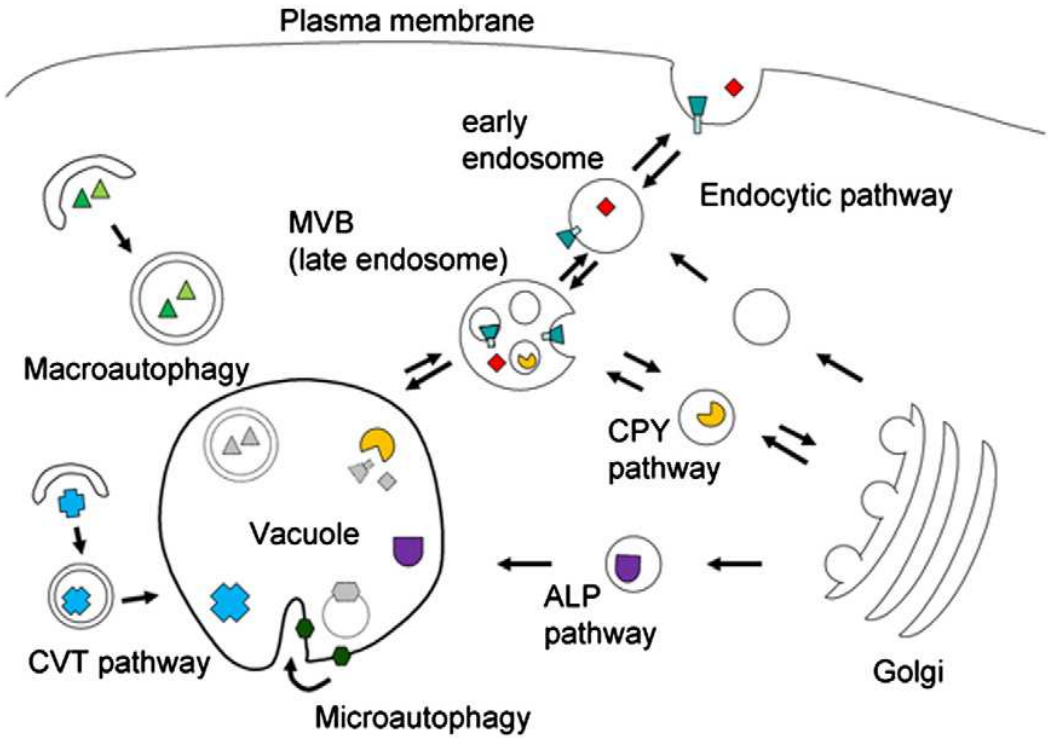

Fig.3 **An overview of the transport routes to the vacuole** – All the transport routes to the vacuole discussed in this chapter, except PMN, are resumed here. The illustrated biosynthetic pathways are the Prc1 (CPY), the Pho8 (ALP) and the Cvt pathways. The routes for degradation represented are endocytosis and macroautophagy. The Prc1 pathway uses part of the secretory pathway compartments, and from the Golgi proteins are delivered to the MVB, where it converges with the endocytic pathway before fusion with the vacuole. The Pho8 pathway is a direct route from the Golgi to the vacuole and the Cvt pathway shares the molecular components with autophagy.

4 The degradation of nucleic acids in the vacuole

In the vacuole, proteins, lipids and carbohydrates can certainly be degraded because hydrolases involved in this specific catabolism have been identified. If there is degradation of nucleic acids, DNA and RNA, is not exactly known. However, there is transport of material to the vacuole that contain DNA or RNA such as ribosomes (RNA) and mitochondria (RNA and DNA) via autophagy or fractions of the nucleus (RNA) through PMN. As mentioned above, the vacuole is capable of degrading a very wide range of cell components, but how it catabolizes the nucleic acids? To be able to perform this enzymatic reaction, exo- and/or endo-nucleases should be present in the vacuole. We have performed a computational amino acid sequence homology search in the genome of *S. cerevisiae* to find potential vacuolar nucleases.

The search for DNases and RNases was preformed using the information collected in one of the *Saccharomyces* genome databases (www.yeastgenome.org). In this database, the entire genome of the yeast *Saccharomyces cerevisiae* is recorded and gives a lot of literature-based information about each gene, including its systematic name, function, eventual localization and known interactions. This database can be searched using the gene name as a key word, but also words such as “nuclease” or “RNase”. The database also contains the nucleotide and amino acid sequence of each gene and protein, respectively. To identify which known domains are present in a protein, sequences can be analyzed using the SMART program (Simple Modular Architecture Research Tool; http://smart.embl-heidelberg.de). This site uses different programs to predict and reveal particular domains, coiled-coil regions, signal peptides, transmembrane domains (TMD) or regions that show low complexity. All this information together can give an idea if a protein is able to fulfill a nuclease function in the vacuole. The important characteristics for such a protein are that it possesses a nuclease activity and should reach the vacuole. To do that, this factor should have a TMD and/or a signal peptide.

4.1 The results obtained searching the databases

The search has started with the use of key words linked to the function that the gene/protein should have, like RNase and (endo-/exo-)nuclease. In tables 4 to 9 (see appendix), all identified genes have been listed indicating their gene name, their systematic name, their RNase or DNase activity, subcellular location, their function and if they contain a TMD or a signal peptide. The genes that are potentially interesting are *VDE1*, *RNY1* and *REX4* (Table 2).

After this initial search, all the DNase or RNase that are listed in tables 4 to 9 where used to perform BLAST analysis within the yeast genome database and all the putative proteins, proteins of unknown functions and some known genes involved in delivery to the vacuole where listed in a tables and again checkedfor their location in the cell and the presence of a TMD or signal peptide

(tables 10 to 15, see appendix). This additional search added few genes to the list of potential vacuolar nucleases (Table 3).

4.2 Potentially interesting nucleases/proteins

The database search only gave a few potentially interesting genes that can act as nucleases in the vacuole. The characteristic of containing a TMD, a signal peptide and a nuclease domain are not always found in the listed genes. Here we will discuss these genes and the probability that they could be a vacuolar nuclease.

4.2.1 The homing endonuclease Vde1

Vde1 (Vma1-derived endonuclease) is a homing nuclease that is encoded within the gene *VMA1* encoding the V-ATPase subunit A (Gimble 1992). The extein gene product of *VMA1* localizes to the vacuolar membrane, what could be interesting for its intein (internal protein sequences) product. *VDE1* is produced via an autocatalytic protein splicing reaction of *VMA1* (Hirata 1990; Kane 1990), but Vde1 seems not to act at the vacuole because of the domain it contains. Vde1 belongs the group I of intron endonucleases and contains the homing endonuclease domain LAGLI-DADG (Lambowitz 1993). Homing is a process of lateral transfer of an intronic or intein sequence to another allele that lacks the sequence leading to a duplication of the sequence. Vde1 creates a double strand break at specific extein *VMA1* alleles, which contains the Vde1-recognition sequence that does not contain this intron to initiate homing of its own sequence during meiosis (Gimble 1992). The abilities and function of the gene in homing gives an indication that Vde1 probably will not function in the degradation of nucleic acids.

4.2.2. T2 endoribonuclease Rny1

The T2 endoribonuclease family includes unspecific RNases that are secreted into the extracellular space or are present in endomembrane compartments. Several T2 RNases are found in lysosomes or lysosome-like structures (Irie 1999), but yeast T2 RNases are often found extracellularly, probably because they have a function in nutrition (Deshpande 2002). A general common function for the conserved family of T2 endorinucleases has not been defined. In a review of Irie, acid RNases are described and all the isolated acid RNases belong to the T2 protein family (Irie 1999). The fact that those are enzymes working in acidic conditions is an interesting feature because the vacuole has a low pH. There are indications that Rny1 is secreted into the extracellular space when overexpressed, but the active enzyme was found intracellularly (MacIntosh, 2001).

One of the conserved characteristics of the T2 RNases is the presence of two conserved active-site segments (CAS), CAS I and II, which contain important catalytic residues, three histidines and a glutamate (Irie 1999), required for RNase activity. Rny1 is the only T2 RNase found in *S. cerevisiae*, just like the other acid T2 RNases, it contains these two CAS sequences (Kobayashi 2003).

Table 2. **Interesting RNase and DNase from the searches with key words**

Yeastgenome.org					SMART		
Gene name (alias)	**Systematic name**	**RNase/ DNase**	**Location (GFP database)**	**Function**	**TMD**	**Signal Peptide**	**Domains**
SCEI/VDE (VDE1, YDL184w-a, YDL185W2)		DNase	?	Site-specific homing endonuclease; derived from the self-splicing of the TFP1 gene product	No[#]	No[#]	Hom_end[#]*, Hom_end_ hint[#]**
VMA1[!] (CLS8, TFP1)	YDL185W		Vacuolar membrane	V-ATPase V1, subunit A; protein precursor undergoes self-catalyzed splicing: extein Tfp1p and intein Vde	No	No	HintN, HintC***
RNY1	YPL123C	RNase	?, Extracellular region	Member of the T2 family of endo-ribonucleases	No	Yes	Ribonuclease _T2****
REX4	YOL080C	RNase	Nucleus and nucleolus	Putative RNA 3'-5' exonuclease possibly involved in pre-rRNA processing and ribosome assembly	No	No	EXOIII

! codes for VDE1; # retrieved from the complete sequence of VMA1
* stands for homing endonuclease, it is able to bind DNA, lies between the Hint domains***
** the protein splicing domain of VDE
*** splitted domain to accommodate large insertions of endonucleases
**** RNase domain of the T2 family containing CASI and CASII, conserved sequences of T2 endoribonucleases

Another interesting feature is that Rny1 is regulated under stress conditions, as its promoter region contains sequences for stress-related transcription factors essential to adapt to heat shock factors, oxidative stress, and hypoxia, and two regions that respond to a number of other stress conditions (MacIntosh 2001). It also contains a signal peptide in its sequence; these features implicate that Rny1 could be an acid RNase located in the vacuole. There are other features in the gene that could be in contrast with this hypothesis. Rny1 is larger then other T2 RNases, just like two other T2 ribonucleases found in two *Basidiomycetes* (Kobayashi 2003) and the C-terminal tail is different from that of other T2 ribonucleases because it contains two putative nuclear localization signals (MacIntosh 2001). In a search using SMART, however, these targeting signals were not found.

4.2.3. The exoribonuclease Rex4

The RNase Rex4 is, according to the SGD database, localized in the nucleus and nucleolus. The function assigned to this RNase is a possible involvement in the pre-rRNA processing and ribosome assembly (Faber 2004), what would fit with its localization. In a paper of Norambuena and co-workers, *REX4* is mentioned for being influenced by a synthetic compound, Sortin2, causing the vacuolar sorting defects of Pcr1 (Norambuena 2008). What this means for the function of Rex4 and if there is an indirect effect on the vacuolar sorting is not addressed. Sortin2 mainly affected the endomembrane system because inhibitorialy binding proteins and that causes the sorting defects. Because it cannot be excluded at priori that a small pool of this RNase could localize to the vacuole and consequently it could be potentially involved in a vacuolar degradative process.

4.2.4. The interesting putative proteins and proteins of unknown function

In table 3, 5 genes are listed and divided in 2 different groups. The first group includes the two most interesting genes, Q0255 and Q0075 (*AI5_BETA*), as they both contain a TMD and/or signal peptide plus the nuclease domain LAGLI_DADG. Q0255 also contains an intron encoded nuclease repeat (IENR1) motif, the function of which is unknown but is probably able to bind DNA and a NUMOD1 domain, which is a helix-turn-helix-containing DNA-binding domain found in homing nucleases. The presence of these features within these genes is relevant, because it shows that they could fulfill a nucleic acid hydrolysis function and can potentially be targeted to the vacuole. Unfortunately, there is no subcellular localization data present in the SGD database and there are indications that these genes are located to the mitochondria, the *AI5_BETA* gene is even encoded within an intron of the mitochondrial *COX1* gene. As the DNase Vde1, mentioned above, they also contain this LAGLI_DADG domain that is a site-specific endonuclease involved in homing. Interestingly, the database states about AI5_BETA that the translational initiation codon is predicted to be ATA rather than the ATG SMART intron-encoded DNA endonuclease. This indicates that the reading frame of this gene might be different and not encodes for an endonuclease.

In the second group, 4 interesting genes are listed: *YKL207w*, *YOL166w-a*, *YFR045w* and *YKR013w* (*PRY2*). These genes all contain a TMD and/or signal peptide, and a domain that can be interesting because the function of these different domains, indicated in table 3, are not yet known or the found domains are under the significance threshold of SMART. *PRY2* and *YFR045w* are of special interest because of the subcellular location and the potential domains, respectively. *YFR045w* potentially contains a DDT domain that could bind DNA; an EXOIII domain, which is an exonuclease domain and a G-alpha domain, which contains a guanine nucleotide binding site. The last two mentioned domains are probably inactive when present as catalytic residue(s) within the domains are missing, as is indicated by SMART. The localization of this protein is predicted to be mitochondria, where it could fulfill a transporter function, as it has similarity with the mitochondrial transporter Rim2 and contains one TMD. So, it indeed seems that the potential domains with DNA binding and nuclease functions in *YFR045w* are not present or inactive. *PRY2* shows similarities with the Ant1 protein, a peroxisomal adenine nucleotide transporter. Importantly, Pry2 is present in the vacuole according to the SGD database, which indicates that it could possibly mediate the nucleotide transport in and/or out of the vacuole, but the protein only contains a signal peptide and no TMDs. Also, the description of the SCP domain in the protein indicates that it is only present in a family of extracellular proteins. The precise function of the domain, however, is not known.

About the domains of the other two genes, *YKL207w* and *YOL166w-a*, no information can be found with SMART. In addition, there are no data about these genes present in the database, including their subcellular localization.

Tabel 3. **Interesting genes obtained with the BLAST searches**

Yeastgenome.org					SMART		
(Reserved) gene name (alias)	**Systematic name**	***BLAST from***	**Location (GFP database)**	**Function**	**TMD**	**Signal peptide**	**Domain(s)**
Interesting genes containing a TMD and/or signal peptide and a nuclease domain							
	Q0255	*MMS4*	? **mitochondrion	Maturase-like protein	No	Yes	LAGLIDADG_1, site-specific DNA endonu-cleases and IENR1, intron encoded nuclease repeat motif and NUMOD1, DNA-binding domain in homing endonucleases
AI5_BETA	Q0075	*AI4*	? **mitochondrion	Protein of unknown function, encoded within an intron creating an endonucease	No	Yes	LAGLIDADG_1, domain of site-specific DNA endonucleases
Interesting genes containing a TMD and/or signal peptide and a potentially interesting domain							
(LRC3)	YKL207W	*RAT1*	?	Putative protein of unknown function; non-essential	Yes, 3	Yes	DUF850, putative TM proteins of unknown function
	YOL166W-A	*SEN2*	?	Identified by gene-trapping, microarray-based expression analysis, and genome-wide homology searching	Yes, 1	No	DUF468, family of uncharacterised yeast proteins
	YFR045W	*RIM2*	? **mitochondrion	Putative mitochondrial transport protein	Yes,1	No	Below treshold multiple domains like: EXOIII, G_alpha, DDT and AICARFT_ IMPCHas
PRY2 (YFW12)	YKR013W	*ANT1*	Vacuole	Protein of unknown function	No	Yes	SCP, family of extra-cellular domains, precise functions still unclear

***sebcellular location decribed by the yeast genome database*

Conclusions and discussion

The yeast vacuole is an important organelle for the degradation of a wide range of cellular compounds. Via multiple routes, the organelle receives not only the substrates targeted for destruction but also the hydrolases necessary to degrade them. In the vacuole, under influence of its low pH, the hydrolases become active and degradation can occur. Autophagy is one of the delivery pathways to the vacuole. Macroautophagy of organelles like mitochondria but also PMN can deliver DNA and/or RNA into the vacuole. When the DNA and RNA are in the vacuole, they are probably degraded and nucleotides may be recycled. This raises the question that there could be one or more vacuolar nucleases and nucleotide transporters. Therefore, a database search was preformed using the information collected in the *Saccharomyces* genome databases (www.yeastgenome.org) to identify such enzymes.

To try to identify all the potential nucleases, first key word-based searches where done to find all the known nucleases (table 4 to 9). Simultaneously, they where all checked for the presence of

TMD(s) and/or a signal peptide because this are the important properties for the potential transport to the vacuole. Almost all the known vacuolar hydrolases contain at least one of these features, except for Ape1 and Ams1. After this first search, all the nucleases retrieved were used to perform a BLAST search (table 10 to 15). During this search, next to the presence of a TMD and/or a signal peptide, also the presence of a nuclease domain was an important selection factor.

The first search gave only three potential vacuolar nuclease genes (table 2) and *RNY1* appears to be a very interesting RNase. Rny1 belongs to the family of T2 endoribonucleases and is the only one found in *S. cerevisiae* (MacIntosh 2001). Like all the T2 nucleases, Rny1 contains two CAS sequences with important catalytic residues (Irie 1999). It also contains a signal peptide indicating that this gene could also be located at the vacuole. The non-site-specific T2 RNases are often found in lysosome-like structures and are therefore called acid RNases (Irie 1999), but yeast T2 RNases are often found extracellularly (Deshpande 2002). Consequently, it could also be possible that Rny1 is present in the extracellular space. Although the functions of the T2 nucleases are not exactly known and Rny1 is larger then other T2 RNases and the C-terminal tail is differently from that of other T2 ribonucleases, this gene is still interesting for further experimental research.

The BLAST search gave only five potential interesting genes. From this search, 2 found genes appear to be interesting, *YFR045w* and *PRY2*. The SMART program indicates that there are a subset of domains present in *YFR045w*, but they are not significant and may be inactive due to the loss of the catalytic residues. When we looked at other aspects of the protein, it contains one TMD and it is predicted to localize to mitochondria, where it could fulfill a transporter function. The protein has similarities with the mitochondrial transporter Rim2. Pry2 also shows similarities with the transporter protein Ant1, a peroxisomal adenine nucleotide transporter. Pry2 localizes in the vacuole according to the SGD database, but no TMDs are present and consequently a transporter function can be excluded. The only interaction of Pry2 is with the RNA-binding protein She2 (Oeffinger 2007), which binds specific mRNAs and is part of the mRNA localization machinery that restricts accumulation of certain proteins to the bud. It is complicated to state something about the function of Pry2 in the vacuole and obviously more experimental data is required.

In conclusion, the database search permitted to identify only a few potential genes involved in nucleic acid hydrolysis or transport. *RNY1* is the most interesting RNase that emerged because of what it is known about other acid T2 RNases but it exact localization and function are not yet clear. It would be interesting to explore that experimentally to see the relevance of this computational search. *YFR045w* can potentially fulfill a nucleotide transporter function, but there are strong indications that the protein localizes to mitochondria. In contrast, *PRY2* localization is known but its function is not due to its domain and no TMDs are present. The transport of DNA or RNA transport to the vacuole is possible, but the presence of vacuolar nucleases is still unclear.

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Appendix

Table 4. **RNase genes found with RNase as keyword search**

Yeastgenome.org					SMART	
Gene name (alias)	**Systema-tic name**	**RNase/ DNase**	**Location (GFP database)**	**Function**	**TMD**	**Signal peptide**
RNY1	YPL123C	RNase	?	Member of the T(2) family of endoribonucleases	No	Yes
POP2 (CAF1)	YNR052C	RNase	Cytoplasm, mitochondrion	RNase of DEDD superfamily, subunit of Ccr4-Not complex, mediates 3' to 5' mRNA deadenylation	No	No
POP3	YNL282W	Rnase ?	Nucleus	Subunit of RNase MRP, cleaves pre-rRNA, and nuclear RNase P, cleaves tRNA precursors to mature 5'ends	No	No
POP5 (FUN53)	YAL033W	RNase	Cytoplasm and nucleus	"	No	No
POP6	YGR030C	RNase	Nucleus	"	No	No
POP7 (RPP2)	YBR167C	RNase	Nucleus	"	No	No
POP8	YBL018C	RNase	Nucleus	"	No	No
RPP1	YHR062C	RNase	Nucleus	"	No	No
SNM1	YDR478W	Rnase?	Nucleus	Subunit of RNase MRP complex, cleaves pre-rRNA and cell cycle-regulated degradation of daughter mRNAs; not shared between RNase MRP and nuclear RNase P	No	No
RPR2	YIR015W	RNase	Nucleus	Subunit of nRNase P; not shared between RNase MRP and RNase P	No	No
RPM2	YML091C	RNase	Mitochondrion	Subunit of mitochondrial RNase P, roles: cytoplasmic + mitochondrial RNA processing and translation	No	No
POP1	YNL221C	RNase	Cytoplasm and nucleus	Subunit of both RNase MRP and nRNase P; binds to the RPR1 RNA subunit in RNase P	No	No
POP4	YBR257W	RNase	?	"	No	No
NUC1	YJL208C	Both	Mitochondrion	Major mitochondrial nuclease, RNAse and DNA endo- and exonucleolytic activities	No	Yes
RNH1	YMR234W	RNase	Cytoplasm and nucleus	Ribonuclease H1; binds double-stranded RNAs and RNA-DNA hybrids (hydrolyse RNA portion)	No	No
RNH201 (RNH35)	YNL072W	RNase	Cytoplasm and nucleus	Ribonuclease H2 catalytic subunit A, removes RNA primers during Okazaki fragment synthesis; cooperates with Rad27p	No	No
RNH202	YDR279W	RNase	Nucleus	Ribonuclease H2 subunit B, required for RNase H2 activity	No	No
RNH203	YLR154C	Rnase	Cytoplasm and nucleus	Ribonuclease H2 subunit C, "	No	No
NGL2	YMR285C	RNase	Cytoplasm	Ccr4p-like RNase required for correct 3'-end formation of 5.8S rRNA at site E	No	No
MTR3	YGR158C	RNase	Cytoplasm and nucleus	3'-5' exoribonuclease, exosome subunit; involved in export of mRNA and ribosomal subunits	No	No
DIS3 (RRP44)	YOL021C	RNase	Cytoplasm, nucleus and nucleolus	3'-5' exonuclease activity, catalytic component of the exosome, involved in RNA processing and degradation	No	No
REX3	YLR107W	RNase	Cytoplasm and nucleus	3'-5' exonuclease, member of RNase D family of exonucleases, required for maturation of RNase MRP RNA component	No	No
DSS1(MSU1)	YMR287C	Rnase	Mitochondrion	3'-5' exoribonuclease, component of the mitochondrial degradosome, mediates turnover of aberrant or unprocessed RNAs	No	No
REX2 (YNT20)	YLR059C	RNase	Mitochondrion, nucleus	3'-5' RNA D exonuclease; involved in 3'-end processing of U4 and U5 snRNAs and 5S and 5.8S rRNAs	No	No

The dark grey rows indicate genes with the same properties or which are in a complex, green rows indicate genes with known other function then nuclease (involves all tables)

Table 5. **Search via known interaction partners of Dnases or RNases started with the known EXO1**

Yeastgenome.org					SMART	
Gene name (alias)	**Systema-tic name**	**RNase/ DNase**	**Location (GFP database)**	**Function**	**TMD**	**Signal peptide**
EXO1 (DHS1)	YOR033C	DNase	Nucleus	5'-3' exonuclease and flap-endonuclease, recombination, DBS repair and DNA mismatch repair	No	No
RAD2	YGR258C	DNase	Cytoplasm and nucleus	Single-stranded DNA endonuclease, cleaves single-stranded DNA in NER to excise damaged DNA	No	No
HO	YDL227C	DNase	Nucleus	Site-specific endonuclease required for gene conversion at the MAT locus (homothallic switching)	No	No
MRE11 (RAD58 , XRS4, NGS1)	YMR224C	DNase	Cytoplasm and nucleus	Subunit of MRX complex, mediates the structural specific endo- and 3'-5'exonuclease nuclease activities	No	No
RAD27 (ERC11, RTH1, FEN1)	YKL113C	DNase	Nucleus	5'-3' exonuclease and 5' flap endonuclease; Okazaki fragment processing, maturation and long-patch base-excision repair	No	No
APN1	YKL114C	DNase	Mitochondrion, nucleus	Apurinic/apyrimidinic endonuclease, 3'-repair DNA damage repair and a 3'-5' exonuclease to repair 7,8-dihydro-8-oxodeoxyguanosine	No	No
KEM1(DST2,RAR5, SEP1, SKI1, XRN1)	YGL173C	RNase	Cytoplasm	5'-3' exonuclease; component of P bodies involved in mRNA decay	No	No
PSO2 (SNM1)	YMR137C	Dnase	Cytoplasm and nucleus	5'-3' exonuclease, repair of DNA single and DBS	No	No
RAD1 (LPB9)	YPL022W	DNase	Nucleus	Single-stranded DNA endonuclease, NER and DBS repair	No	No
RAD10	YML095C	DNase	Cytoplasm and nucleus	Single-stranded DNA endonuclease, "	No	No
SAE2 (COM1)	YGL175C	DNase	Cytoplasm and nucleus	Endonuclease, processes hairpin DNA structures with MRX complex in meiotic + mitotic DSB repair	No	No
RAT1 (HKE1, TAP1)	YOR048C	RNase	Nucleus	5' to 3' single-stranded RNA exonuclease, rRNA and snRNA processing, mRNA transcription termination	No	No
MUS81 (SLX3)	YDR386W	DNase	?	Structure-specific endonuclease, involved in DNA repair and replication fork stability	No	No
MMS4 (SLX2, YBR100W)	YBR098W	DNase	?	Subunit of Mms4p-Mus81p endonuclease, cleaves branched DNA	No	No
MTR3	YGR158C	RNase	Cytoplasm and nucleus	Exosome subunit, for 3'-5' exoribonuclease activity	No	No
MTR4 (DOB1)	YJL050W	RNase	Nucleus and nucleolus	Co-factor of nuclear exosome complex, exonuclease for 3' end formation; dead-box helicase	No	No
SKI3 (SKI5)	YPR189W	RNase	Cytoplasm	Involved in exosome mediated 3'- 5' mRNA degradation, translation inhibition of non-poly(A) mRNAs	No	No
SKI8 (REC103)	YGL213C	RNase	Cytoplasm	"	No	No
RNH70(REX1 ,RNA82)	YGR276C	RNase	?, **nucleus	3'-5' exoribonuclease; required for maturation of 3' ends of 5S rRNA and tRNA-Arg3	No	No
PAN2	YGL094C	RNase	Cytoplasm	RNase, control of poly(A) tail length, regulates stoichiometry, activity of postreplication repair complexes	No	No
NOP58 (NOP5)	YOR310C	RNase	Nucleolus	RNase, endonuclease involved in pre-rRNA processing, 18S rRNA synthesis, and snoRNA synthesis	No	No
CDC39 (NOT1, ROS1 , SMD6)	YCR093W	RNase	Cytoplasm	Component of CCR4-NOT complex, multiple roles in regulating mRNA levels including 3'-5' exoribonuclease activity, regulation of transcription and destabilizing mRNAs by deadenylation	No	No

Table 6. **DNase and RNase genes found with a nuclease keyword search**

Yeastgenome.org					SMART	
Gene name (alias)	**Systema-tic name**	**Rnase/ DNase**	**Location (GFP database)**	**Function**	**TMD**	**Signal peptide**
DIN7 (DIN3)	YDR263C	DNase	?	Mitochondrial nuclease functioning in DNA repair and replication	No	No
TATD (from Lit)	YBL055C	Dnase	Cytoplasm	3'-5' exonuclease and endonuclease with a possible role in apoptosis	No	No
FCF1	YDR339C	Rnase	?	Putative PINc domain nuclease; cleavage of 35S pre-rRNA and maturation of 18S rRNA	No	No
DNA2	YHR164C	DNase	Nucleus	DNA replication factor: single-stranded DNA-dependent ATPase, ATP-dependent nuclease and helicase activities	No	No
	YGL085W	?	Mitochondrion	Putative protein with homology to Staphylococcus aureus nuclease	Yes	No

Table 7. **RNases and DNases found with exonuclease search**

Yeastgenome.org					SMART	
Gene name (alias)	**Systema-tic name**	**RNase/ DNase**	**Location (GFP database)**	**Function**	**TMD**	**Signal peptide**
RRP6	YOR001W	RNase	Nucleus and nucleolus	3'-5' exonuclease, component of the nuclear exosome	No	No
REX4	YOL080C	RNase	Nucleus and nucleolus	Putative RNA 3'-5' exonuclease maybe involved in pre-rRNA processing, ribosome assembly	No	No
*RRP42**						
*RRP43**						
TRZ1	YKR079C	RNase	Cytoplasm and nucleus	tRNA 3'-end processing endonuclease tRNase Z	No	No
YME2 (RNA12, PRP12)	YMR302C	?	Mitochondrion	Some sequence similarity to exonucleases; Integral inner mitochondrial **membrane** protein	No	No
From function description						
RRP42	YDL111C	RNase	Nucleus, nucleolus, cytoplasm	Component of the exosome 3'-5' exonuclease complex; involved in rRNA processing	No	No
RRP43	YCR035C	RNase	Nucleus and nucleolus	"	No	No
RRP4	YHR069C	RNase	Cytoplasm, nucleus and nucleolus	3'-5'exoribonuclease, exosome non-catalytic core-component; processing of rRNA and mRNA		
RRP41 (SKI6, ECM20)	YGR195W	RNase	Nucleus and nucleolus	3'-5' phosphorolytic exoribonuclease, subunit of the exosome	No	No
DIS3 (table 4)						

**Described later with its other complex genes*

Table 8. **Rnases and Dnases found with endonuclease search**

Yeastgenome.org					SMART	
Gene name (alias)	**Systema-tic name**	**RNase/ DNase**	**Location (GFP database)**	**Function**	**TMD**	**Signal peptide**
AI4	Q0065	?	?, **mitochondrion	Endonuclease, encoded by mobile group I intron within the mitochondrial COX1 gene	Yes	Yes
AI3	Q0060	?	?, "	"	Yes	Yes
AI5ALPHA	Q0070	?	?, "	"; involved in intron mobility	Yes	Yes
SCEI	Q0160	DNase	?, **mitochondrion	I-SceI DNA endonuclease, mediates gene conversion that propagates the intron into intron-less copies of the 21S_rRNA gene	No	No
NGL3	YML118W	RNase ?	?	Putative endonuclease, domain similar to magnesium-dependent endonuclease motif in CCR4	No	No
NGL1	YOL042W	RNase ?	Mitochondrion	"	No	No
NGL2(table 4)		RNase	Cytoplasm	Ccr4p-like RNase		
VDE1(YDL184w-a, YDL185w2,SCEI/VDE)	?	?	?	DSB repair site-specific homing endonuclease; derived from the self-splicing of the Tfp1/Vma1 gene product	?	?
SCEI/ENS2		DNase	?	Mitochondrially-encoded subunit of Endo.SceI, dimeric multi-site-specific endonuclease, introduces DBS at well-defined sites on mitochondrial DNA	?	?
CCE1 (MGT1)	YKL011C	DNase	Mitochondrion	Mitochondrial cruciform cutting endonuclease, cleaves Holliday junctions	No	No
SEN2	YLR105C	RNase		"; contains the active site for tRNA 5' splice site cleavage	No	No
SEN34 (FUN4)	YAR008W	RNase	?	"; contains the active site for tRNA 3' splice site cleavage	No	No
APN2 (ETH1)	YBL019W	DNase	Nucleus, cytoplasm	Class II abasic (AP) endonuclease involved in repair of DNA damage	No	No
YEN1	YER041W	?	?	Protein of unknown function, has similarity to endonuclease Rad27p	No	No
RPS3 (SUF14)	YNL178W	DNase	?	Apurinic/apyrimidinic (AP) endonuclease; protein component of small (40S) ribosomal subunit	No	No
FAP7	YDL166C	RNase	Cytoplasm and nucleus	Essential NTPase for small ribosome subunit synthesis, mediates processing of 20S pre-rRNA at site D in the cytoplasm, may be the endonuclease for site D	No	No

***the yeastgenome page give the subcellular location mentioned (involves all tables)*

Table 9. **Nucleotide transporters**

Yeastgenome.org				SMART	
Gene name (alias)	**Systematic name**	**Location (GFP database)**	**Function**	**TMD**	**Signal peptide**
RIM2 (PYT1)	YBR192W	?	Mitochondrial pyrimidine nucleotide transporter; imports pyrimidine nucleoside triphosphates and exports pyrimidine nucleoside monophosphates	No	No
ANT1	YPR128C	Peroxisome	Peroxisomal adenine nucleotide transporter; involved in beta-oxidation of medium-chain fatty acid; required for peroxisome proliferation	Yes	no

Table 10. **Genes that show similarity with nucleases from table 4 via BLAST**

Yeastgenome.org					SMART		
(Reserved) gene name (alias)	**Systematic Name**	**BLAST from**	**Location (GFP database)**	**Function**	**TMD**	**Signal peptide**	**Domain**
(AIM1)	YAL046C	*RMP1*	?	Putative protein of unknown function, not essential; null mutant: increased frequency of mitochondrial genome loss	No	No	BolA
	YCL073C	"	?, **membrane	Protein of unconfirmed function; coding sequence 98% identical to that of YKR106W	Yes, 11	No	MFS
	YKR106W	"	"	", coding sequence 98% identical to that of YCL073C	Yes, 11	No	"
FAT1	YBR041W	"	lipid particle	Fatty acid transporter and very long-chain fatty acyl-CoA synthetase	Yes, 2	Yes	AMP-binding
	YGR251W	*REX4*	nucleus and nucleolus	Putative protein of unknown function, required for maturation 18S rRNA; essential gene	No	No	No
ATG23(CVT23)	YLR431C	"	?, **PAS	Peripheral membrane protein required for Cvt pathway; cycles between the PAS and non-PAS	No	No	No
(AIM2)	YIR003W	"	Actin	Protein of unknown function; may interact with ribosomes; null mutant displays increased frequency of mitochondrial genome loss	No	No	No
LDB16	!?YCL005W	"	lipid particle	Protein of unknown function; null mutants have decreased net negative cell surface charge; native protein is detected in purified mitochondria	Yes, 2	No	No
PHM8	YER037W	*POP2*	Cytoplasm and nucleus	Protein of unknown function, expression is induced by low phosphate levels and by inactivation of Pho85p	No	No	Hydrolase
	YIR004C	"	?	Possible pseudogene in strain S288C; YIR044C and YIR043C, together may encode a non-functional member of the conserved, often subtelomerically-encoded Cos protein family	No	No	No
	YGR201C	"	?	Putative protein of unknown function	No	No	GST_C
	YLR049C	*POP5*	?	Putative protein of unknown function	No	No	No
	YGR021W	*POP6*	Mitochondrion	Putative protein of unknown function	No	No	DUF28
	YLR419W	"	Cytoplasm	Putative helicase; the authentic, non-tagged protein is detected in highly purified mitochondria in high-throughput studies; not an essential gene	No	No	UBA, RWD, AAA orDEXDc HELICc, HA2, DUF1605
PBI2 (LMA1)	YNL015W	POP7	Cytoplasm and nucleus	Cytosolic inhibitor of vacuolar proteinase B, required for efficient vacuole inheritance; protein complex LMA1, which assists in priming SNARE molecules and promotes vacuole fusion	No	No	No
	YJL123C	*POP8*	(Early) Golgi and cytoplasm	Protein of unknown function, may interact with ribosomes; synthetically lethal with cdc13-1	No	No	No
	YOL164W-A	*RPP1*	?	Putative protein of unknown function	No	No	No
	YDR374C	"	?	Putative protein of unknown function	No	No	YTH
SOV1	YMR066W	*RPM2*	Mitochondrion	Mitochondrial protein of unknown function	No	No	No
NUR1	YDL089W	"	Nuclear periphery	Protein of unknown function	Yes, 2	No	No

	YML053C	"	Cytoplasm and nucleus	Putative protein of unknown function; not an essential gene	No	No	No
	YBR201C-A	"	?	Putative protein of unknown function	Yes, 1	No	No
	YPR097W	"	ambigous; mitochondrion	Protein that contains a Phox homology (PX) domain and binds phosphoinositides	No	No	PX
	YER079W	"	Cytoplasm and nucleus	Putative protein of unknown function	No	No	No
	YHL012W	"	?	Putative protein of unknown function, some homology to Ugp1p, encodes UDP-glucose pyrophosphorylase	No	No	UDPGP
VPS53	YJL029C	"	Early Golgi and punctate composition	Component of the GARP complex, which is required for the recycling of proteins from endosomes to the late Golgi; required for vacuolar protein sorting	No	No	Vps53_N
PEP3 (VAM8 , VPS18,VPT18)	YLR148W	"	Endosome and vacuolar membrane	Component of CORVET complex; vacuolar peripheral membrane protein, promotes vesicular docking/fusion in conjunction with SNARE proteins, required for vacuolar biogenesis	No	No	Pep3_Vps18, CLH, RING
VPS15 (GRD8 , VAC4 , VPL19)	YBR097W	"	Endosome	Myristoylated serine/threonine protein kinase involved in vacuolar protein sorting; functions as a membrane-associated complex with Vps34p	No	No	STYKc, HEAT, WD40
MON1 (AUT12)	YGL124C	"	late Golgi; cytosol, vacuole membrane	Protein required for fusion of cvt-vesicles and autophagosomes with the vacuole	No	No	Mon1
	YLR211C	*POP1*	Cytoplasm	Putative protein of unknown function; not an essential gene; ORF contains an intron	No	No	No
SWC3 (SWC1)	YAL011W	*POP4*	Nucleus	Protein of unknown function, component of the SWR1 complex; required for formation of nuclear-associated array of smooth endoplasmic reticulum known as karmellae	No	No	No
	YDL159W-A	"	?	Putative protein of unknown function	No	No	No
	YGR071C	*RNH202*	Nucleus	Putative protein of unknown function; deletion mutant: increased glycogen accumulation	No	No	ZnF_BED
FUN19	YAL034C	"	?	Non-essential protein of unknown function, expression induced in response to heat stress	No	No	SWIRM
	YCR090C	"	Cytoplasm, nucleus	Putative protein of unknown function; not an essential gene	No	No	No
	YLR149C	"	ambigous	Putative protein of unknown function; not an essential gene	No	No	No
MAG2	YLR427W	*RNH201*	Cytoplasm	Protein of unknown function predicted to encode a DNA-3-methyladenine glycosidase II that catalyzes the hydrolysis of alkylated DNA	No	No	RING
	!YOR293C-A	*NGL2*	?	Putative protein of unknown function, identified by expression profiling and mass spec	No	Yes	No
VAM7 (VPS43)	YGL212W	*MTR3*	Vacuole and cytoplasm	Component of the vacuole SNARE complex involved in vacuolar morphogenesis; functions with a syntaxin homolog Vam3p in vacuolar protein trafficking	No	No	PX
	YLR162W-A	*DIS3*	?	Putative protein of unknown function	No	No	No
SWT1	YOR166C	"	?, **nucleus	Protein of unknown function; involved in transcription associated process	No	No	Pinc
	YLR031W	"	?	Putative protein of unknown function	No	No	No
SWT1	*(see DIS3)*	*REX3*					
	YML119W	*DSS1*	?	Putative protein of unknown funtion; not an essential gene; potential Cdc28p substrate	No	No	No
	YBR138C	"	Cytoplasm	Protein of unknown function, potentially phosphorylated by Cdc28p; not an essential gene	No	No	No

VPS8 (FUN15, VPT8)	YAL002W	"	Endosome	Membrane-associated protein, interacts with Vps21p to facilitate soluble vacuolar protein localization; component of the CORVET complex; contains RING finger motif	No	No	WD40 and RING
(PAU12)	YGR294W	"	?	Putative protein of unknown function	No	Yes	SRP1_TIP1
(PAU8)	YAL068C	"	?	", not essential	No	Yes	"
(PAU10)	YDR542W	"	?	"	No	Yes	"
(PAU11)	YGL261C	"	?	", not essential; mRNA expression appears to be regulated by SUT1 and UPC2	No	Yes	"
(PAU13)	YHL046C	"	?	", not essential; expression is induced after ethanol shock	No	Yes	"
(PAU14)	YIL176C	"	?	"	No	Yes	"
(PAU18)	YLL064C	"	?	"	No	Yes	"
(PAU20)	YOL161C	"	?	"	No	Yes	"
	YKL075C	"	Cytoplasm	Putative protein of unknown function	No	No	No
FYV6	YNL133C	*RNH1*	Nucleus	Protein of unknown function, required for survival upon exposure to K1 killer toxin; proposed to regulate DBS repair via non-homologous end-joining	No	No	No
ATG11 (CVT9)	YPR049C	"	Punctate composite	Adapter protein for pexophagy and Cvt pathway; directs receptor-bound cargo to PAS for packaging into vesicles; required for recruiting other proteins to the PAS	No	No	No
ATG10	YLL042C	"	?	Conserved E2-like conjugating enzyme that mediates formation of the Atg12p-Atg5p conjugate, which is a critical step in autophagy	No	No	No
	YMR102C	"	Ambigous	Protein of unknown function; transcription is activated by paralogous transcription factors Yrm1p and Yrr1p along with genes involved in multidrug resistance; mutant shows increased resistance to azoles; not essential	No	No	WD40
	YMR031C	"	Punctate composite; cytoplasm, mitochondrion	Protein of unknown function with similarity to Ykl050cp and Uso1p; not an essential gene	No	No	No
MTC4	YBR255W	"	Punctate composite	Protein of unknown function, required for normal growth rate at 15 degrees C; is synthetically sick with cdc13-1	Yes, 1	No	No
	YPR148C	"	Punctate composite	Protein of unknown function that may interact with ribosomes	No	No	No
	YNR004W	"	Nucleolus	Putative protein of unknown function	No	No	No
(AML1)	YGR001C	"	Cytoplasm	Putative protein of unknown function with similarity to methyltransferase family members; required for replication of Brome mosaic virus in S. cerevisiae	No	No	No
YBL100W-C	YBL100W-C	"	?	Putative protein of unknown function	No	No	No
	YGL242C	"	?	Putative protein of unknown function; deletion mutant is viable	No	No	ANK
CCC1	YLR220W	"	Vacuole	Putative vacuolar Fe2+/Mn2+ transporter; suppresses respiratory deficit of yfh1 mutants, which lack the ortholog of mammalian frataxin, prevents mitochondrial iron accumulation	Yes, 3	No	DUF125
	YOR362W	*REX2*	Nucleus, cytoplasm	Putative protein of unknown function; expression levels regulated by Arg5,6p	No	No	No
PBI2	*(see POP7*	"					
	YGR226W	"	ER	Protein of unknown function, predicted to contain a single transmembrane domain; localized to both the mitochondrial outer membrane and the plasma membrane	No	No	No

Table 11. **Genes that BLAST with the genes from table 5 with interacting RNases and DNases**

Yeastgenome.org					SMART		
(Reserved) gene name alias	Systematic name	*BLAST from*	Location (GFP database)	Function	**TMD**	**Signal peptide**	**Domain**
	YLR412C-A	*EXO1*	?	Putative protein of unknown function	No	No	No
HER1	YOR227W	"	Cytoplasm	Protein of unknown function that may interact with ribosomes; authentic, non-tagged protein is detected in highly purified mitochondria in high-throughput studies	No	No	No
SMY2	YBR172C	"	Cytoplasm	Protein of unknown function involved in COPII vesicle formation; interacts with the Sec23p/Sec24p subcomplex	No	No	GYF
(see POP1)	YLR211C	"					
	YKL023W	"	Cytoplasm	Putative protein of unknown function; predicted by computational methods to be involved in mRNA degradation	No	No	No
SPS18 (SPX18)	YNL204C	"	?	Protein of unknown function	No	No	ArfGAP, ARF
FYV8	YGR196C	*RAD2*	?, **cytoplasm	Protein of unknown function, required for survival upon exposure to K1 killer toxin	No	No	No
VPS72 (SWC2)	YDR485C	"	Nucleus	Htz1p-binding component of the SWR1 complex, required for vacuolar protein sorting	No	No	YL1, YL1_C
	YFR016C	"	Cytoplasm and bud	Putative protein of unknown function; interacts with Spa2p; not an essential gene	No	No	No
	YOL019W	"	Vacuole, cell periphery	Protein of unknown function	Yes, 4	Yes	PAII
	YLR460C	"	?	Putative protein of unknown function, possibly up-regulated by iodine	No	No	ADH_N and AHD_zinc_N
	YKL088W	"	Cytoplasm	Predicted phosphopantothenoylcysteine decarboxylase, may be involved in coenzyme A biosynthesis	No	No	Flavoprotein
	YMR317W	"	?	Putative protein of unknown function; not an essential gene	No	No	No
SQS1	YNL224C	"	Cytoplasm and nucleus	Protein of unknown function; overexpression antagonizes the suppression of splicing defects by spp382 mutants	No	No	G-patch
VMA10	YHR038C-A	"	Vacuolar membrane	Vacuolar H+ ATPase subunit G of the catalytic (V1) sector, involved in vacuolar acidification	No	No	V-ATPase_G
PHM8	YER037W	"	Cytoplasm and nucleus	Protein of unknown function, expression is induced by low phosphate levels and by inactivation of Pho85p	No	No	Hydrolase
	YMR144W	"	Nucleus	Putative protein of unknown function; not an essential gene	No	No	No
	YGR130C	"	Punctate composite	Putative protein of unknown function; phosphorylated in vitro by mammalian IP7	No	No	No
(see POP8)	YJL123C	"					
SYH1	YPL105C	"	Cytoplasm	Protein of unknown function, may interact with ribosomes; authentic, non-tagged protein is detected in highly purified mitochondria in high-throughput studies	No	No	GYF
IRC8	YJL051C	"	?, yeastgenome: bud tip	Protein of unknown function; mRNA is targeted to the bud by She2p dependent system; mRNA is cell cycle regulated; null mutant increased levels of spontaneous Rad52p foci	Yes, 4	No	No
	YOR287C	"	?	Putative protein of unknown function; may play a role in the ribosome and rRNA biosynthesis based on expression profiles and mutant phenotype	No	No	DUF947
(see REX4)	YIR003W	"					

	YMR086W	"	Cell periphery	Protein of unknown function that may interact with ribosomes; cAMP repress expression	No	No	No
	YCR102C	"	?	Putative protein of unknown function; involved in copper metabolism; similar to C.carbonum toxD gene; not an essential gene	No	No	ADH_N, AHD_zinc_N
NBA1	YOL070C	*MRE11*	Bud neck, cytoplasm, cell periphery	Protein of unknown function; interacts with Nap1p; potential Cdc28p substrate	No	No	No
PAM1	YDR251W	"	Bud and bud neck	Essential protein of unknown function; exhibits variable expression during colony morphogenesis	No	No	No
(see RAD2)	YMR317W	"					
"	YGR130C	"					
FAB1 (SVL7)	YFR019W	"	Vacuolar membrane	1-phosphatidylinositol-3-phosphate 5-kinase; vacuolar membrane kinase that generates phosphatidylinositol (3,5)P2, which is involved in vacuolar sorting and homeostasis	No	No	FYVE, PIPKc
(AIM41)	YOR215C	"	Mitochondrion	Putative protein of unknown function; null mutant displays altered rate of mitochondrial loss and reduced growth rate in minimal glycerol media	No	No	YqeY
	YLR257W	"	Cytoplasm	Putative protein of unknown function	No	No	No
	YCL058W-A	"	?	Protein of unknown function	No	No	No
	YPL150W	"	Ambigous	Putative protein kinase of unknown cellular role	No	No	S_TKc
	YMR124W	"	cell periphery, cytoplasm, bud, bud neck	Protein of unknown function; interacts with Crm1p in two-hybrid assay; not essential gene	No	No	No
VMA10	(see RAD2)	*RAD27*					
NCE101(NCE1, YJL206C-A, YJL205C-A)	YJL205C	"	?	Protein of unknown function, involved in secretion of proteins that lack classical secretory signal sequences	Yes,1	Yes	No
	YDL007C-A	*APN1*	?	Putative protein of unknown function	No	Yes	No
	YPR147C	"	Cytoplasm	Putative protein of unknown function, induced in response to DNA-damaging agent MMS	Yes,1	No	No
	YER158C	*KEM1*	?	Protein of unknown function, similarity to Afr1p; potentially phosphorylated by Cdc28p	No	No	No
(See EXO1)	YLR412C-A	"					
	YHR138C	"	Ambigous	Putative protein of unknown function; similarity to Pbi2p; double null mutant lacking Pbi2p and Yhr138p exhibits highly fragmented vacuoles	Yes, 1	Yes	No
NST1	YNL091W	"	Cytoplasm	Protein of unknown function, mediates sensitivity to salt stress; interacts physically with the splicing factor Msl1p and also displays genetic interaction with MSL1	No	No	No
ATG29	YPL166W	"	Nucleus, cytoplasm, punctate composite	Protein specifically required for autophagy; may function in autophagosome formation at the pre-autophagosomal structure in collaboration with other autophagy proteins	No	No	No
(See RAD2)	YMR086W	"					
	YJR098C	"	Cytoplasm	Putative protein of unknown function; the authentic, non-tagged protein is detected in highly purified mitochondria in high-throughput studies	No	No	No
	YIL161W	"	Cytoplasm	Putative protein of unknown function; mRNA is enriched in Scp160p-associated mRNPs; non-essential gene	No	No	No
MAG2	(seeRNH201)	"					

	YHL039W	*PSO2*	Cytoplasm	Putative protein of unknown function	No	No	SET
(AIM31)	YML030W	"	Mitochondrion	Putative protein of unknown function; null mutant is viable and displays decreased frequency of mitochondrial genome loss and severe growth rate in minimal glycerol media	Yes, 2	No	HIG_1_N
	YGR017W	"	Cytoplasm and nucleus	Putative protein of unknown function	No	No	No
	YDL160C-A	"	?	Putative protein of unknown function	No	No	DUF2008
(see: RAD2)	YGR130C	*RAD1*					
SWC3	(see POP4	"					
CUE3	YGL110C	"	Cytoplasm	Protein of unknown function, domain may facilitate intramolecular monoubiquitination	No	No	CUE
(CMR1)	YDL156W	"	Cytoplasm and nucleus	Putative protein of unknown function	No	No	WD40
(See RAD2)	YOR287C	"					
	YBR255C-A	"	?	Putative protein of unknown function	Yes, 1	No	No
EDC3 (DCP3, LSM16)	YEL015W	*RAD10*	Punctate composite	Non-essential conserved protein of unknown function, role in mRNA decapping by specifically affecting the function Dcp1p; localizes to cytoplasmic mRNA processing bodies	No	No	DFDF
	YBR074W	"	?	Putative metalloprotease	Yes, 9	Yes	Peptidase _M28
(RCN2)	YOR220W	"	Cytoplasm	Protein of unknown function; induced in response to the DNA-damaging agent MMS	No	No	No
	YDR186C	*SAE2*	Cytoplasm	Putative protein of unknown function; may interact with ribosomes	No	No	No
(COM2)	YER130C	"	?	Protein of unknown function	No	No	ZnF_C2H2
(see RAD2)	YOR287C	"					
	YOR072W-B	"	?	Putative protein of unknown function; identified by expression profiling and mass spec	No	No	No
(see MRE11)	YMR124W	"					
(RTC1)	YOL138C	"	Vacuole	Putative protein of unknown function; may interact with ribosomes; null mutation suppresses cdc13-1 temperature sensitivity	No	No	WD40 and RING
	YNL193W	"	?	Putative protein of unknown function; two-hybrid interaction with Yhr151cp	No	No	No
	YFR016C	"	Cytoplasm and bud	Putative protein of unknown function; not an essential gene	No	No	No
VPS5	YOR069W	"	Endosome	Nexin-1 homolog for localizing membrane proteins from a prevacuolar/late endosomal compartment back to the late Golgi apparatus; structural component of the retromer membrane coat complex; forms a retromer subcomplex with Vps17p	No	No	PX
(See: RNH1)	YMR031C	"					
(See: RNH1)	YPR148C	*RAT1*					
(See: RAD2)	YFR016C	"					
"	YOR287C	"					
ENP2	YGR145W	"	?,**preribosome	Essential nucleolar protein of unknown function; interacts with Mpp10p +Bfr2p,homology to Spb1	No	No	WD40,NUC153
DOS2	YDR068W	"	Cytoplasm	Protein of unknown function	No	No	BSD

(See: RNH1)	YGL242C	"					
(See: DNA2)	YKL050C	"					
SYH1	(see RAD2)	"					
NST1	(see KEM1)	"					
(See: RAD2)	YGR130C	"					
NOP13	YNL175C	"	Nucleus and nucleolus	Protein of unknown function; similarity to Nop12p, required for pre-18S rRNA processing	No	No	RRM
(URN1)	YPR152C	"	Nucleus	Putative protein of unknown function; overexpression: accumulation of cells in G1 phase	No	No	WW and FF
SMY2 (See: EXO1)		"					
	YER128W	"	Ambigous	Putative protein of unknown function	No	No	DUF1742
	YDR306C	"	?	F-box protein of unknown function; interacts with Sgt1p via a Leucine-Rich Repeat domain	No	No	No
(LRC3)	YKL207W	"	?	Putative protein of unknown function; non-essential gene	Yes, 3	Yes	DUF850
SET4	YJL105W	*MUS81*	?	Protein of unknown function	No	No	PHD, SET
(See: DNA2)	YEL043W	"					
	YPL141C	"	Cytoplasm	Putative protein kinase; similar to Kin4p; not an essential gene	No	No	S_TKc
(CMR2)	YOR093C	"	?	Putative protein of unknown function; deletion causes sensitivity to unfolded protein response-inducing agents	No	No	DMAP_ binding
TMA22	YJR014W	"	Cytoplasm	Protein of unknown function; associates with ribosomes and has a putative RNA binding domain	No	No	SUI1
(See: RAT1)	YFR016C	"					
	YDR222W	"	Punctate composite	Protein of unknown function	No	No	Svf1
	YKR045C	"	Ambigous	Putative protein of unknown function; epitope-tagged protein localizes to the cytoplasm	No	No	No
	YLR177W	*MMS4*	Cytoplasm	Putative protein of unknown function; phosphorylated by Dbf2p-Mob1p in vitro; not an essential gene	No	No	PSP1
	YBL113C	"	?	Helicase-like protein encoded within the telomeric Y' element	No	No	No
VPS16 (SVL6, VAM9,VPT16)	YPL045W	"	Vacuolar membrane	Subunit of the vacuole fusion and protein sorting HOPS complex and the CORVET tethering complex; part of the Class C Vps complex	No	No	Vps16_N, Vps16_C
VMA7	YGR020C	"	Vacuolar membrane and ambigous	Subunit F of the eight-subunit V1 peripheral membrane domain of V-ATPase; required for the V1 domain to assemble onto the vacuolar membrane	No	No	ATP_synt_F
NST1	(see KEM1)	"					
(see MUS81)	YEL043W	"					
(ESL2)	YKR096W	"	Cytoplasm and nucleus	Protein of unknown function that may interact with ribosomes	No	No	PINc
(see MRE11)	YMR317W	"					
(TSR4)	YOL022C	"	Cytoplasm	Cytoplasmic protein of unknown function; essential gene in S288C, and non-essential with reduced growth rate in CEN.PK2; Null mutant accumulates 20S pre-rRNA	No	No	PDCD2_C
	YBL029W	"	Cytoplasm and nucleus	Non-essential protein of unknown function	No	No	No

	YIL169C	"	?	Putative protein of unknown function; serine/threonine rich and highly similar to YOL155C, a putative glucan alpha-1,4-glucosidase; non-essential gene	No	Yes	No
SQS1 (see RAD2)		"					
VBA4	YDR119W	"	Vacuolar membrane	Protein of unknown function with proposed role as a basic amino acid permease; physical interaction with Atg27p suggests a possible role in autophagy; non-essential gene	Yes, 14	No	MFS_1
	Q0255	"	?, **mitochondrion	Maturase-like protein	No	Yes	LAGLIDADG_1
SGM1	YJR134C	"	(Early) Golgi	Protein of unknown function, required for wild-type growth rate on galactose and mannose; localizes to COPI coated vesicles and the Golgi apparatus	No	No	No
ACF4	YJR083C	"	Ambigous	Protein of unknown function, possible role in actin cytoskeleton organization; potential Cdc28p substrate	No	No	No
	YDL063C	"	Cytoplasm and nucleus	Putative protein of unknown function; not an essential gene	No	No	No
JJJ3 (DPH4)	YJR097W	"	Cytoplasm and nucleus	Protein of unknown function, contains a J-domain	No	No	DnaJ, zf_CSL
	YHL050C	"	?	Putative protein of unknown function, potential Cdc28p substrate	No	No	DEAD
APM2	YHL019C	"	Late Golfi and ambigous	Protein of unknown function, homologous to the medium chain of mammalian clathrin-associated protein complex; involved in vesicular transport	No	No	Adap_comp_sub
	YBR197C	"	Cytoplasm and nucleus	Putative protein of unknown function; not an essential gene	No	No	No
	YJL049W	"	?	Putative protein of unknown function; a non-essential gene	No	No	Snf7
(EMC2)	YJR088C	"	ER	Putative protein of unknown function	No	No	TPR_2
(AIM20)	YIL158W	"	Vacuole	Putative protein of unknown function; overexpression causes a cell cycle delay or arrest	Yes, 1	No	No
SWC3 (see: POP4)		"					
	YDR003W-A	"		Putative protein of unknown function; identified by expression profiling and mass spec	No	No	No
	YMR018W	*SKI3*	?	Putative protein of unknown function with similarity to human PEX5Rp; transcription increases during colony development similar to genes involved in peroxisome biogenesis; not an essential gene	No	No	TPR
	YIR021W-A	"	?	Putative protein of unknown function; identified by expression profiling and mass spec	Yes, 1	No	No
	YNL313C	"	Cytoplasm and nucleus	Essential protein of unknown function	No	No	TPR
SHE10	YGL228W	"	?	Putative GPI-anchored protein of unknown function; overexpression causes growth arrest	No	Yes	No
ATG23	(see REX4)	"					
	YKL096C-B	"	?	Putative protein of unknown function; identified by gene-trapping, microarray-based expression analysis, and genome-wide homology searching	Yes, 1	No	No
	YAR061W	"	?	Hypothetical protein, a pseudogene	No	Yes	No
	YHR212W	"	?	Putative protein of unknown function; identified by gene-trapping, microarray-based expression analysis, and genome-wide homology searching	No	Yes	No
	YHR050W	"	?	Protein of unknown function; identified by expression profiling and mass spectrometry	No	No	No
	YDR034C	"	?	Putative protein of unknown function; contained within the solo Ty1 LTR element YDRWdelta7	Yes, 1	No	No

	YNL035C	*SKI8*	Nucleus	Putative protein of unknown function; not an essential gene	No	No	WD40
ATG18(NMR1, CVT18,AUT10, SVP1)	YFR021W	"		Phosphoinositide binding protein required for vesicle formation in autophagy and the Cvt pathway	No	No	WD40
	YPL247C	"	Cytoplasm and nucleus	Putative protein of unknown function; overexpression causes a cell cycle delay or arrest	No	No	No
	YBR296C-A	"	?	Putative protein of unknown function; identified by gene-trapping, microarray-based expression analysis, and genome-wide homology searching	No	No	No
(see RAD1)	YDL156W	"					
(see SAE2)	YOL138C	"					
	YLR194C	"	ER	Structural constituent of the cell wall attached to the plasma membrane by a GPI-anchor	No	Yes	No
RTC5	YOR118W	*RRP4*	Cytoplasm	Protein of unknown function; null mutation suppresses cdc13-1 temperature sensitivity	No	No	No
VPS64 (FAR9)	YDR200C	"	Cytoplasm	Protein required for CVT targeting of proteins; involved in pheromone-induced cell cycle arrest; also localized to the ER membrane	No	No	FHA
ATG11	(see RNH1)	*CDC39*					
(see MRE11)	YOR215C						
	YCL057C-A	*RNH70*	?, yeastgenome: mitochondrion	Putative protein of unknown function	Yes, 1	No	DUF543
	YOR381W-A	"	?	Putative protein of unknown function; identified by fungal homology and RT-PCR	No	No	No
	YPR078C	"	?	Putative protein of unknown function; possible role in DNA metabolism and/or in genome stability; expression is heat-inducible	No	No	No
(AIM28)	YKR016W	*PAN2*	Mitochondrion, ambigous	Mitochondrial protein of unknown function; null mutation: synthetic slow growth defect in combination with null mutation in GEM1, which encodes a mitochondrial GTPase	Yes, 1	No	No
	YDL085C-A	"	Cytoplasm and nucleus	Putative protein of unknown function	No	No	4F5
(See RAD1)	YOR287C	"					
PEP1 (VPS10, VPT1)	YBL017C	"	Endosome, punctate composite	Type I transmembrane sorting receptor for multiple vacuolar hydrolases; cycles between the late-Golgi and prevacuolar endosome-like compartments	Yes, 1	Yes	VPS10, BNR
(see SKI3)	YIR021W-A	*NOP58*					
?!ROG1	YGL144C	"	?		No	No	DUF676
ATG11	(see RNH1)	"					
	YJR154W	"	Cytoplasm	Putative protein of unknown function	No	No	PhyH

Table 12. **Genes found from the BLAST of genes from table 6, the nuclease search**

Yeastgenome.org					SMART		
(Reserved) gene name (alias)	**Systematic name**	**BLAST from**	**Location (GFP database)**	**Function**	**TMD**	**Signal peptide**	**Domain**
	YDL233W	*DIN7*	Cytoplasm, nucleus	Putative protein of unknown function; not an essential gene	No	No	No
DID4 (GRD7, REN1, VPL2, VPT14, VPS2, CHM2)	YKL002W	"	Cytoplasm	Class E Vps protein of the ESCRT-III complex, required for sorting of integral membrane proteins into lumenal vesicles of multivesicular bodies, and for delivery of newly synthesized vacuolar enzymes to the vacuole, involved in endocytosis	No	No	Snf7
FMP24	YMR115W	"	Mitochondrion	Putative protein of unknown function	Yes, 1	No	No
	YMR262W	*Tat-D*	?	Protein of unknown function; interacts weakly with Knr4p; not an essential gene	No	No	TatD_Dnase
	YKL065W	"	?	Putative protein of unknown function	Yes, 1	No	No
YIM1	YMR152W	"	ER	Protein of unknown function; null mutant displays sensitivity to DNA damaging agents	No	No	ADH_N
VOA1	YGR106C	"	Vacuole membrane	ER protein (?) that functions, together with other assembly factors, in assembly of the V0 sector of the V-ATPase	Yes, 1	Yes	No
(See: DSS1)	YML119W	*DNA2*					
	YDR179W-A	"	?	Putative protein of unknown function	No	No	No
	YKL050C	"	?	Protein of unknown function; a target of the SCFCdc4 ubiquitin ligase complex and transcription is regulated by Azf1p	No	No	No
VPS45 (STT10, VPL28)	YGL095C	"	Early and late Golgi	Protein of the Sec1p/Munc-18 family, essential for vacuolar protein sorting; essential for fusion of Golgi-derived vesicles with the prevacuolar compartment	No	No	Sec1
	YGR126W	"	Cytoplasm, nucleus	Putative protein of unknown function; induced in response to DNA-damaging agent MMS	No	No	No
	YEL043W	"	ER	Predicted cytoskeleton protein involved in intracellular signalling; may interact with ribosomes	No	Yes	FN3
(see RAD2)	YGR130C	"					
FYV8 (see RAD2)		"					
	YML083C	"	?	Putative protein of unknown function; strong increase in transcript abundance during anaerobic growth compared to aerobic growth	No	No	No

Table 13. **Genes found from the BLAST of genes from table 7, the exonuclease search**

Yeastgenome.org					SMART		
(Reserved) gene name (alias)	**Systematic name**	**BLAST from**	**Location (GFP database)**	**Function**	**TMD**	**Signal peptide**	**Domain**
	YKR078W	*RRP6*	Cytoplasm	Protein of unknown function; potential Cdc28p substrate; specifically binds (PtdIns-3-P)	No	No	PX, Vps5
VPS3 (PEP6, VPL3, VPT17)	YDR495C	"	Punctate composite	Component of CORVET tethering complex; sorting and processing of soluble vacuolar proteins, acidification of the vacuolar lumen, and assembly of the V-ATPase	No	No	No
	YGR016W	"	?	Putative protein of unknown function	Yes, 4	No	No
PET10	YKR046C	"	Lipid particle	Protein of unknown function; expression pattern suggests role in respiratory growth, computational analysis of large-scale interaction data suggests role in ATP/ADP exchange	No	No	No
(IBI1)	YGR273C	"	?	Putative protein of unknown function	No	No	No
	YOR022C	"	Mitochondrion	Protein with similarity to bovine phospholipase A1	No	No	DDHD
	YLR290C	"	Mitochondrion	Putative protein of unknown function; not an essential gene	No	No	No
(see RNH1)	YPR148C	"					
VPS36 (GRD12, VAC3, VPL11)	YLR417W	*TRZ1*	Cytoplasm	Component of the ESCRT-II complex; contains the GLUE domain which is involved in interactions with ESCRT-I and ubiquitin-dependent sorting of proteins into the endosome	No	No	ZnF_RBZ, EAP30
VPS3	(see RRP6)	"					
GSM1	YJL103C	"	?	Putative zinc cluster protein of unknown function; proposed to be involved in the regulation of energy metabolism, based on patterns of expression and sequence analysis	No	No	GAL4
VPS72	(see RAD2)	"					
	YOR051C	"	Nucleus	Nuclear protein that inhibits replication of Brome mosaic virus in S. cerevisiae	No	No	No
ICP55	YER078C	"	Mitochondrion	Protein of unknown function; expression repressed by inositol and choline in an OPI1-dependent manner	No	No	AMP_N, Peptidase_M24
ROG1	(see NOP58)	"					
	YNL033W	"	?	Putative protein of unknown function	Yes, 2	No	No
(ENV7)	YPL236C	RRP41	Vacuole membrane	Putative protein kinase that exhibits Akr1p-dependent palmitoylation	No	No	STYKc
(See: KEM1)	YER158C	"					
	YFR039C	"	Ambigous	Putative protein of unknown function;	No	Yes	No
TMA10	YLR327C	"	Cytoplasm and nucleus	Protein of unknown function that associates with ribosomes	No	No	No
LOH1	YJL038C	"	?	Protein of unknown function with proposed role in maintenance of genome integrity; deletion mutant displays elevated rates of LOH; induced during sporulation; repressed during vegetative growth by Sum1p and Hst1p; sequence similar to IRC1	Yes, 2	Yes	No

Table 14. **Genes found from the BLAST of genes from table 8, the endonuclease search**

Yeastgenome.org					SMART		
(Reserved) gene name (alias)	**Systematic name**	**BLAST from**	**Location (GFP database)**	**Function**	**TMD**	**Signal peptide**	**Domain**
AI5_BETA	Q0075	*AI4*	?, **mitochondrion	Protein of unknown function, encoded within intron of mitochondrial COX1 gene; trans-lational initiation codon predicted to be ATA rather than ATG SMARTIntron-encoded DNA endonuclease	No	Yes	LAGLIDADG_1
(See: MMS4)	Q0255	"					
	YLR283W	"	Mitochondrion	Putative protein of unknown function, not an essential gene	No	No	No
	YCL001W-A	"	?	Putative protein of unknown function; not an essential gene	No	No	No
	YBL107C	"	Cytoplasm	Putative protein of unknown function; not an essential gene	No	No	No
NCE101	(see RAD27)	"					
VPS15	(see RPM2)	"					
	YGR146C-A	"	?	Putative protein of unknown function	Yes, 1	No	No
AI5_BETA	(see AI4)	*AI3*					
(see: MMS4)	Q0255	"					
	YOL013W-A	"	?	Putative protein of unknown function; identified by SAGE	No	No	No
	YOR019W	*AI5_ ALPHA*	?	Protein of unknown function that may interact with ribosomes	No	No	No
NCE101	(see: RAD27)	"					
	YDR366C	"	?	Putative protein of unknown function	Yes, 3	Yes	No
	YMR158C-A	"	?	Putative protein of unknown function, may contain a lipid attachment site; not an essential gene	No	No	No
AI5_ALPHA		*SCEI*					
	YIL014C-A	"	?	Putative protein of unknown function	No	No	No
(AIM22)	YJL046W	"	?	Putative lipoate-protein ligaseA; null mutant viable, respiratory growth defect	No	No	BPL_LipA_LipB
(See: TRZ1)	YJL103C	"	?				
	YJL070C	"	Cytoplasm	Putative protein of unknown function, similarity to AMP deaminases; non-essential gene	No	No	A_Deaminase
	YMR027W	*NGL3*	Cytoplasm, nucleus	Putative protein of unknown function; not an essential gene	No	No	DUF89
(See: NGL2)	YOR293C-A	"					
	YGR125W	"	Vacuole	Putative protein of unknown function; deletion mutant has decreased rapamycin resistance but normal wormannin resistance	Yes, 10	No	Sulfate_transp, STAS, c_NMP_binding
	YML096W	*NGL1*	Cytoplasm	Putative protein of unknown function; not an essential gene, partially overlaps RAD10	No	No	Asn_synthase
(See: RAD2)	YGR130C	*CCR4*					

	YOR376W-A	"	?	Putative protein of unknown function; identified by fungal homology and RT-PCR	No	Yes	No
	YGL188C-A	"	?	Putative protein of unknown function	No	No	No
AHC2	YCR082W	*CCE1*	Cytoplasm, nucleus	Protein of unknown function; proposed Ada Histone acetyltransferase complex component	No	No	No
	YJL147C	"	Mitochondrion	Protein of unknown function; a non-essential gene	No	No	No
VPS53	(see RPM2)	"					
(see: PSO2)	YML030W	"					
YPT53	YNL093W	"	?,**late endosome	GTPase, similar to Ypt51p and Ypt52p and mammalian rab5; required for vacuolar protein sorting and endocytosis	No	No	RAB or Miro and Ras
KRE28	YDR532C	"	Spindle pole, ambigous	Protein of unknown function; forms a complex with Spc105p	No	No	No
(See: RAD2)	YFR016C	"					
	YOL166W-A	*SEN2*	?	Identified by gene-trapping, microarray-based expression analysis and genome-wide homology searching	Yes, 1	No	DUF468
	YPL216W	*SEN34*	?	Putative protein of unknown function; not an essential gene	No	No	DDT
(See: AI4)	Q0255	"					
SLP1	YOR154W	"	?	Integral membrane protein of unknown function; member of SUN-like family of proteins	Yes, 1	Yes	Sad1_UNC
YSH1(BRR5)	YLR277C	"	Nucleus	Putative endoribonuclease, subunit of the mRNA cleavage and polyadenylation specificity complex required for 3' processing of mRNAs	No	No	Lactamase_B, RMMBL
	YLR345W	*YEN1*	Cytoplasm	Similar to 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase enzymes; mRNA expression is repressed by Rfx1p-Tup1p-Ssn6p repressor complex; not an essential gene	No	No	6PF2K, PGAM
(See: TRZ1)	YJL103C	"					
	YBR225W	"	Ambigous	Putative protein of unknown function; non-essential gene	No	No	No
(See: TRZ1)	YOR051C	"					
PAI3	YMR174C	"	Cytoplasm, nucleus	Cytoplasmic proteinase A inhibitor, dependent on Pbs2p and Hog1p protein kinases for osmotic induction; intrinsically unstructured, N-terminal half becomes ordered in the active site of proteinase A upon contact	No	No	No
VID27	YNL212W	"	Cytoplasm	Cytoplasmic protein of unknown function; possibly involved in vacuolar protein degradation	No	No	VID27
PHM8	(See: RAD2)	"					
	YGR042W	*RPS3*	Cytoplasm, nucleus	Putative protein of unknown function; involved in maintenance of proper telomere length	No	No	No
	YOR296W	*FAP7*	Cytoplasm	Putative protein of unknown function; expressed during copper starvation; not an essential gene	No	No	C2
	YPL152W-A	"	?	Identified by gene-trapping, microarray-based expression analysis + genome-wide homology searching	No	No	No
	YMR155W	*YSH1*	?	Putative protein of unknown function	Yes, 10	No	No
	YGL006W-A	"	?	Putative protein of unknown function; identified by SAGE	No	No	No
(See: SAE2)	YOR072W-B	"					

Table 15. **Genes found from the BLAST of genes from table 9, the nucleotide transporter search**

Yeastgenome.org					SMART		
(Reserved) gene name (alias)	**Systematic name**	**BLAST from**	**Location (GFP database)**	**Function**	**TMD**	**Signal peptide**	**Domain**
YEA6 (NDT2)	YEL006W	*RIM2*	Mitochondrion	Putative mitochondrial NAD+ transporter, member of the mitochondrial carrier subfamily (see also YIA6); has putative human ortholog"	Yes, 3	No	Mito_carr
	YPR011C	"	Mitochondrion	Putative transporter, member of the mitochondrial carrier family	No	No	Mito_carr
	YDL119C	"	Mitochondrion	Putative mitochondrial transport protein; GFP-fusion protein is induced in response to the DNA-damaging agent MMS	No	No	Mito_carr
	YMR166C	"	Mitochondrion	Predicted transporter of the mitochondrial inner membrane; has similarity to human mitochondrial ATP-Mg/Pi carriers; YMR166C is not an essential gene	No	No	Mito_carr
!	YFR045W	"	?,**mitochondrion	Putative mitochondrial transport protein; null mutant is viable, exhibits decreased levels of chitin and normal resistance to calcofluor white	Yes,1	No	Below treshold: o.a. EXOIII
(See: RRP41)	YJL038C	"					
CRG1	YHR209W	"	?	Putative S-adenosylmethionine-dependent methyltransferase; mediates cantharidin resistance	No	No	Methyltransf_11/12
	YLR281C	"	Mitochondrion	Putative protein of unknown function; not an essential gene	No	No	No
	YLR050C	"	ER	Putative protein of unknown function; not an essential gene	Yes, 3	No	No
YEA6 (NDT2)	(see RIM2)	*ANT1*					
(See: RIM2)	YDL119C	"					
(See: RIM2)	YPR011C	"					
(See: RIM2)	YMR166C	"					
(See: RIM2)	YFR045W	"					
UGO1	YDR470C	"	?,**mitochondrion	Protein of unknown function; outer membrane component of the mitochondrial fusion machinery; Ugo1p bind directly to Fzo1p and Mgm1p and thereby link these two GTPases during mitochondrial fusion	Yes, 3	No	No
PRY2 (YFW12)	YKR013W	"	Vacuole	Protein of unknown function	No	Yes	SCP
	YGR111W	"	Cytoplasm and nucleus	Putative protein of unknown function	No	No	No