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Review

Studies on oligosaccharyl transferase in yeast★

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In yeast, OT consists of nine different subunits, all of which contain one or more predicted transmembrane segments. In yeast, five of these proteins are encoded by essential genes, Swp1p, Wbp1p, Ost2p, Ost1p and Stt3p. Four others are not essential Ost3p, Ost4p, Ost5p, Ost6p. All yeast OT subunits have been cloned and sequenced (Kelleher *et al.*, 1992; 2003; Kelleher & Gilmore, 1997; Kumar *et al.*, 1994; 1995; Breuer & Bause, 1995) and the structure of one of them, Ost4p, has been solved by NMR (Zubkov *et al.*, 2004). Very recently, the preliminary crystal structure of the lumenal domain of an archaeal Stt3p homolog has been reported (Mayumi *et al.*, 2007). Homologs of all OT subunits have been identified in higher eukaryotic organisms (Kelleher *et al.*, 1992; 2003; Kumar *et al.*, 1994; Kelleher & Gilmore, 1997).

Keywords: oligosaccharyl transferase, protein glycosylation, yeast

FUNCTIONS OF THE OT SUBUNITS

It is unclear why the catalysis of the N-glycosylation reaction requires 9 different subunits. However, studies in the last two decades have provided clues to the possible functions of the OT subunits in the N-glycosylation reaction.

Stt3p: Three lines of evidence clearly indicate that Stt3p may bear the active site of the OT reaction:

(1) Extensive studies were carried out in our laboratory using photoreactive peptide acceptor probes to identify the OT subunit(s) that recognize(s) the -Asn-X-Ser/Thr- sequence. We prepared labeled peptides containing a photoreactive benzoyl phenylalanine (Bpa). Yeast microsomes were incubated with labeled peptide, and immunological procedures were then used to determine if a subunit of OT had been labeled. Although studies indicated that Ost1p was labeled, extensive mutagenesis studies on Ost1p and Stt3p suggested that the lumenal domain of Stt3p bears the peptide recognition site and/or the catalytic site for oligosaccharide transfer (Yan *et al*, 1999; Yan & Lennarz, 2002a; 2002b).

(2) A homolog of *STT3* (pglB) has been identified in a bacterium and has been shown to be essential for glycosylation observed in this species (Wacker *et al.*, 2002). None of the other OT subunits are present in this bacterium.

(3) In the mammalian system, the STT3 protein was found to be the only OT subunit that could be cross-linked to the glycosylation consensus sequence of the translocating nascent chain (Nilsson *et al*., 2003).

Wbp1p: There are several lines of evidence pointing toward Wbp1p as the subunit that recognizes dolichol-PP-oligosaccharide. It was shown in mammalian system that a chemically reactive hexapeptide could be covalently linked to both a [^{14}C]oligosaccharide and either ribophorin I (mammalian homolog of Ost1p) or OST48 (mammalian homolog of Wbp1p) (Bause *et al.*, 1997). Chemical modification of cysteine residues also suggested that Wbp1p may be involved in the recognition of the dolichol-linked oligosaccharide (Pathak *et al*., 1995). Wbp1p possesses a GIFT domain (Beatson & Ponting, 2004), which when present in other proteins is

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Abbreviation: OT, oligosaccharyl transferase.

known to bind to oligosaccharides. We have shown that the sequence of the lumen-oriented half of the transmembrane domain is important for the function of the Wbp1 protein (Li *et al*., 2003). Recently, it has been reported that the lumenal domain of Wbp1p may possess the divalent metal ion binding site (Li *et al*., 2003).

Ost1p: Ost1p was found to cross-link with all other OT subunits and Alg1p, an enzyme in Dol-P-P-oligosaccharide biosynthesis (Dempski & Imperiali, 2004). Interestingly, in very early studies the mammalian homolog of Ost1p (ribophorin I) was found to crosslink to the ribosomes. Antibodies to epitopes located within the cytoplasmic domain of ribophorin I, but not antibodies to epitopes in the lumenal domain of this protein, were effective in inhibiting translocation (Yan *et al*., 2003). In another study, ribophorin I was found to cross-link to a subset of membrane proteins, irrespective of their glycosylation status, after their integration into the Sec61 translocon (Wilson *et al*., 2005). In addition, because Ost1p was able to cross-link to the glycosylatable substrate-based peptide photoaffinity probe, it was proposed that Ost1p bears the peptide-binding site of the OT complex (Yan *et al*., 1999). However, extensive mutagenesis studies later disproved this proposal, and showed that the functional domain of Ost1p is its membrane-anchored lumenal domain (Yan & Lennarz, 2002b). All these observations indicate that Ost1p possesses an extraordinary ability to be cross-linked, presumably by virtue of its function in the N-glycosylation process. Therefore it may be that the lumenal domain of Ost1p is involved in funneling the newly synthesized polypeptides into the active site on Stt3p for the glycosylation OT reaction.

Swp1p: Although a product of an essential gene, the function of this gene product remains unclear. It has been found to interact and cross-link to Wbp1p (te Heesen *et al*., 1993), Sss1p (Chavan *et al*., 2005) and Stt3p (Dempski & Imperiali, 2004). Swp1p probably possesses three transmembrane segments, with its N-terminus in the lumen and C-terminus in the cytosol (Yan *et al*., 2003). Since the lumenal domain of Swp1p was found to interact with that of Ost1p (Li *et al*., 2003), it may aid Ost1p in funneling nascent polypeptide chains into the active site of the OT complex.

Ost2p: It has been predicted that the Ost2 protein possesses three transmembrane segments, with its N-terminus in the cytosol and C-terminus directed towards the lumen. Our studies using the split-ubiquitin two hybrid system have demonstrated that the N-terminus is in the cytosol (Yan *et al*., 2005). The amino-acid sequence of Ost2p is highly conserved, especially in the region of the transmembrane regions, thus suggesting that its functional properties may reside in these segments. Since Ost2p interacts strongly with Wbp1p (Dempski & Imperiali, 2004; Yan *et al*., 2005), which is implicated in the recognition of the Dol-P-P-oligosaccharide, Ost2p may aid Wbp1p in recognition of the Dol-P-P-oligosaccharide.

Ost3p and Ost6p: Ost3p and Ost6p are believed to perform redundant function(s) in the OT reaction because the two proteins exhibit sequence similarity and strikingly similar hydropathy plots (Chavan *et al*., 2006). Although disruption of either of the genes coding for the two proteins results only in a minor defect in glycosylation, an *ost3Δost6Δ* double mutant yields a severe underglycosylation phenotype, affecting both membrane and soluble glycoproteins (Chavan *et al*., 2006). The lumenal domains of both Ost3p/Ost6p are predicted to have a thioredoxin-like fold (Knauer & Lehle, 1999). Our recent studies indicate that two OT isoforms exist in the ER membrane, both containing all remaining subunits plus Ost3p or Ost6p (Yan & Lennarz, 2005). When we investigated specific interactions of Ost3p or Ost6p with Sbh1p (the β-subunit of the Sec61 translocon) as well as with Sbh2p (the β-subunit of the Ssh1 translocon) we found a specific interaction between Ost3p (but not Ost6p) and Sbh1p. In the case of Ost6p (but not Ost3p) an interaction with Sbh2p was detected (Yan & Lennarz, 2005). All other OT subunits displayed an identical interaction pattern with Sbh1p and Sbh2p. This suggests that two isoforms of the OT complex generated by virtue of Ost3p and Ost6p specifically associate with the two structurally similar translocon complexes.

Ost4p: Ost4p is a minimembrane protein containing only 36 amino acids (Chi *et al*., 1996). Although it is product of a non-essential gene, an *ost4* null strain exhibits a temperature-sensitive phenotype and a severe underglycosylation defect (Chi *et al*., 1996). Our studies indicate that Ost4p functions to bind Ost3p and Stt3p together in a subcomplex of OT *via* interactions with its amino-acid residues near the cytosolic leaflet of the ER membrane (Kim *et al*., 2000; 2003). Ost4p is also proposed to be responsible for recruiting Ost3p or Ost6p into the OT complex (Spirig *et al*., 2005).

Ost5p: Ost5p is a membrane protein of 9.5 kDa present in highly purified OT preparations (Reiss *et al*., 1997). Ost5p is not essential for growth but its depletion results in a minor defect in OT activity (Reiss *et al*., 1997). An earlier study suggested that Ost5p directly interacts with other OT components, most likely with Ost1p and/or Stt3p (Reiss *et al*., 1997). At the present time no function can be proposed for this protein in the OT enzymatic reaction.

The maximal rate of N-glycosylation requires the presence of all the subunits. Those subunits encoded by essential genes (Ost1p, Ost2p, Wbp1p,

Swp1p and Stt3p) are proposed to carry out critical functions and directly participate in the N-glycosylation reaction. Subunits encoded by the non-essential genes (Ost3p, Ost6p, Ost4p and Ost5p) may perform important accessory functions.

Different isoforms of the OT complex

As noted above, two OT isoforms exist in yeast as well as in the mammalian ER membrane. These studies suggest that multiple OT isoforms with different translocon and ribosome affinities, as well as heterogeneous subunit composition, may provide a means of regulating the rate of protein N-glycosylation. However it is important to emphasize that with the exception of Stt3p, the function of none of the other OT subunits in the N-glycosylation reaction is well understood. As noted earlier, we know the structure of the Ost4p subunit and the crystal structure of a portion of the lumenal domain of the Stt3p subunit is anticipated (Mayumi *et al*., 2007). In addition, we have carried out a series of experiments designed to elucidate interactions of the various subunits with each other.

Organization of the OT complex

The interactions of the subunits of the OT complex have been studied using two techniques:

A) Studies on OT complex organization using a bifunctional, hydrophobic crosslinker.

In one approach, we utilized a membrane-permeable thio-cleavable crosslinking reagent, dithiobis (succinimidyl-propionate) (DSP), and a series of 20 yeast strains in which different OT subunits were HA- or Myc-tagged. The crosslinker spans 12 Å and it should be possible to detect proximities between subunits that are 12 Å or less apart. Based on the observations made during this study, an interaction map was drawn. The map indicates that the OT subunits can interact with each other through their transmembrane domains, rather than their lumenal or cytosolic domains, since crosslinking was performed using DSP, a hydrophobic crosslinker (Dempski & Imperiali, 2004).

B) In another approach *in vivo* interactions of OT subunits were determined using the split-ubiquitin system.

We carried out pair-wise analyses of *in vivo* interactions among all OT subunits using the split-ubiquitin system (Yan *et al*., 2005). It was demonstrated that OT subunits display specific interactions with each other *in vivo* that are quite similar to the interactions detected by chemical cross-linking. However, certain interactions that were not evident using the chemical cross-linking technique were observed using the split-ubiquitin method. One likely explanation for the observed differences is that the techniques utilized are inadequate in providing accurate conformational details of the arrangement of subunits in a protein complex. Another possibility is that the observed differences reflect a distinct conformational rearrangement that takes place when the enzyme complex changes from a non-functional state to the metabolically active functional state (Dempski & Imperiali, 2004; Yan *et al*., 2005). This conformational change is supported by previous kinetic data on yeast OT, which showed substantial deviations from a typical Michaelis–Menten equation, proposed to be likely due to allosteric regulation at the binding site of the donor substrate (Karaoglu *et al*., 2001). Based on our findings using these two techniques, we conclude that the association of these two complexes is stabilized *via* multiple protein–protein contacts.

Effort to carry out structural analysis of OT:

In collaboration with Dr. Huilin Li (Brookhaven National Laboratory and Stony Brook University) we are attempting to structurally characterize OT by electron microscopic (EM) techniques. In addition in collaboration with Dr. Hermann Schindelin (University of Würzburg, Germany) we will continue to carry out an X-ray crystallographic analysis of the OT complex. Although each technique possesses advantages and limitations, information from one technique can complement that from the other.

As a structural tool, EM encompasses two different but related approaches, i.e. electron crystallography (EC) and single particle cryo-electron microscopy. We have employed the single particle method of determining the OT structure at 12 Å. EC depends on obtaining two-dimensional crystals of membrane proteins in a lipid bilayer. Lipid confers a large stabilization effect and increases the chances that the protein will be crystallized in its native state. In this regard, our planned EC effort on OT will complement that of the X-ray crystallography. In addition, atomic detail can be achieved by EC in favorable cases, for examples, the bacteriorhodopsin (Henderson *et al*., 1990), the light harvesting complex II (Kuhlbrandt *et al*., 1994) and aquaporin (Murata *et al*., 2000; Gonen *et al*., 2004). Typically, however, the attainable resolution is between 6–10 Å (1 Å = 0.1 nm), which is only sufficient to resolve secondary-structural elements such as TM α-helices.

In contrast to the EM techniques, X-ray crystallography has met with success, albeit limited, for membrane proteins. However recent advances has improved the success rate. X-Ray crystallography relies on obtaining high quality crystals in detergent. We have a successful and active collaboration with

the Schindelin lab, which recently resulted in the crystal structure analysis of the ER lumenal resident yeast enzyme, protein disulfide isomerase (Murata *et al*., 2000) and mouse peptide *N*-glycanase (unpublished). Now we are trying to obtain crystals of OT.

In summary, limited knowledge about the function of the many subunits of OT is available. Our current efforts are focusing on determining the 2D and 3D structures of oligosaccharyl transferase. In the long term, we hope to visualize its structure in the lipid bilayer of the endoplasmic reticulum.

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