**Supplementary File 4 to Gutierrez et al.**

**Overview of the Secretory Pathway in animal cells**

Historically, most of the knowledge on the secretory pathway was obtained by studying protein transport processes and secretion in *Saccharomyces cerevisiae*1. Albeit quite similar in core functions, the secretory pathways of mammalian cells and fungi differ significantly in some of the steps which have been evolved based on species-specific secretion phenotypes2. The following paragraphs briefly overview the mammalian secretory pathway and highlights pathways exclusive to animals not present in fungi. The last section provides an in-depth comparison of the yeast and animal secretory pathways while highlighting the most important differences between both.

**Translocation and processing in endoplasmic reticulum**

Proteins destined to the secretory pathway generally bear a signal peptide at the amino-terminus which targets the proteins to the endoplasmic reticulum (ER) where the initial post-translational modifications (PTMs) take place. This transport requires translocating the target protein across the ER membrane through two general pathways: co-translational translocation (GTP dependent) and post-translational translocation (ATP dependent)3. An additional pathway for tail-anchored (TA) proteins into the ER membrane has also been discussed in the literature and included in our iCHO1921s reconstruction4,5. Once inside the ER lumen, the target proteins are folded by the action of several transmembrane ER proteins, including calnexin, calreticulin, and other luminal chaperones6–8. In the event of protein misfolding, a target protein may go through a “quality control” system (exclusive in the mammalian secretory pathway) that attempts to correct for folding errors9,10. However, if the misfolded state of the protein is sustained for too long, the protein then enters the ER associated degradation pathway, or ERAD, which involves retrotranslocation of the misfolded protein back to the cytosol, ubiquitination and proteasomal degradation11–13.

Besides folding, a target protein may acquire additional PTMs while inside the ER such as attachment of a glycosylphosphatidylinositol (GPI) anchor14,15, formation of disulfide bonds16, and N-linked glycosylation17–20. After these PTMs are successfully completed , the target proteins are transported to the Golgi apparatus via COPII-coated vesicles that bud from the ER21,22 whereas misfolded proteins are retro-translocated to the cytoplasm23,24 for proteasomal degradation via the ER-associated degradation pathway (ERAD)25,26. In the Golgi apparatus, N-glycans are processed into branched and complex glycoforms and proteins are further glycosylated with O-linked glycans27–29 and then sorted to their final destination (e.g. lysosome, extracellular medium) via clathrin-coated secretory vesicles30–33.

**A note on translocation pathways**

In co-translational translocation, proteins destined to the secretory pathway bear a hydrophobic signal sequence at the amino-terminus that promotes the targeting of ribosome-nascent chain (RNC) complexes to the ER via binding to the signal recognition particle (SRP). The SRP recognizes the signal peptide as soon as it emerges from the ribosome during translation. Then, the newly formed SRP-RNC complex is recognized by the SRP receptor on the ER membrane where translocation is initiated by interaction with the Sec61 complex (Sec61C) and assisted by the chaperone BiP to increase the efficiency and ensure the unidirectionality of this process30.

Post-translational translocation, in contrast to co-translational translocation, occurs independently of SRP and its receptor34. Furthermore, this process does not rely too heavily on the Sec61C to translocate the target protein and instead utilizes the protein Sec62 as a safe route that guarantees the efficient translocation of small proteins (<160 amino acids in length)35.

Finally, the pathway for inserting TA proteins into the ER membrane also occurs post-translationally due to the fact that the ER targeting signal of TA proteins is located very close to the carboxy-terminus, which allows the ribosome to release the protein before it is recognized and localized to the ER36. This pathway depends on ATP and one of the main players in the process is a transmembrane recognition complex known as TRC40 or Asna137.­­­

**Important differences between the yeast and animal secretory pathways**

As mentioned above, core functions of the secretory pathway are conserved between mammalian and yeast cells. These core functions (see Table SF4.2) are:

* Translocation through endoplasmic reticulum
* Primary glycosylation in ER (N-linked glycans) and Golgi (N-linked and O-linked glycans)
* Protein folding and quality control in ER
* Anterograde and retrograde vesicular transport between ER and Golgi via COPII and COPI vesicles, respectively.
* Dolichol pathway for N-linked core glycan translocation through the ER membrane
* Endoplasmic reticulum associated degradation (ERAD)
* GPI biosynthesis
* Unfolded protein response (UPR)

Nevertheless, minor and major differences exist between the yeast and mammalian secretory pathways. Some of these differences have been thoroughly reviewed before in an excellent review by Delic and colleagues2 and are summarized in Table SF4.1 below. Here, we highlight the major differences between both secretory pathways that are relevant for modeling purposes using the secretory reconstructions.

**Table SF4.1 – Summary of differences between mammalian and yeast secretory pathways as described by Delic et al.**2

|  |  |  |  |
| --- | --- | --- | --- |
| **Description of difference** | **Mammalian secretory pathway** | **Yeast secretory pathway** | **Importance for modeling purposes** |
| Chaperones involved in translocation | The main chaperone is BiP | The main chaperone is Kar2 | Minor |
| Presence of heat-shock proteins (HSPs) in ER | Mainly presence of proteins in the Hsp90 family | Not found in yeast | Minor |
| Enzymes for detoxification of reactive oxygen species in ER | Contains several enzymes such as Ero1 and glutathione peroxidases | Not found in yeast | Major |
| Oxidation state of Protein disulfide isomerase (PDI) | PDI is mainly reduced | PDI is mainly oxidized | Minor |
| Components of calnexin-calreticulin cycle | Includes an enzyme coded by the UGGT gene to transfer glucose residues to core N-linked glycans in misfolded proteins | Lacks UGGT and instead directs misfolded proteins to ER exit | Major |
| ERAD pathway branches for degrading misfolded proteins | Capable of directing misfolded proteins towards the ERAD pathway by trimming N-linked glycan residues in the A, B and C branches | Capable of directing misfolded proteins towards the ERAD pathway by trimming N-linked glycan residues only in B and C branches | Major |
| Components of COPII vesicles | Contains four isoforms of Sec24 | Expresses Sec24 with three cargo binding sites as well as Sec24 homologs Sfb2-3 | Minor |

Finally, the table below summarizes the differences between the mammalian and the fungal secretory pathway reconstructions in terms of components, reactions, and subsystems.

**Table SF4.2 – Overview of main differences between the mammalian and yeast secretory pathway reconstructions**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Secretory pathway reconstruction** | **Number of components** | **Number of reactions** | **Number of Subsystems** | **Core subsystems (in both mammalian and yeast secretory pathways)** | **Unique subsystems** |
| Mammalian | 271 | 144 | 12 | A total of 9 core subsystems: COPI, COPII, Dolichol pathway, ER glycosylation, ERAD, Golgi processing, GPI biosynthesis, Protein folding, and Translocation | Clathrin vesicles, GPI transfer |
| Yeast | 165 | 137 | 16 | ALP pathway, CPY pathway |

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