

Role of Regulation of the RGS protein Sst2p on Polarization and Mating in Yeast

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ABSTRACT

RGS proteins stimulate the deactivation of heterotrimeric G-proteins. Why is this class of proteins necessary when instead a $G\alpha$ subunit possessing a faster intrinsic deactivation (GTPase) rate could have evolved? One hypothesis is that the regulation of the RGS proteins plays a critical role in fine-tuning the dynamics of G-protein signaling. The yeast RGS protein Sst2p is regulated at both the transcriptional (i.e. pheromone-induced expression) and post-transcriptional (i.e. phosphorylation, protein binding interactions, degradation, etc.) levels. We investigated the role of this regulation by replacing the *SST2* gene (encoding 698 amino acids) with the human *RGS4* gene (*hRGS4*, encoding 231 amino acids), which consists of the catalytic domain and an N-terminal membrane attachment peptide, and by replacing the native promoter (P_{SST2}) with the tetracycline-repressible promoter (P_{TET}), which is not pheromone-inducible. We then measured the effect of the substitution on pheromone sensitivity, mating, polarization, and gradient-sensing. Many of the differences were modest (e.g. reduced mating efficiency), but significant. The exception was polarization in which there was a dramatic change; RGS4-substituted strains did not form multiple mating projections at high levels of alpha-factor, but instead formed a single malformed “amoeboid-like” mating project (shmoo), which frequently gave rise to a bud (“shmoo-bud” phenotype). We demonstrated that this phenotype was the result of the fact that unlike Sst2p, RGS4 did not localize to the shmoo. Reporters of pheromone response indicated that the RGS4 strain relative to the wild-type strain displayed a reduced mating response at earlier time points ($t < 10$ min), but at later time points ($t = 3$ hr) showed an enhanced response arising from the mislocalization of the RGS functionality away from the shmoo. We used mathematical modeling to interpret the data and examine quantitatively how regulation of Sst2p may contribute to the robustness of the spatial dynamics of G-protein signaling necessary for proper polarization and mating and concluded that localization of Sst2p to the shmoo mediated by an interaction with alpha-factor receptor Ste2p prevents excess G-protein activation during the pheromone response.