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Grant Title: Role of Cytoplasmic Glycans in Protozoan Oxygen Sensing

Abstract

Objectives: All aerobic cells continuously monitor environmental O_2 and integrate this information with other cues to regulate their metabolism, growth and development. We have identified a novel paradigm in unicellular protozoa, in which O_2 levels regulate the proteome via activation of the SCF class of E3 polyubiquitin ligases that selectively target proteins for degradation in the 26S-proteasome. Key to the mechanism is an O_2-dependent prolyl 4-hydroxylase, described in the social amoeba *Dictyostelium*, whose modification of the SCF subunit Skp1 enables its glycosylation resulting in assembly of the novel pentasaccharide illustrated in the figure below. Biochemical studies in *Dictyostelium* show that full glycosylation maximally promotes Skp1's association with another SCF subunit - substrate receptor F-box proteins (FBPs), which is a prerequisite for E3 activity. Genetic analysis and proteasome inhibition studies are consistent with a model in which the posttranslational modification pathway acts solely through Skp1 and involves proteasomal degradation to control landmark developmental transitions. Genomic searches suggest the occurrence of related versions of this posttranslational modification pathway in many other protozoa.
Methods: Using a combination of high sensitivity/resolution mass spectrometry, structural genomics, gene editing and enzymology, we investigated the existence and characteristics of the predicted Skp1 glycosylation pathway in the agent for human toxoplasmosis, *Toxoplasma gondii*.
Results: We found homology between the two species for the first three sugars and the mechanism of their attachment, but a striking divergence for the final two sugars (see figure). Genetic analysis showed that all 5 sugars are important for proliferation in fibroblast monolayers at normoxia. The distinct sequence and mechanism of formation of the non-reducing terminal disaccharide enables a new strategy to remodel the Skp1 glycan in either organism, which will help define the mechanism by which the glycan promotes Skp1 conformational organization, inhibits its dimerization, contributes to E3(SCF)ubiquitin ligase assembly, and promotes cell growth and development. The evolutionary confinement of the Skp1 modification pathway to protozoans implies relevance to the single cell mode of existence, and might be exploitable for future development of anti-parasite drugs.