Phosphorylation of Sec2 by Cdc28-Hgc1 is Required for Transport of Secretory Vesicles to the Spitzenkörper during the Hyphal Growth of *Candida albicans*

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Candida albicans hyphae grow in a strongly polarised fashion from the tip driven by a Spitzenkörper, a subapical body rich in secretory vesicles. It is thought that the Spitzenkörper acts as a supply centre ensuring that more vesicles fuse the plasmamembrane at the hyphal apex than fuse subapical regions. At sites polarised growth in *S. cerevisiae* a multiprotein complex called the exocyst mediates the docking secretory vesicles with the cell surface, while a second complex called the polarisome nucleates the formation of actin cables along which secretory vesicles are transported by a class V myosin, Myo2, partnered by its regulatory light chain Mlc1. Cytological studies using protein fusions to GFP show that Mlc1, Sec4 and Sec2 localise to a distinct intracellular apical spot resembling a Spitzenkörper, while exocyst and polarisome components localise to surface crescent. Thus during hyphal growth the Spitzenkörper is clearly a separate structure from the polarisome and exocyst. Transport of post-Golgi secretory vesicles to sites of polarised growth and their fusion with the cell surface requires the action of the Rab-family GTPase Sec4. Activation of Sec4 to its active, GTP-bound form is mediated by its guanine exchange factor Sec2.

We reasoned that the accumulation of secretory vesicles in the Spitzenkörper during the switch to hyphal growth may be promoted by Sec2. In order to test this hypothesis we have dissected the regulation of the Sec2. Deletion mapping identified an 8-amino acid window between residues 583-591 that was required for hyphal growth, the localization of SVs to the Spitzenkörper and the hyphal-specific pattern of phosphorylation. Truncation alleles $Sec2_{1\text{-}550}$-YFP and $Sec2_{1\text{-}583}$-YFP were still capable of supporting growth of yeast cells, but not hyphal development. Truncations which extended beyond 550, which deleted the region corresponding to the 58 amino acid localization domain in *Sc*Sec2 were not capable of supporting viability. There are two serines 584 and 588 that are potential phospho-acceptor sites within the 8 amino acid region which contains the boundary of the region critically required for hyphal growth. A non-phosphorylatable alanine substitution for serine at 588 (S588A) formed hyphae normally, but the *sec2* S584A mutant was apparently non-functional because we were unable to construct a strain in which this allele provided the sole source of Sec2. However, phosphomimetic substitution of glutamate for serine at 584, S584E, resulted in a functional protein that still supported hyphal growth, but no longer displayed the hyphal specific pattern of phosphorylation in band shift

assays. Thus S584 is likely the key phospho-acceptor site and phosphorylation of this residue is required for hyphal development. We obtained convincing evidence that Cdc28 partnered by the cyclins Hgc1 and/or Ccn1 was responsible for the phosphorylation of S584. We constructed a Cdc28-1as allele that allows Cdc28 activity to be conditionally repressed by the addition of 1NM-PP1 . When Cdc28 activity was so inhibited, hyphal development was abnormal, Sec2 was mislocalized and failed to show the hyphal-specific pattern of phosphorylation. It may be argued that the failure of Sec2 localization and phosphorylation was an indirect result of the failure of hyphae to develop normally. However, it should be noted that Sec2 showed the hyphal-specific pattern of phosphorylation when cells were grown hyphal-inducing conditions in the absence of Cbk1 or Tpk1/2 activity, which in both cases results in the complete failure of hyphal development. To demonstrate that the cyclin dependent kinase, Cdc28 directly targets Sec2 we used co-immunoprecipitation and showed that Sec2 and Cdc28 physically associate with each other. To demonstrate that Cdc28 targets S584 we showed that the phosphomimetic S584E allele rescues the phenotype resulting from Cdc28 inhibition. Finally we showed that the hyphal-specific cyclin Hgc1 physically interacts with Sec2 and is required for the hyphal specific pattern of phosphorylation.

These data show that the accumulation of secretory vesicles in the Spitzenkörper requires phosphorylation of Sec2 S494 by the cyclin-dependent kinase Cdc28 partnered by its Hgc1 cyclin that is only expressed during hyphal growth.