Chapter 5

Secretion Mechanism across Wall

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http://dx.doi.org/10.5772/intechopen.70783

Abstract

Yeast organisms are widely explored by humans for different biotechnological applications. During their growth, they need to adapt and interact themselves with the environment medium. For this purpose, organisms uptake nutrients and at the same time secrete different molecules include proteins to extracellular medium. This phenomenon requires the use of specialized structures to regulate entry and exit of molecules called transporters. Two transporters, namely Proteins and Vesicles, are specialized in translocating molecules in and out across the wall. The knowledge of these systems is important and served to bring novel applications of yeast. Taking together, this book chapter is divided into two parts: at first, it primarily accounts on few examples of protein (carbohydrates and peroxisome proteins) and vesicle (intracellular and extracellular vesicles) transporters of yeasts. Second, it deals with the recent advances of yeast applications in diverse area of science.

Keywords: vesicles, symporter, induction, repression, transporters

1. Introduction

For decades, *Saccharomyces cerevisiae* has been the model organism of the lower eukaryotes. The available complete genome favors access to perform possible molecular and genetics researches to understand cell activities of this yeast. The composition and concentration of molecules that conform the outer medium dictates the transporters pattern present in the cellular membrane. The role of molecular transport mechanisms is highly recognized and well-studied. Basically, transport systems can be drawn in two ways; the barrier systems- cell membrane and cell wall. (1) Transport of compounds from inside to outside, (2) And outside to inside. There are many compounds involucrated in the phenomenon: carbohydrates, peptides, some proteins (mucin-type glycopeptides and gycoproteins) and ions (**Figure 1**). As organisms

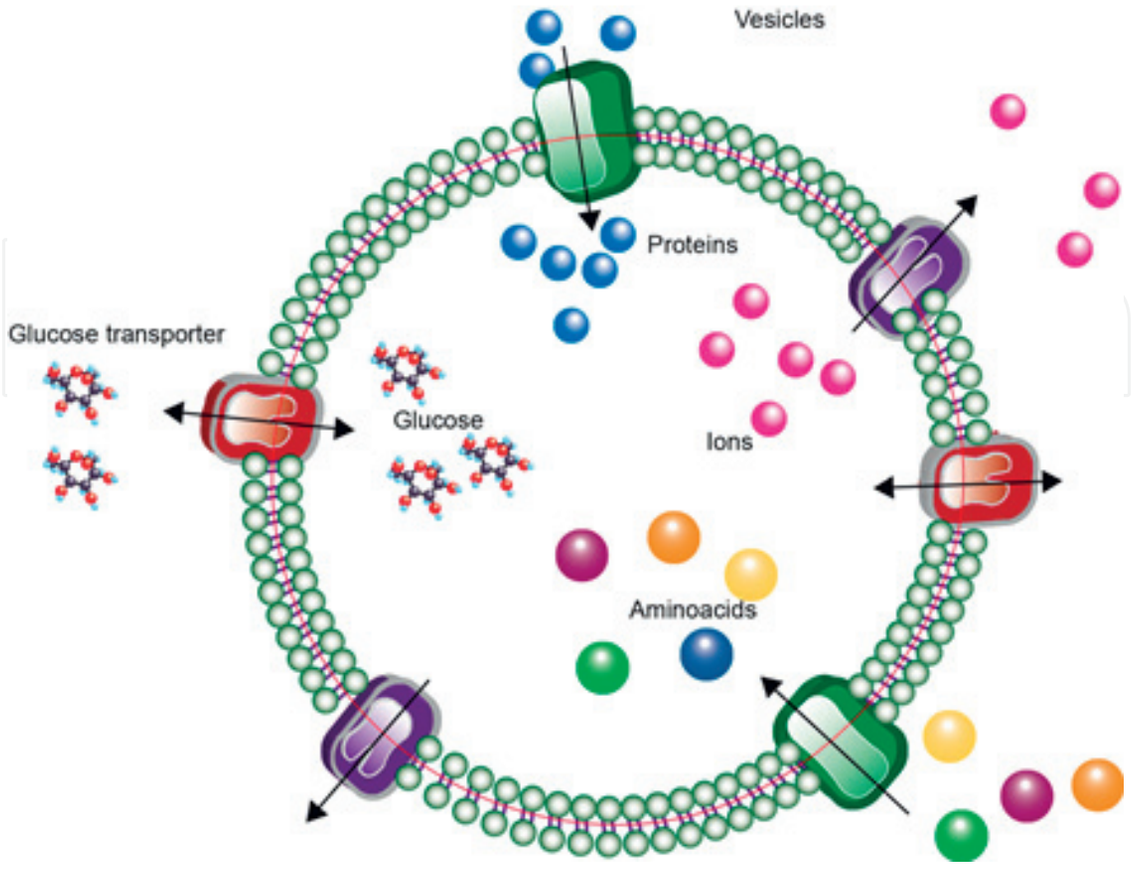

Figure 1. Pictorial representation of diverse transport systems present in the yeast organisms. It includes protein machinery for the transport of biomolecules such as glucose, amino acids, enzymes, ions. Other transporters include vehicles such as vesicles, which are bi-layered liposomes.

like *S. cerevisiae* contains multiple membranous organelles, it is expected to conduct similar types of transport, from cytosol to intracellular organelles across the membrane. Organelle could be any of the following: Nucleus, Mitochondria, Golgi bodies, Endoplasmic reticulum, Peroxisomes. Besides diffusion, various types of transport systems are shown to be involved to either import or export molecules across membranes; we categorize different types reported in the literature. Membrane transporters: Embedded in the membrane are demonstrated to carry out different mechanisms such as a) Protein transport, b) Carbohydrate transport, c) Bilayered membranous vesicles (both intracellular and extracellular vesicles).

2. Hydrocarbons transporters of yeast

There is a great diversity of yeasts and they all require a carbon source to maintain metabolic, physiological and cell growth processes. One of the main nutrients is glucose because it plays a key role regulating the expression of sugar-carrying genes. Yeasts can also consume different types of sugars like xylose, arabinose and under very specific conditions glycerol. These nutrients need to be introduced into the cell whereby yeasts have developed numerous transporters proteins, with similar structures, but with very specific substrate functions and affinities.

http://dx.doi.org/10.5772/intechopen.70783

2.1. Glucose regulation

Glucose is a substrate of easy metabolism and can act as a signaling molecule depending of its extracellular/intracellular concentration to adjust diverse cellular activities. In *Saccharomyces cerevisiae*, there are two pathways to control glucose consume by regulation of HXT transporter [1].

2.1.1. Pathway of glucose induction Rgt2/Snf3, responsible for its consumption.

Snf3, and Rgt2 are important sugar sensors (not glucose transport) in the *S. cerevisiae*, they are in cell membrane and play key roles, selecting what nutrient to utilize and coordinating expression of sugar transporters. Under low glucose concentration Snf3 sensor elevates the transcription of high-affinity hexose transporter genes, while at high concentrations, Rgt2 sensor promotes low-affinity hexose transporter expression [2]. In both cases, glucose binding to sensors leads their conformation switch and activate a casein kinase I (Yck1/2) which phosphorylates regulators of the glucose-sensing signal transduction pathway Mth1 and Std1 to subsequently be ubiquitylated and degraded [3]. The degradation of Mth1 and Std1 interrupts the interaction between a transcriptional factor Rgt1 and Cyc8/Tup1 to form a general co-repressor complex of expression of HXT genes. Once liberated Rgt1 is phosphorylated therefor HXT genes are expressing [4].

2.1.2. Pathway of glucose repression Snf1/Mth1 negatively regulates genes involved in glucose oxidation and the use of alternative sugars.

Under glucose limitation, there is transcriptional inhibition of hexose transporter genes (HXT) by blocking of their promoter by a repressor complex conformed with Snf1, the complex Cyc8-Tup1 and the Mth1/Std1 [1, 5]. This mechanism is required for the yeast to adapt to glucoses limitation medium.

In *Candida albicans* membrane, the sugar sensor Hht4 (homolog to Snf3 and Rgt2) responses to different levels of sugar by inactivation of a transcriptional repressor Rgt1 that regulates multiple HGT genes encoding hexoses transporters (Hgt2, Hxt10 and Hgt7), this process plays a key role in systemic infections [6]. Hxt4 expression is repressed by high levels of glucose. Snf3p in *Candida glabrata* is essential for growth in low glucose media and plays a role in the induction of several hexoses transporters [7]. Kluyveromyces lactis possesses a system of glucose signaling that also depends of intercellular glucose metabolism, demonstrated in glycolytic mutants whose affection has a direct correlation with the repression of one of its main transporters of glucose Rag1. [8]. **Figure 2** shows the expression pattern of the carbohydrates transporters depending on the glucose concentration.

2.2. Hexoses transporters

The vast majority of yeast carbohydrate transporters belong to the Major Facilitator Superfamily (MFS) and the hexoses transporters (HXT) of *S. cerevisiae* have been extensively studied. HXT family is conformed of 17 putative membrane proteins with a high similarity, but different affinities, [9]. A deletion assay has demonstrated that HXT1–4, HXT6–7 are the mayor functional transporters in glucose or fructose [10]. Some transporters can transport xylose efficiently but there is inhibition by glucose presence because they have a clear preference for this sugar [11].

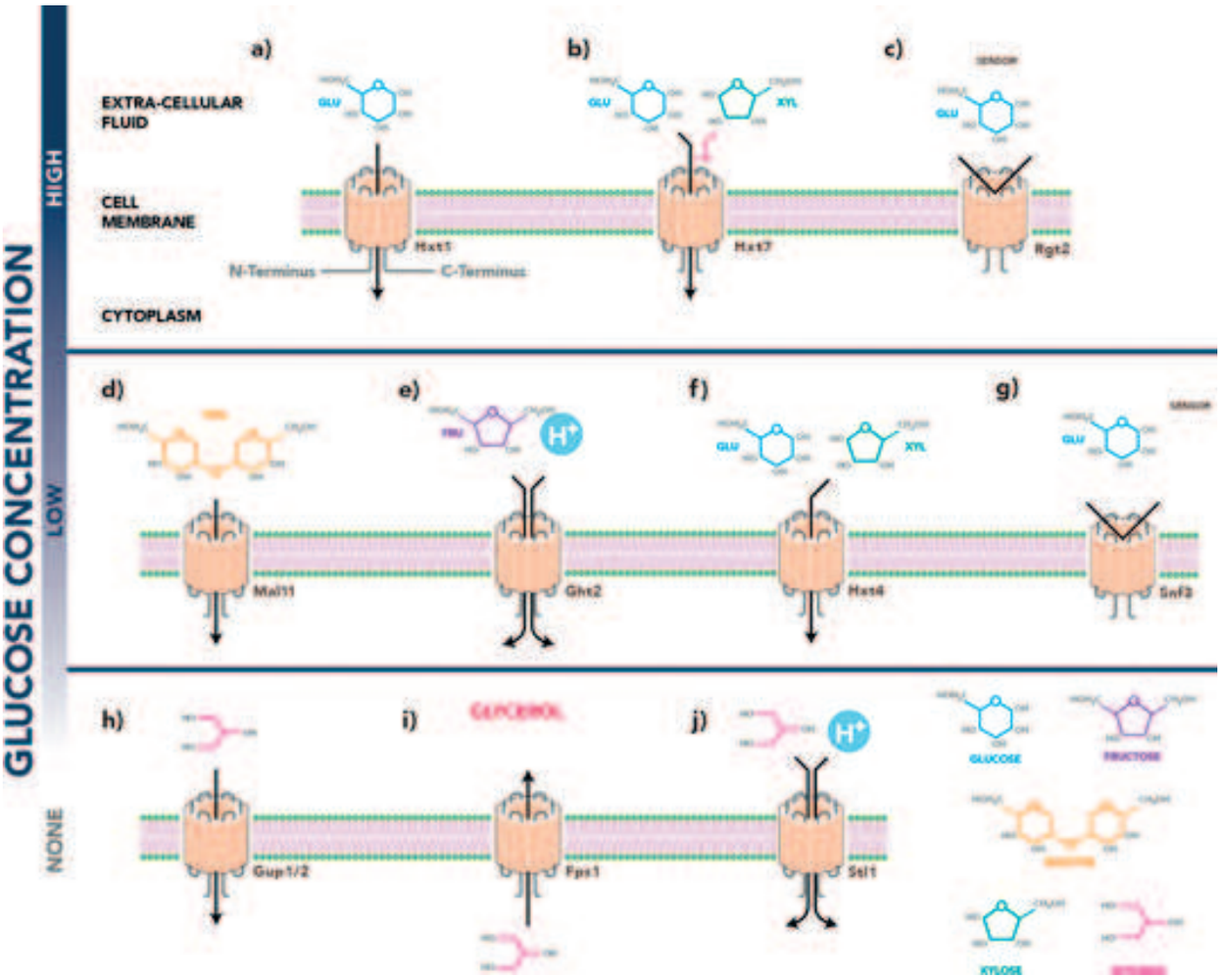

Figure 2. Expression of transporters in yeasts dependent on the concentration of glucose in the medium. At high concentrations of Glu: a) Hxt1 main transporter of Glu, b) Hxt7 has inhibition in xylose transport; c) Rgt2 is a sensor that induces expression of transporters required at high concentrations of glucose. At low concentrations of Glu: d) Mal11 transports α-glusides as maltose, e) Ght2 a symporter proton allows the consumption of fructose, f) decreases inhibition of xylose transport and initiates its consumption, g) Snf3 is a sensor that induces expression of required transporters at low glucose concentrations. In the absence of sugars, yeasts use alternative carbon sources via carriers h) Gup1 or 2 and j) St1. Under osmotic stress, equilibrium is maintained by expelling glycerol via carrier i) Fps1.

All transporters are expressed in several specific conditions. Hxt1 has a low affinity and is expressed in high glucose levels to control carbon flux; in some cases the affinity of the transporters can be modulated to adapt to consumption needs, as Gal2 and Hxt2, they switch affinity to regulate specific transport. Some transporters (Hxt 8–17) are transcribed at low levels and cannot support the demand of nutrients by themselves and in the particular case of Hxt12 does not transport glucose. HXT family has the ability to translocate other sugars such as fructose, which also covers a role of expression regulation [9, 10]. In Schizosaccharomyces pompe, the symporter Ght2 has better affinity for fructose instead of glucose [12].

Specific fructose symporter (Fsy1) has been described to function as a proton symporter; this transporter is able to discriminate between fructose and other hexoses in *Saccharomyces pastorianus* [13]. The symport transport of fructose occurs when cells growth in low fructose (0.5%) medium and exhibits an unusual fructose:H^+ stoichiometry of 1:2 [14].

Frt1, from *Kluyveromyces lactis* encoding a fructose transport protein of high affinity to fructose that acts as a proton-coupled symporter dependent of energy and is rather specific for fructose. This transporter is also induced by galactose, although in a lesser extent. It remains to be determined whether Frt1 transcription is under control of the Rag4 glucose sensor [15].

Yeast *Zygosaccharomyces bailii* uptake fructose mainly at high sugar concentrations by a specific transporter system with high capacity and low affinity and a non-specific transporter with low-capacity and high-affinity that also transports glucose, similar results were also found for *Z. rouxii* [16]. Galactose is another sugar that is uptake by yeast, transporter Gal2 who shows homology with HXT but is not a specific-transporter and presents two conformational state for low and high affinity. In mutant of transporter Lac2 of *K. lactis* lost the capacity of lactose consumption and also for galactose indicating that Lac2 can transport galactose [17].

2.3. Pentoses transporters

Glucose is preferentially transported into the cell due to a 100-fold lower affinity of xylose for the transporters. In *S. cerevisiae* some non-specific Hxt transporters are able to transport xylose only when glucose is absent or in concentrations below 5 g/L. Hxt7 has a low affinity to xylose and presents efficient transport for this sugar [11].

Candida intermedia shows to grow well on xylose, the transport of this sugar is carried on by two different transport systems: a Gxf1 glucose/xylose facilitator 1 with low affinity, it is constitutive expressed and on the other hand a Gxs1 glucose/xylose symporter 1 whit a high affinity to xylose is repressed in the presence of glucose [18]. In [19] was detected a very weak growth in complementation of xylose for YHT1 and YHT6 (genes from *Yarrowia lipolytica*) in an hxt-null mutant of *S. cerevisiae* engineered for use of xylose.

S. cerevisiae lacks of arabinose specific transporters, however Gal2 can be transported at a slow rate, other yeast as *Scheffersomyces stipitis* have an AraT to uptake arabinose and apparently does not facilitate hexoses transport [20].

2.4. α-glucosides transporters

MAL loci contains genes necessary for the transport and consumption of maltose as MALx1 which encodes a maltose permease with low affinity and MALx3 encoding a positive regulatory protein of these genes in the presence of maltose, a clear example would be maltotriose/maltose: symporter Mal61 encoded by MAL61 and a positive regulatory protein encoded by MAL63 [21]. In yeast there are maltose transporters with high and low affinity, for example MAL11, MAL21 and Mal 61 have high affinities to maltose (Michaelis constant (Km): 2–4 mM) and can carry other sugars as turanose but cannot convey maltotriose. Atg1:H^+ is a symporter transporter capable of transporting a wide variety of α-glucosides (trehalose, sucrose, maltose, α-methyl-glucoside, maltotriose) in *S. cerevisiae* [22].

2.5. Glycerol transporters

Polyols like glycerol are used as osmoprotectants by many organisms; yeasts accumulate glycerol under high osmolality conditions. Fps1 glycerol efflux facilitator in *S. cerevisiae* is essential

maintaining the balance in hypoosmotic changes, this transmembranal protein contain a cytosolic terminal domains that is important regulating glycerol flux through the channel [23].

Another glycerol:H^+ proton symport transporters like Stl1 are expressed transitorily and activated when all sugar is consumed and the yeast enters into diauxic shift, during this, major changes in gene expression alter the fermentative to oxidative metabolism, allowing to utilize the produced ethanol and glycerol before entry into the stationary phase. Stl1 was inactive in the presence of glucose [24]. By homology analysis with Stl1 from *S. cerevisiae*, the Gt1 *of Scheffersomyces stipitis* demonstrated to be a glycerol transporter that is active when the medium contains ethanol and absence of sugars [25].

2.6. Inositol transporter

Inositol transporters ITR1 and ITR2 (from *S. cerevisiae*) are located in the plasma membrane and accept myo-inositol, both have similar affinities (ITR1: Km = 100 μM, ITR2: Km = 140 μM). However, there is insufficient inositol uptake when only ITR2 is present and there for ITR1 appears to be responsible for inositol uptake because ITR1 is highly transcripted. The ITR2 transporter of Schizosaccharomyces pombe (inositol auxotroph) is essential for regular cell growing, this transporter contains 12 intermembranial domains whit two sugar-transport motifs typical for HXT and shows similarity whit *S. cerevisiae* inositol transporters. The mRNA levels of itr2 gene are also repressed by glucose [26].

All transporters mentioned have the transport of carbohydrates in common, but they present variation on substrate affinity that can be classified in low (Km: >40 mM) and high (Km <40 mM) affinity, this feature leads to control carbon flux; therefore at high substrate concentrations the expression of low affinity transporters is induced. One way to measure carbohydrate transport rates (uptake) is by scintillation assay, where studied strains that express the transporter of interest. It is harvested and transferred to a substrate-free buffer to subsequently expose them to a solution of known concentration of the radioactively labeled carbohydrate of interest for a defined period of time, then, the cells are filtered and washed with the same buffer, after, the remanent is analized by a liquid scintillation counter. The difference between the radioactivity data of the initial substrate and the remaining concentrations, allows substrate consumption quantification per unit time; this information can also be integrated into an enzymatic modeling or nonlinear regression analysis to obtain kinetic parameters of Km and maximal initial uptake speed (Vmax). **Table 1** presents a list of diverse characterized yeast and transporters.

3. Protein transport

Membranous and non-membranous proteins are the indispensable machinery for the cells life. Membranous proteins are integral and peripheral membrane proteins that include transporters (sugars, ions), GTP binding proteins, cell wall synthesizing proteins. While, non-membranous proteins are metabolic proteins, transcription factors and so on. Most proteins are usually encoded in the nucleus and synthesized in the free ribosomes of cytoplasm [39].

Species	Transporter	Hexoses	Km	Pentoses	Km	Other	Km	Regulation	Reference
Saccharomyces cerevisiae									
	Hxt1	Glu > Fru	Low	Xyl	Low			Induced by high glucose level Does not transport xylose as unique carbon source	[9]*
	Hxt2	Glu	Mod					Induced by low glucose levels	
								Repressed by high glucose levels	
	Hxt3	Glu > Fru	Low					Induced by high glucose levels	
	Hxt4	Glu > Fru	Low	Xyl	Low	As(OH)3	NR	Induced by low glucose levels	
								Repressed by high glucose levels	
	Hxt5	Glu > Fru	Low	Xyl	Low			Regulated by cellular growth	
	Hxt6	Glu > Fru > Man	High	Xyl		Maltose	NR	Induced slightly at low glucose concentrations Highly induced in non-fermentable substrates	
	Hxt7	Glu	High	Xyl	Low			Repression by high glucose levels. It varies only in 2 amino acids with Hxt6	[11]
	Atg1					Treha/Sucr:H + > Malt/ α-met-gluc: H* Maltose	High Low	High levels of expression of this gene during wort fermentation	[22]
	Gal2	Glu > Gal	Mod	Xyl/Ara	low			Induces by presence of galactose. Repressed at high glucose concentrations	[20]
	Mal11, 61					Maltose > Turanose	High	MAL loci of constitutive expression and induced by presence of maltose.	[21]
	Mph2, 3					Maltose/ Maltotriose			[22]
	Irt1–2					Inositol	Low	Repression by the presence of glucose	[26]
	Stl1: H+					Glycerol		Active when found in a system with nonfermentable carbon sources, inducible in saline conditions, transient expression, inactive in the presence of glucose	[24]

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Species	Transporter	Hexoses	Km	Pentoses	Km	Other	Km	Regulation	Reference
	Fps1					Glycerol efflux		Form protein channel, essential in maintaining the balance in changes hypoosmotic	[23]
	Fsy1 EC1118	Fru Glu	High Low					Repressed by high concentrations of glucose or fructose and was highly expressed on ethanol as the sole carbon source	[27]
	Gup1 / 2					Glycerol		They allow medium growth with glycerol as the sole carbon source and stabilize cell under salinity conditions, Membrane-bound O-acyl transferases family.	[28]
Scheffersomyces stipitis	Xut1/3	Glu/Fru	NR	Xyl	High			Preference for xylose over glucose but moderate transport efficiency	[29]
	Qup2	Glu/Fru	NR	Xyl	NR				
	Hxt2.6	Glu/Fru	NR	Xyl	NR				
	Arby			Ara				Repression by the presence of glucose	[20]
	Gt1					Glycerol	NR	Is active when the medium contains ethanol and absence of sugars	[25]
	Sut1	Glu > Fru	High	Xyl	Low			Induced by glucose presence	[9]
	Sut2	Glu	High	Xyl	Low			Constitutive expression under aerobic conditions and are independent of carbon source	
	Sut3	Glu > Fru	High Low	Xyl > Gal	Low			Constitutive expression under aerobic conditions and are independent of carbon source	
Candida intermedia	Gxs1	Glu: H+		Xyl: H+	High			Repression by the presence of glucose	[18]
	Gxf1	Glu	Low	Xyl	Low			Constitutive expression	
Candida albicans	Hgt1/Hgt2	Glu	High					Repression by high glucose levels	[30]
	Hgt7	Glu	NR					Induced by low glucose levels	
	Hgt12	Glu	NR					Induced by low glucose levels	
	Stl1					Glycerol: H+	High	Induced by salt stress	[31]

Species	Transporter	Hexoses	Km	Pentoses	Km	Other	Km	Regulation	Reference
Yarrowia lipolytica	Yht1	Glu/Fru/Man	NR	Xyl	NR			Main hexose transporters Induced by presence of glucose and galactose	[19] [32]
	Yht2	Fru	NR					Detected in stationary phase of growth	
	Yht3	Fru	NR					Detected in stationary phase of growth	
	Yht4	Glu/Fru/Man	NR					Main hexose transporters Induced by presence of glucose and galactose	
	Yht6			Xyl	NR			Detected in stationary phase of growth	
Schizosaccharomyces pombe	Ght1	Glu > Fru: H+ / Fru	High						[12]
	Ght2	Glu: H+ Fru: H+	Low High						
	Ght3	Glu: H+	High			Gluconate		Transitory expression Gluconate transport inhibited by glucose presence	
	Ght5	Glu > Fru	High Low					Constitutive expression in different carbon sources	
	Ght6	Fru > Glu: H+	High	Fru	High			Constitutive expression in different carbon sources	
	Sut1					Mal > Suc: H+	High	No specific induction. Glucose repression	[33]
Kluyveromyces lactis	Hgt1	Glu > Gal	High					Constitutive expression with 26–31% identity with Hxt in *S. cerevisiae*.	[17]
	Ftr1	Fru: H+	High					Induced by presence of glucose, fructose and galactose	[34]
	Lac2	Gal	Low			Lac	High		[17]
	Rag1	Glu	Low	Fru	NR			Induced by high levels of glucose, fructose and other sugars. Repressed by absence of glucose	[35]
	Kht1	Glu	Low					Induced by high levels of glucose, fructose and other sugars	

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Species	Transporter	Hexoses	Km	Pentoses	Km	Other	Km	Regulation	Reference
	Kht2	Glu	High					Induced by low glucose levels	
								Repressed by high glucose levels	
	Kht3	GFal	NR						[36]
Debaryomyces hansenii	Xylh			Xyl: H+	High			Induced by the presence of xylose	[37]
Saccharomyces pastorianus and bayanus	Fsy1	Glu Fru: H+	High			Sorbose	Low	Induced by low Fru levels	[14]
Zygosaccharomyces rouxii	Ffz1	Fru: H+	High					Induced by High levels of Fru	[38]
	Ffz2	Fru	Low						
	Fsy1	Glu Fru: H+	Low High					Induced by low Fru levels	[16]

NR: No reported, Glu: Do not glucose transport, Mod: Affinity modulated by substrate concentrations.
*Modified of Leandro 2009.

Table 1. Yeast transporter with affinity substrate ratios and expression regulation.

Once synthesized, they must have to be transported to different compartmentalized organelles such as Nucleus, Endoplasmic Reticulum (ER), Mitochondria (Mt), Golgi bodies, Vacuoles and Peroxisomes [39–41]. These compartmentalized organelles are constituted by multiple sites like outer membrane, intermembrane space, inner membrane and matrix as shown in **Figure 3**. The proteins should be transported to all specified sites of organelle(s) and across the wall (cell wall) to the extracellular medium [39–41]. The order of events that leads the protein to get transported are protein recognition and its subsequent translocation into the organelle. Despite the organelle specific transport, multiple steps of protein transport are briefly generalized here.

3.1. Signal sequence

Most proteins synthesized in the cytosol are mostly precursors or preproteins carrying signal sequences [39]. The signal sequences, present in each protein molecule, are organelle specific. They can be found either at the N-terminal or C-terminal ends of proteins [39–41]. The signal sequence has three conserved general domains: A N-terminal region that varies widely in length, but typically, contains amino acids which contribute a net positive charge: a central hydrophobic region made up of seven to 16 amino acids; followed by a signal cleavage site (**Figure 4**). For instance, Mt. preproteins are rich in positively charged amino acids, arginine and lysine, and hydroxyl bearing ones, serine and threonine. In nuclear preproteins, the sequence region of first 10–90 N-terminal residues, exhibiting a high composition of arginine and near absence of

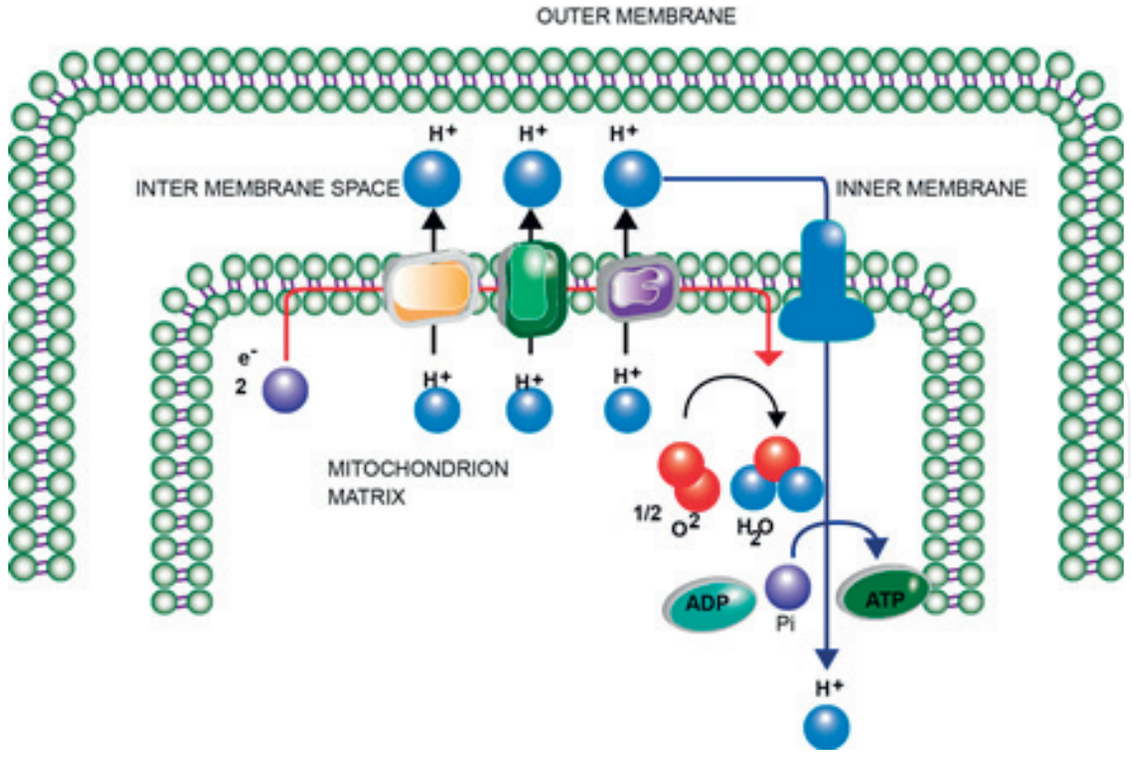

Figure 3. Compartmentalization of organelles like nucleus, endoplasmic reticulum, mitochondria, peroxisomes into multiple layers include outer membrane, intermembrane space, inner membrane and matrix. A mitochondria layers example is given here.

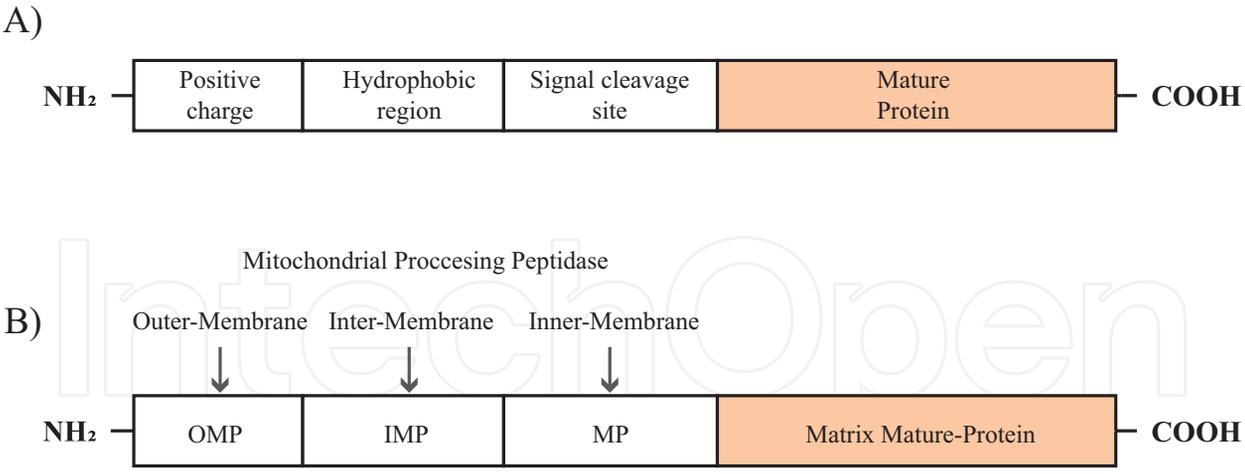

Figure 4. Representation of signal peptide regions present in the various preproteins. (a). The classic preproteins that are entering ER organelle consists of three separate regions in signal peptide include a segment of positively charged amino acids followed by a stretch of hydrophobic amino acids. A protease cleavage site is found next to the mature protein segment. (b). More similarly, mitochondrial matrix proteins contain three peptide regions that corresponds to specific peptidases located on the outer membrane, intermembrane space and inner membrane. Each region is highlighted by arrow. The successive cleavage of peptide regions at respective sites moves the proteins across the membrane to reach matrix.

negatively charged residues, is considered as signal peptide [42]. Regarding membrane proteins, the targeting signals have so far only been identified for a small subset of proteins [43]. In general, non-membranous proteins carry signal peptides at N-terminal, whereas signal peptides are located at the carboxyl termini of membranous proteins [43]. Additional signal sequences found in the proteins conceive multiple entries across the membrane layers of organelles. The example is shown in **Figure 4**, where Mt. luminal proteins contain three signal sequences as follows: (1). A N-terminal protein signal required to gain access into organelle, (2). A stretch of amino acids signalizing the intermembrane space and (3). The mature part of the precursor protein signal that allows the protein to locate themselves into the Mt. lumen [44, 45].

As proteins contain unique signals to each organelle, various bioinformatics databases are developed to facilitate the search process of signals in the proteins. The databases are listed at the end of the book chapter. The enlisted bioinformatic databases will assist the researchers to study and explore the signal peptides appropriate for the organelles of interest.

3.2. Protein recognition and entry into organelle

The signal sequences present in the protein molecules are recognized by signal receptors or signal recognition particles and outer-membrane translocases [42, 44, 45]. They are usually found either in the cytosol or on the membrane of the organelles'. Pex5p is a remarkable example of cytoplasmic receptor protein [46]. Some examples of membranous receptors are exportins and importins (nuclei), translocase outer-membrane complex (Tom70; Mt) [44, 45]. The receptor always function by coupling with other accessory proteins to import and export proteins. For instance, Tom 70 binds to a subset of mitochondrial precursor proteins, with Tom70, are Tom22, Tom5, Tom6, Tom7, Tom20 and Tom70 [44, 45]. These binding partners cooperate and facilitate the targeting of mitochondria proteins. Usually, receptors contain binding sites for signal sequence in the precursor proteins. After gaining access to the organelle specific receptors, precursor proteins are either further processed and deposited into the

respective compartments, or translocated directly through the membrane pore complexes [39–41]. In the case of processing precursors, the function of multiple peptidases locating in the respective compartment is required. Especially, in the transport of Mt. luminal proteins, three peptidases: Mitochondrial processing peptidase, Mitochondrial intermediate peptidase, Mitochondrial inner membrane peptidase and their complex proteins are involved to translocate protein from the outer - membrane to the matrix [44, 45] (**Figure 3**).

In the following section, we account on the examples of two typical protein transport systems based on the presence (peroxisome protein) and absence of signal sequences (vesicle-associated protein).

3.3. Transport of peroxisome proteins

Peroxisomes are ubiquitous eukaryotic cell organelles that compartmentalize a large variety of oxidative metabolic reactions. Peroxisome proteins play essential roles in glycolate recycling, amino acid biosynthesis and in fatty acid degradation. Since, it does not contain any genetic material, all the peroxisome proteins are encoded in the nuclear genome. Two types of Peroxisome transport sequence (PTS) have been discovered: type I (PTS1) and type II (PTS2) to translocate proteins from cytoplasm [46, 47]. Some of the identified peroxisome signal peptides are listed in **Table 2**. The PTS1 is found in most of the peroxisome matrix proteins and is located at the C-terminus as a tripeptide SKL20. It generally fits the consensus sequence (S/A/C)-(K/R/H)-(L/M). The PTS2 is a conserved sequence which is located near the N-terminus of a protein and is comprised in some species within a pre-sequence that is cleaved off after import into the peroxisomal matrix. Sequence comparisons showed the conserved nonapeptide of PTS2 as (R/K)-(L/V/I)-X5-(H/Q)-(L/A/F). Some proteins which do not contain neither a PTS1 nor a PTS2 have been identified and well known examples are acyl-CoA oxidase, catalase from *S. cerevisiae* and *Y. lipolytica*, the alcohol oxidase from *Hansenula polymorpha* [48].

Pex5p protein, the cytoplasmic receptor, shuttles between a soluble form and an integral membrane-bound form [46, 49, 50]. They guide free-ribosomal-synthesized peroxisome proteins to translocate across the peroxisome membrane to matrix. It has been characterized that this protein has the capacity to translocate folded, and even oligomeric proteins. The C-terminal domain comprises of seven tetratricopeptide (TPR) repeats, in which 1–3 and 5–7 TPRs adopt extended conformation to link other three TPRs [49]. This conformation produces a funnel shaped binding site for the proteins containing PTS1 signal sequence. Once the receptor recognizes the cargo in the cytosol, a set of proteins Pex13p, Pex14p, Pex17p associate to it forming a docking

Yeasts	Protein	Sequence
C. tropicalis	Catalase	ILELSPRK
S. cerevisiae	Catalase	ELSSNSLF
C. tropicalis	Acyl-CoA oxidase	EYAAILSK
H. polymorpha	Dihydroxyacetone synthase	NHDKVNKL
C. tropicalis	Trifunctional enzyme	LVGDLAKI
S. cerevisiae	Trifunctional enzyme	LSQAKSKL

Table 2. List of peroxisome protein signal sequences.

complex [46, 50]. This establishes a possible link to cargo-receptor complex with peroxisome membrane. At the peroxisome membrane, Pex5p would act as intrinsic membrane protein forming a stable complex with the docking proteins. This complex is shown to exhibit the main conductance of a pore with 3.8 nm in diameter [46, 50]. Also, they can transiently expand to more than 9 nm, when they are importing large oligomeric cargo proteins. The formed pore might at some stage import and translocate the proteins to the lumen [46, 50]. After the luminal protein is released, Pex5p is recycled and translocated to the cytosol by an ATP dependent ubiquitination machinery [46, 49, 50]. In summary, in the cytosol, Pex5p functions as PTS1-receptor in cargo recognition and at the peroxisome membrane where it contributes to pore formation and presumably translocation (**Figure 5**).

3.4. Transport mechanism of a transmembrane protein, Snc1p/2p

Here, we give an example of transport of a transmembrane protein associated to vesicles (discussed below in the following section). Synaptobrevin (Snc1p/Snc2p) is a soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) transmembrane protein. These proteins bind onto vesicles and interact with t-SNARE proteins on the plasma membrane, by which they provide specificity for the targeting and fusion of vesicles with the plasma membrane [51]. It consists of a variable N-terminal domain, a central coiled-coil domain, and, in most cases, of a single C-terminal transmembrane domain (TMD) that is thought to be α-helical. The conserved region in the SNARE proteins was predicted to contain two amphipathic alpha helices [51]. Helix 1, from 39 to 53, is unusually hydrophobic and Helix

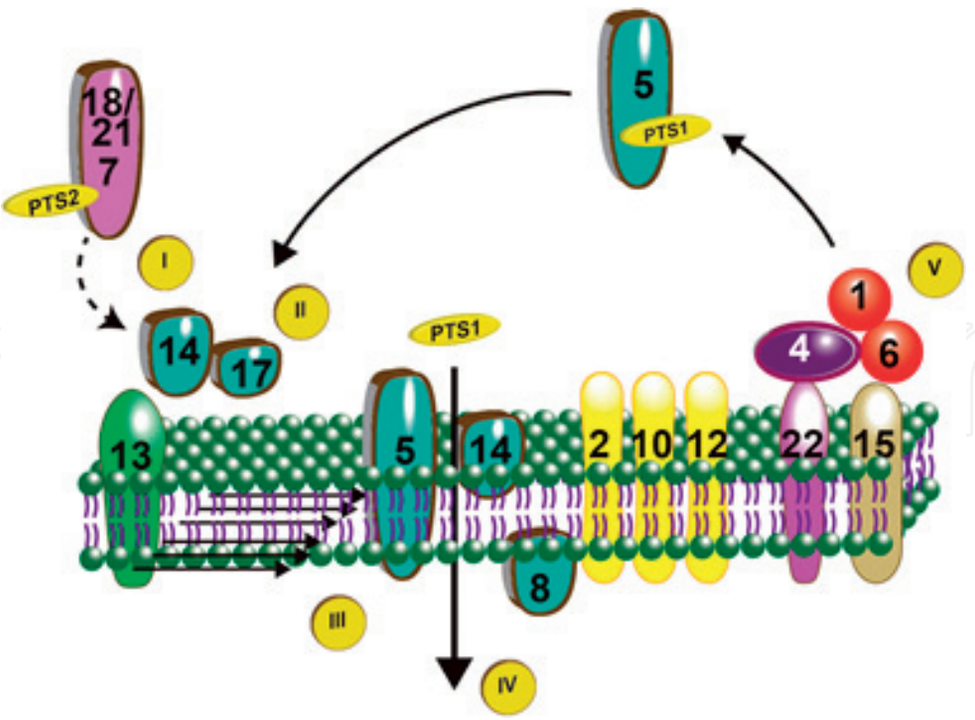

Figure 5. Outline of transportation of matrix proteins of peroxisome mediated by Pex5p and its associated proteins. The protein transport involves five steps: (I) cargo-receptor recognition (II) docking of cargo-receptor complex to the membrane (III) translocation of cargo into matrix across membrane (IV) disassembly and (V) recycling of receptor to cytosol (modified from [50]).

2, from 60 to 88, predicted to be interacted with other hydrophobic segments of membrane proteins t-SNAREs (Syntaxin) during the fusion of vesicles. Other than helices, it carries a variable domain in the N-terminal, a carboxy trans-membrane domain (TMD) region of 96–110 amino acids is usually hydrophobic and some amino acids present intravesicular in vesicles [51].

Just like the other class of membrane proteins, it lacks a signal sequence and contains a single hydrophobic segment close to their C-terminus, leaving most of the polypeptide chain in the cytoplasm (tail-anchored) [43]. The initial targeting of these proteins to the ER is mediated by hydrophobic signal sequences, which are recognized during translation by the signal recognition particle. This hydrophobic stretch near the C termini of membranous protein do not bind to signal recognition particles and are inserted into membranes post-translationally. Once after getting entry into ER, it wasn't clear about the regions responsible in targeting them to secretory vesicles. Deletion and mutational studies were made in the SNARE proteins to investigate the region possessing the ability to target it (**Table 3**). From the targeting studies of Grote et al. [52] and Gerst [53], it was clear that in the absence of helical loops, it is not possible to target the Snc proteins onto secretory vesicles. Thus, deletion or gross substitutions in either of the predicted H1or H2 segments result either in the loss of targeting or in a complete loss of functions. This shows that conserved amphipathic alpha helical region (32–85) is essential for the confinement of snare proteins.

Regions deleted		**Effects**		**Reference**
		Presence	**Absence**	
VAMP	2–30	++		[52]
	2–60		−−	
	31–38		−−	
	41–50		−−	
	61–70	++		
	71–80	++		
Snc1	2–27	++		[53]
	31–50		−−	
	51–82	N.D.		
VAMP	1–90		−−	
Snc1	91–116		−−	
Snc1	1–65	++		
VAMP	65–84			
Snc1	85–116			

++ confers the targeting of SNARE proteins.
−− confers the non-targeting of SNARE proteins.
VAMP—vesicles associated membrane protein/ortholog of Snc1.

Table 3. Deletion mutational study reveals the regions required for the targeting of Snc1 and its ortholog proteins.

In the other hand, deletions of both variable domain and transmembrane domain do not produce a more deleterious effect in the fusion of vesicles. That is, their localization onto vesicles is not affected by these mutations [52–54]. These results substantiate that the TMD of Snc protein is tuned to conduct its delivery into ER, while the helices take it over from ER to Golgi. Besides, the targeting of SNAREs to vesicles, TMD plays a key role in their sorting and fine tunes their distribution within the secretory pathway. That is, TMD sorts Sncp proteins and let them to undergo a dynamic cycle of transport to and retrieval from the plasma membrane to vesicles. Thus, it is understood that TMD serves both, as a key factor in the membrane distribution and as the targeting signal for initial insertion of protein to ER domain. Taking together, it was concluded that the sequence-specific information present in the membrane proteins is important for the respective localization to specific organelles and its subsequent protein function.

4. Vesicular transport

Despite the appreciable functionality of various transporters and protein machinery, there is another existing sophisticated source to transport materials across the walls. They are "naturally existing liposomes" which are made up of an outer hydrophobic lipid bi-layer and an inner aqueous hydrophilic core. Two vesicle types depending on their localization: intracellular and extracellular vesicles are identified and extensively studied in the literature. This section briefly describes the role of such vesicles in the transport of biological materials in yeast organisms.

4.1. Intracellular vesicles

In *S. cerevisiae*, two types of intracellular vesicles: - early secretory and post secretory vesicles are involved to transport cargos (proteins) [39–41]. Early secretory vesicles (ESVs), derived from ER membrane, carry cargoes to Golgi complex, where at this stage post translational modifications such as glycosylation, mannosylation, acetylation, methylation, phosphorylation and acylation are done [41]. While post secretory vesicles (PSVs), shed from trans-Golgi membrane, transport selective cargos destined to extracellular medium and plasma membrane [40]. First, Golgi complex cargoes, secretion and cargoes of other organelles requiring post translational modification are selected via the signal peptide. Later, they are transferred from the ER and packaged as cargo into COPII coated vesicles (ESVs) bound for the stacks of the Golgi complex [39, 41]. The ER vesicles fuse with the cis-Golgi membrane to deposit the cargo into the Golgi complex. The deposited proteins are post translationally modified according to their functional requirements [39, 40]. Then, the proteins for secretion and plasma membrane, that are sorted away from the rest of the cargo, pass into the trans-Golgi network (TGN) and are packed into clathrin coated vesicles, which are called early as PSVs [39].

The PSVs move vectorially towards sites of polarized growth (the bud and mother/daughter neck). They move to arrive at the target membrane dock and subsequently fuse to transfer

their contents to extracellular medium [39, 55]. This complete process is termed as polarized exocytosis. It consists of at least three stages. First, PSVs are targeted to the vicinity of designated plasma membrane domains via microtubule- and/or actin-based transport systems [55, 56]. Second, after vesicles arrive at their sites of active exocytosis, where a exocyst complex consisting of eight components: Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70, and Exo84 mediate the targeting and tethering of PSVs for subsequent membrane fusion [56–58]. Two proteins Sec15p and Sec10p bridge Sec4p, a Rab Gtpase, to other exocyst components. On the plasma membrane, Sec3 and Exo70 interact with PIP2 and with other family members of Rho Gtpases (Cdc42, Rho1p) [58]. Finally, the fusion between PSVs and plasma membrane takes place allowing the secretion of vesicle contents and the incorporation of membrane proteins at specific plasma membrane domains. This specific fusion event is mediated by interaction of proteins present in PSVs membrane (v-SNAREs, snc1p/2p) (SNARE, soluble N-ethylmaleimide-sensitive fusion attachment protein receptors) and plasma-membrane (t-SNAREs; sso1p/2p) [51].

Wild type *S. cerevisiae* strains generate PGVs from 50 to 70 nm in diameter. In contrast, some mutant strains deficient in vesicular transport accumulates PSVs within the cell in different size ranges. For example, exo70–35 and exo70–38 mutant cells accumulated PSVs from were 80–100 nm in diameter [58]. Forsmark et al. [59] have determined the protein composition of PGVs obtained from the sec6–4 and sro7 mutant strains for isolation. The protein content identified are mainly involved in vesicle transport, molecules transportation, metabolism of carbohydrates and protein biosynthesis and degradation. Major dominant lipids constituents of membrane are phosphatidylcholine (PtdCho), phosphatidylethanolamine (PtdEtn) and phosphatidylinositol (PtdIns). It has been speculated that the above mentioned mutants serve as an outstanding source of vesicles for lateral biotechnological applications.

4.2. Exosomes or extracellular vesicles

In all the three kingdoms of life, Exosomes or Extracellular Vesicles (EVs) are one of the most protective sources of conducting trans-cell wall transfer of macromolecules to the recipient cells. EVs (Ø 50–120 nm) are secreted from cells as membranous vesicular organelles by a wide variety of cells, from lower to higher eukaryotic organisms, i.e., from fungi to mammals [60, 61]. Unlike intracellular vesicles, they act as extracellular carriers of proteins and/or nucleic acids, particularly microRNAs and mRNAs, between cells and serve as shuttle vectors and mediators of intercellular communication, immune responses, and antigen presentation [60]. The biogenesis of exosomes begins in the last stage of endocytosis, during which the endocytic membrane undergoes budding to form intraluminal vesicles (ILVs). The accumulated ILVs within the original endocytic membrane, at this stage, is named the multi-vesicular body. These bodies, then fuse with either lysosomes for degradation or the plasma membrane for extracellular release of ILVs, i.e., exosomes or EVs. EVs are released from cells, either constitutively or upon activation of a secretory pathway [60]. The machinery involved in the biogenesis of exosomes varies in different cell types [60, 61]; however, in most cells, the ESCRT (endosomal sorting complex required for transport) machinery plays a major role in EVs biogenesis [60, 61]. The roles of both the ESCRT-dependent and -independent mechanisms in

exosome biogenesis remain largely unknown and are yet to be fully elucidated. Similarly, the mechanisms underlying the packaging of cargo into exosomes and the transport of these exosomes across cellular membranes have been described both *in vivo* and *in vitro*, but remain to be fully elucidated [60, 61].

EVs have been conserved and distributed widely in many different fungal species, including yeast cells and hyphae [23]. Pathogenic fungus and opportunistic fungus are the well-recognized candidates for the release of EVs. Some of the examples are as follows: *Paracoccidioides brasiliensis*, *Sporothrix schenckii*, *Candida albicans*, *Candida parapsilosis*, *Malassezia sympodialis*, *Histoplasma capsulatum*, *Cryptococcus neoformans*, *Malassezia sympodialis* [61]. Non-pathogenic *S. cerevisiae* mutants (sec4–2, sec6–4, sec4–8, sec23–1, exo70–35, and exo70–38) have also been demonstrated to excrete EVs in the extracellular medium [62]. Several EV proteome studies revealed the presence of multiple organelle specific proteins which are derived from the cytoplasm, plasma membrane, mitochondrial, vacuolar and even nuclear proteins. Sterols, phospholipids and pigments are also present in the EVs. Quite recently, the presence of small RNAs in the fungal EVs was addressed [63].

4.2.1. Diverse roles of transport cargoes of EVs

The EVs derived from pathogenic fungus are natural born carriers of cargo responsible for fungal pathogenesis. Several components of fungal EVs are potent elicitors of immunological activities [64]. For instance, the very common protein HSP60 carried by EVs acts as immunogen and induces protective antibodies [65]. The main virulence factor of EVs derived from *Cryptococcus neoformans* is a polysaccharide capsule coating glucuronoxylomannan, which activates immune-suppressive and anti-phagocytic properties [65]. The incubation of cryptococcal vesicles with murine macrophages induced high levels of extracellular tumor necrosis factor alpha (TNF-α), interleukin-10 (IL-10), and transforming growth factor β (TGF-β) [65]. *M. sympodialis* releases extracellular vesicles carrying allergen were shown to induce IL-4 and TNF-α responses in PBMC patients [66]. The immunogenic galactosyl epitopes distributed on the surface of large EVs of *Paracoccidioides brasilensis* generated robust immune response in the paramycoccidiodomycosis patients [67]. They bind to host lectins and induce immunological type 2 suppressive response. Also, *C. albicans* EVs stimulated dendritic cells (DCs) to produce IL-12p40, IL-10, and TNF-α, and induced upregulation of CD86 and MHC-II [68]. The change in protein composition of THP-1 macrophage-derived EVs was studied during the interaction with *C. albicans* [69]. This study revealed the changes in the abundance of proteins relating to immune response, signaling, or cytoskeletal reorganization. The interaction significantly increased the secretion of proinflammatory cytokines and the candidacidal activity. More likely, the treatment of *H. capsulatum* cells with monoclonal antibodies (MAbs) affected the sizes, enzymatic contents, and proteomic profiles of the vesicles released by fungal cells [70]. The coating of Hc with cryptococcal glycans (Cn-gly) resulted in higher pulmonary fungal burden in co-infected animals relative to control. Co-cultivation or addition of Cn-gly resulted in enhanced pellicle formation with a hybrid polysaccharide matrix with higher reactivity to GXM mAbs [71].

5. Methods to determine the secretion-proteins across yeast wall

Numerous established techniques are already available in literature to detect, characterize and demonstrate the phenomenon of secreting proteins, towards the extracellular medium, across yeasts wall. At cellular level, usually, the proteins destined to secretion are always preserved intact into the secretory vesicles of yeasts. Taking advantage of this nature, many fluorescent methods detect the proteins presence througth the secretion route in cells by fluorescence. The availability of several fluorescent proteins (FPs): Green-FP, Red-FP, Yellow-FP and Blue-FP has made the detection process simple and effective [72]. To this end, tagging proteins of interest with FPs, using genetic engineering techniques, will come handy and serve the purpose of locating them into the cells. In the other hand, immunofluorescent technique makes use of antibodies to demonstrate the integrity of secretion proteins inside vesicles [53]. For this purpose, various temperature sensitive sec-mutant strains, with the ability to accumulate vesicles, are highly recommended [73].

Once the proteins are secreted outside, they can be characterized by molecular techniques like SDS-PAGE and Western blotting to identify specifically the proteins of interest in the extracellular medium [53, 74]. By other hand enzyme activity studies are suitably advantageous to determine the proper functioning of the secreted protein The design of such experiments generally varies with respect to the enzymes and must be handled appropriately and the experiments can be performed either by using the whole extracellular medium containing secreted proteins or by using the purified proteins of interest (see protein purification section in applications below). Combining all together, we conclude that one of the abovementioned techniques could be suitable for realizing adequate studies on the proteins secretion.

6. Applications in biotechnology

This is an overview of the main trends reported within the last years in current research on applications related to transport proteins in some yeast, which has not yet discussed in detail. Major advances, of the role of different biological transporters in *S. cerevisiae* are focused in carbohydrates related to obtain value-added bioproducts. Mainly discuss the expression of carbohydrates transporters in yeast are focused to improve different substrates and in the modification of specific aminoacides into transporters to regulate the affinity, order to alleviate transport inhibition by sugar concentration. The capacity to co-transport glucose and xylose into yeast has remained a technical challenge in the field [11, 29]. Due to the lack of an endogenous xylose transporter in *Saccharomyces cerevisiae*, the xylose uptake depends on transporter engineering to increase transport rates avoiding glucose-based inhibition, thus enhancing the potential of using lignocellulosic biomass as a feedstock for yeast [11, 29]. Besides of to the generation of fuels, the production of value-added chemicals from renewable biomass has been widely studied. According to [75], *S. cerevisiae* could be exploited for the production of other non-ethanol fuels and chemicals from byproducts through metabolic engineering expressing specific sugar transporter. Some other efforts to use *S. cerevisiae* strains as a cell

factory to obtain valued-added products which no involves the use of genetic tools, but metabolic activators [76] and ultrasound [77].

Some other works involves the study of trafficking mechanisms of small and large compounds to regulate biosynthesis of appreciated biochemical products. Also, mitochondrial transport mechanisms are relevant due to its use in future comparative studies aiding explorations of human mitochondrial diseases and to improve biochemical process. Because energy is a fundamental enabler of the economy, energy security and environmental safety are two major issues in the current world that have boosted the demand for an alternative and eco-friendly energy source.

6.1. Protein purification mediated by heterologous expression

Using genetic engineering techniques, recombinant proteins can be synthesized in anyone of three compartments of heterologous hosts: cytoplasm, periplasm and the extracellular medium. The natural ability of secreting proteins is captivated by many researchers as a medium for the large-scale industrial production of foreign proteins and simplifying downstream processes [78]. The secretory expression requires a simple tagging of recombinant proteins of interest with three essential components: (1). A signal peptide sequence targeting secretion, followed by (2) a purification tag and (3) a protease cleavage site [78–80]. Some of the examples of these three essential components are enlisted and the recommendable design of a gene fusion cassette for recombinant protein secretion is shown in **Figure 6**. The expression of this gene fusion cassette in the following hosts enables the secretion of protein towards extracellular medium. The purification tag serves as an anchor and allows the recombinant protein to separate from rest of the media culture, which is subsequently recovered by using protease enzyme [80]. Some of the valuable hosts as recommended by Food and Drug Administration are *S. cerevisiae*, *P. pastoris*, *Y. lipolytica*, *K. lactis*, and *H. polymorpha* [78]. Though technology ages 3 decades, the growth and value of applications are still increasing with respective to the demand. Some of the recent heterologous expression and secretion of proteins of biotechnological interest are presented in the **Table 4**. It is important to note that the α-MF signal sequence has proven to be most effective in directing protein through the secretory pathway in host organisms [81, 84–91, 93, 94]. Such expression and purification of recombinant proteins are widely applied in the industries of textile, food processing, therapeutic applications. In the other hand, the natural ability of yeast hosts to provide, post translational modifications was highly utilized to express, modify and further secrete eukaryotic proteins, especially for therapeutic applications, in the extracellular medium [99]. The expression and secretion of full length IgGs, insulin, glucagon, growth hormone, in yeast hosts is a proof-of-concept in this context [52]. The glycolate form of antibodies and human glycoproteins with fully complex terminally sialylated N-glycans were also synthesized in the engineered *Scchefersomyces stipitis* (formerly *P. pastoris*) [97, 98].

6.2. Peroxisome production of valuable bioproducts

Here, we highlight the use of signal peptides and transporter system of Peroxisome for the synthesis of valuable bioproducts. Mostly, researchers took advantage of the active fatty acid

(a)

Proteins	Signal Peptide
Galactosidase	MFAFYFLTACISLKGVFG:VSPSYNGLGL
Acid phosphatase	MFKSVVYSILAASLANA:GTIPLGKLAD
Carboxypeptidase Y	MKAFTSLLCGLGLSTTLAKA:ISLQRPL
Invertase	MKIYHIFSVCYLITLCAAATTAREEFF
Mating factor a-1	MLLQAFLFLLAGFAAKISA:SMTNETSDRP
Glucoamylase	MVGLKNPYTHTMQRPFLLAYLVLSLLF NSALGFPTALVPRGS
Mating factor alpha-2	MKFISTFLTFILAAVSVTASSDEDIAQVPA

(b)

Purification Tags
His-tag or His6
Myc
Glutathione S-transferase (GST),
Maltose binding protein
Calmodulin binding peptide
Intein-Chitin binding domain
Streptavidin/Biotin tags
FLAG
Halo
Small ubiquitin-like modifier

(c)

Protease cleavage site
Chymotrypsin
Thrombin
Trypsin
Enterokinase
TEV protease
Enteropeptidase
3C protease
Carboxypeptidase
Aminopeptidase
Factor Xa

(d)

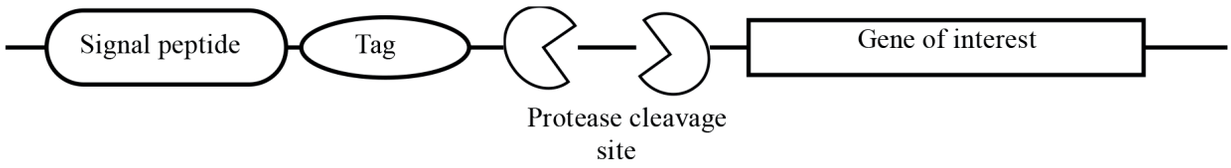

Figure 6. Overview of genetic elements used for the recombinant secretion of proteins towards extracellular medium (a, b, and c). The most significant elements are signal peptide sequence, protein tags and protease cleavage sites. Some examples of well-recognized and highly used components are listed. (d). The design of a gene construct that is in practice and essential for the heterologous expression and secretion of recombinant proteins in yeast hosts in presented.

pathways and PTS1 signals to generate polyhydroxyalkanoates (bioplastics) and biofuels (fatty-acid-derived fatty alcohols, alkanes and olefins) [99–106]. From literature, a simple modification of polyhydroxyalkanoate synthase with PTS was sufficient for targeting and

Organism	Protein	Signal	Applications	Reference
S. cerevisiae				
	Human β-defensin-2, (hBD2)	MFα1 (mating factor alpha) leader	Antimicrobial activity	[81]
	Beta glucosidase	Sed1, glucoamylase, alpha mating leader	Cellulolytic activity	[82]
	endoglucanase II			
	Cel3A	Native secretion signal	Lignocellulosic	[83]
	Cel7A		ethanol production	
	Cel5A			
Pichias pastoris				
	Trx-HPV16-L2 immunogen	alpha-factor signal peptide	Vaccine	[84]
	Horseradish peroxidase	MATα prepro secretion signal		[85]
	Candida antartica lipase			
	Human Pro-relaxin L2	alpha-factor signal peptide	Therapeutic applications	[86]
	FSL2, Lipase	*S. cerevisiae* α-factor signal sequence	Lipolytic activity	[87]
	Endo-polygalacturonase	alpha-factor signal peptide	Textile scouring	[88]
	Camel Hepcidin	*S. cerevisiae* α-factor signal sequence	Antimicrobial activity, Hormone	[89]
	Human anti-αIIbβ3 antibody	alpha-factor signal peptide	Atheroma Targeting	[90]
	Subtilisin QK	alpha-factor signal peptide	Thrombolytic activity	[91]
Yarrowia lipolytica				
	Glucoamylase	preLip2, preXpr2, and preSuc2	Starch degradation	[92]
	Xylanase			
Kluyveromyces lactis				
	Fructosyltransferase	alpha-factor signal peptide	Hypocaloric sweeteners	[93]
	Arylsulfatase	alpha-factor signal peptide	Milk processing	[94]
	Interferon-Beta	Glucoamylase signal sequence	Therapeutic applications	[95]

Table 4. Recent heterologous expression and secretion of recombinant proteins from mid2016–Feb2017.

synthesizing PHAs in peroxisome of *S. cerevisiae* and *P. pastoris* [99–102]. The authors did an addition of carboxyl 34 amino acids from the *Brassica napus* isocitrate lyase to *Pseudomonas aeruginosa* PHAC1 synthase for peroxisome targeting, which was further expressed under the

control of the promoter of the *P. pastoris* acyl-CoA oxidase gene [96, 97]. By this expression, PHAs was accumulated as inclusions within the peroxisomes and synthesized up to 1% medium-chain-length PHA per g dry weight was obtained using oleic acid as substrate in the medium. In another study, a medium-chain-length-PHA (mcl-PHA) polymer was synthesized in the cytosol of *S. cerevisiae* utilizing the ß-oxidation intermediates, key peroxisome proteins, including Faa2p, Fox1p, and Fox2p, together with PHA synthase [101]. A Pex5p mutant was made in *S. cerevisiae* to retain peroxisome proteins in the cytoplasm. This retention led the peroxisome proteins to take part actively in the generating the mcl-PHA monomers. Accumulated PHA up to approximately 7% of its cell dry weight with a monomeric composition of C12 (3-hydroxydodecanoic acid), C10 (3-hydroxydecanoic acid), C8 (3-hydroxyoctanoic acid), and C6 (3-hydroxyhexanoic acid).

Another effective exploration is targeting synthetic pathways to peroxisomes to produce medium fatty alcohols and long fatty alcohols [103–106]. The targeted expression of fatty acyl-CoA reductase TaFAR to the peroxisome of *S. cerevisiae* has produced medium chain fatty alcohols [103]. The genes Pex7p and acetyl-CoA carboxylase are overexpressed together with targeted TaFAR enzyme in the peroxisome. The coexpression improved the synthesis of decanol, dodecanol, tetradecanol and hexadecanol, which have extensive applications as biofuels and detergents. Another heterologous expression of a fatty acyl-CoA reductase from *Arabidopsis thaliana* in a Pex10p mutant *Y. lipolytica* had produced over 500 mg/L of 1-decanol [104]. Likewise, *Rhodospirillum toruloides* was engineered to express a bifunctional fatty acyl-ACP reductase (FaCoAR) from *Marinobacter aquaeolei* VT8 and produced up to over 8 g/L of C16–C18 fatty alcohols in fed-batch condition using sucrose as carbon source [105]. A recent study has shown enhanced the peroxisome production of fatty alcohols by targeting the FaCoAR enzyme using signal per2 (GGGSAAVKLSQAKSKL) [100]. In the same study, the expression of two FFA based enzymes, *Mycobacterium marinum* carboxylic acid reductase (MmCAR)29 and its activation cofactor-4′-phosphopantetheinyl transferase NpgA from *Aspergillus nidulans* in a Pex31p/Pex32p mutant strain have resulted in the high level of alkane production.

6.3. Vesicles in therapeutic applications

The prime role of intercellular communication has motivated researchers to conceive EVs as potential nano-vehicles for biodelivery applications. Recently in 2016, A patent entitled ¨Compositions and Methods for Yeast Extracellular Vesicles as Delivery Systems, US 20160331686¨ was filed and published [107]. The authors have proposed the use of native and modified EVs from yeasts cells as practical drug delivery vehicles. In the case of modified EVs, an exosomal transmembrane peptide of mammalian origin is immobilized onto the outer membrane of EVs for targeted biodelivery applications. Using these yeast EVs, various therapeutic sources of cargoes: therapeutic RNAs (circular RNAs), autonomously replicating cytoplasmic linear mammalian plasmid (express either therapeutic RNAs or proteins), therapeutic peptides, have been tested for delivery applications. Once the cargo loaded EVs are released from cells, they have been isolated from culture supernatants by either centrifugation or ultra/micro filtration. Authors conducted *in vivo* and *in vitro* studies to study the

uptake of EVs by these cells and its effect in the delivery of cargoes. The purified vesicles are recognized by mammalian target cells with the receptors specific for the targeting ligand and take up the vesicles carrying the biologically active therapeutics via endocytosis. Following this inspirational work, we believe that the combination of recombinant DNA techniques and natural loading efficiency of cargoes into EVs would bring potential drug-targeting properties in future.

7. Webserver

Mitochondria

- p://mitf.cbrc.jp/MitoFates/
- MitoII

Subcellular localization program

- PSORT
- TargetP
- NNPSL (neural network-based predictor)
- http://www.signalpeptide.de/
- SignalP 4.1 (http://www.cbs.dtu.dk/services/SignalP/)
- SPdb (http://proline.bic.nus.edu.sg/spdb)

Peroxisome

- PTS1 Predictor - http://mendel.imp.univie.ac.at/mendeljsp/sat/pts1/PTS1predictor.jsp

Peptidase Database

- http://merops.sanger.ac.uk/

Acknowledgements

The authors acknowledge Juan Corona-Hernandez and Alonso Cid Cervantes for their art-work contribution. GKM thank CONACyT (CB-2014-01; 236285 and Fronteras de la Ciencia 2015-1: 016) for financial support. López-vargas acknowledge to scholarship of PNPC-CONACyT program 5353.

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