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Progress Report:

Abstract:

An essential aspect of the maturation of over 200 yeast proteins is the post-translational modification of N-linked glycosylation within the lumen of the endoplasmic reticulum. The transfer of the large glycan tree from a dolichol moiety to the nascent protein is catalyzed by a multisubunit membrane-associated enzyme, oligosaccharyltransferase (OST). Despite intense research into this complex little is known about the function of the auxiliary subunits. Ground breaking work in yeast and mammals has shown that auxiliary components as well as specific sub-complexes can confer substrate specificity. In this work we set out to use a high throughput, unbiased and systematic method to uncover the clients of each subunit to provide the first-ever comprehensive map of the contribution of every OST subunit to glycosylation events and their outcomes in yeast. To do this we started off by tagging every OST substrate with a green fluorescent protein (GFP) so that we can track its fate in the cell in a quantitative manner. The results of this effort are a new N' GFP library that should be useful for a wide variety of other cell biological applications (Yofe et al, Submitted 2015). Our next step is to now use this library to look at all glycosylated proteins on the background of mutants in each OST subunit.