A new family of regulators of G-protein-coupled receptors?

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Organisms as diverse as fungi and humans use G-protein-coupled receptors to control signal transduction pathways responsive to various hormones, neuroregulatory molecules and other sensory stimuli [1]. Continual stimulation of these receptors often leads to their desensitization, which is mediated in part by the consecutive actions of two families of proteins — the G-protein-coupled receptor kinases, which phosphorylate the agonist-occupied receptors [2], and the arrestin proteins, which subsequently bind to the receptors [3]. We now present evidence that a group of proteins — the G0S8/Sst2p family — may be a third class of receptor-desensitizing factors.

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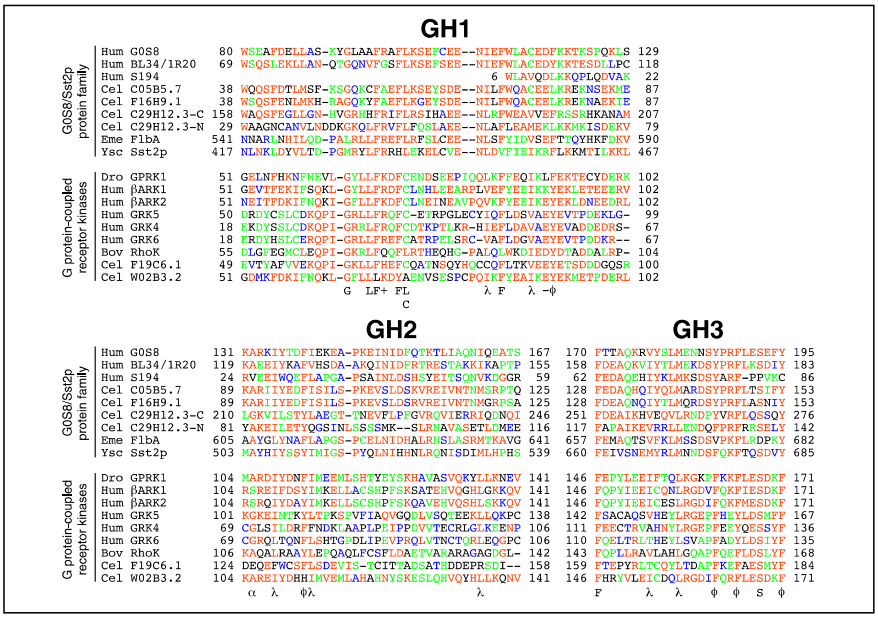
Results and discussion

We have previously reported the cloning of genes thought to regulate the switch from G0 to G1 phase of the cell cycle, including *G0S8*, a gene of unknown function expressed in acute leukemias of both myeloid and lymphoid lineages [4]. We have searched sequence databases using the amino-acid sequence of G0S8 and identified a group of proteins with similarity in three discrete sequence motifs, GH1, GH2 and GH3 (Fig. 1). The GH domain (for G0S8-homology) was also identified in sequences near the amino-terminus of G-protein-coupled receptor kinases, specifically in the region thought to contain recognition site(s) for activated receptor complexes [2]. We did not observe similarity among the G0S8/Sst2p family to other domains of the receptor kinases.

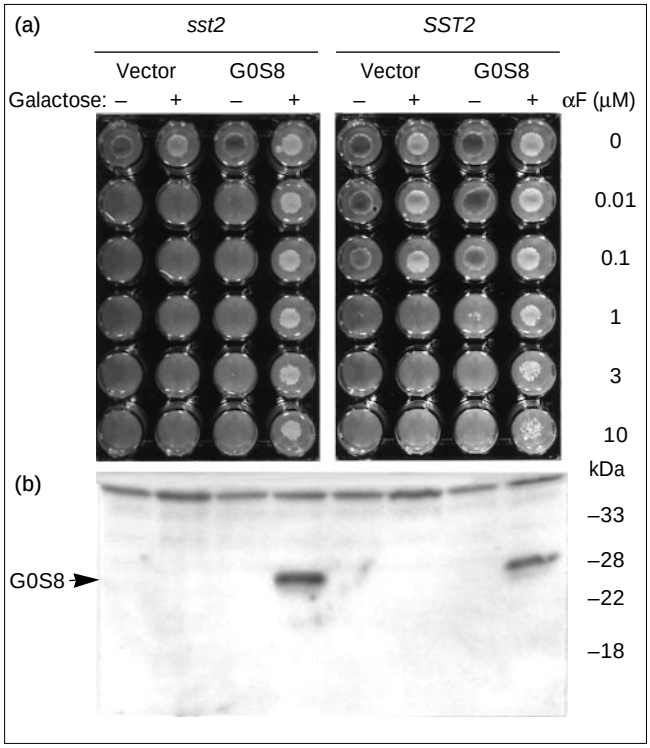
We have identified proteins containing the GH domain in a wide range of eukaryotic organisms, including humans, *C. elegans*, *Aspergillus* (*Emericella nidulans*) and *Saccharomyces cerevisiae*. The protein most closely related to G0S8 is human BL34/1R20, a B-cell-specific immediate-early gene product whose expression is markedly increased by various mitogenic stimuli [5]. Full-length or partial GH domains were also identified within a partial open-reading frame

Figure 1



Protein sequence alignment of G0S8/Sst2p family members and G-protein-coupled receptor kinases. Regions of homology were identified by a BLAST search of the NCBI non-redundant protein sequence database using the BLOSUM60 scoring matrix, aligned by the Wisconsin Package GAP and PILEUP programs, and exported into MALIGNED for output of block alignment (quantify mode) and consensus line (conserved mode). The human S194 sequence is derived from a partial cDNA clone (Genbank accession L40394), presumably missing the amino-terminal 33 amino acids of GH1. The hypothetical open-reading frame C29H12.3 of *C. elegans* contains two GH domains, denoted '–C' and '–N' for carboxy-terminal and amino-terminal, respectively. Percent similarity of sequence motifs to G0S8, as calculated by GAP alignments using the PAM-250 scoring matrix, ranged from 70 % (BL34) to 36 % (βARK1) for GH1, 59 % (BL34) to 27 % (C29H12.3-N) for GH2, and 77 % (BL34) to 38 % (GRK4) for GH3. Colour denotes the prevalence of each amino-acid in a column (orange, most prevalent; green, second most prevalent; purple, third most prevalent). Related amino-acids: A/G (denoted by α); F/Y (ϕ); R/K/H (+); D/E (–); I/L/V/M (λ); S/T; N/Q) were considered equivalent for both block alignment and consensus line. Abbreviations: GPRK/GRK, G-protein-coupled receptor kinase; βARK, β adrenergic receptor kinase; Hum, human; Cel, *C. elegans*; Ysc, *S. cerevisiae*; Eme, *E. nidulans*; Dro, *D. melanogaster*; Bov, bovine. SwissProt (sp), GenPept (gp), and NCBI sequence ID (gi) accession numbers (in order): sp:P41220, sp:Q07918 and Q08116, sp:P34295, gp:L40394, gp:U23169, gi:1066938 and 1065702, sp:P38093, sp:P11972, sp:P32865, sp:P25098, sp:P35626, sp:P34947, sp:P32298, gi:539594, sp:P28327, gi:642184, gi:722362.

Figure 2



Effect of *G0S8* expression on pheromone sensitivity in yeast. (a) Pheromone-supersensitive (*sst2*) and wild-type (*SST2*) yeast strains were grown on media containing indicated concentrations of α factor. Haploid yeast strains BC159 (*MAT*a *leu2-3,112 ura3-52 his3-Δ1 ade2-1*^{oc}) and BC180 (*BC159 sst2-Δ2*) were transformed with the *URA3*$^{+}$, *GAL10*/*CYC1*-promoter expression plasmid pEMBLyex4 (vector) or pEMBLyex4*G0S8* (G0S8), grown in liquid selective medium containing 2 % glucose and lacking uracil, and spotted (2000 cells per well) onto YEP-agar containing either 2 % glucose (–) or 2 % galactose (+) and indicated concentrations of synthetic α factor; plates were incubated for 4 days at 30 °C. The darker hue of colonies grown in 2 % glucose is the result of red pigment produced by adenine auxotrophy. (b) Immunodetection of galactose-induced *G0S8* expression. Yeast strains as above were grown to mid-log phase in liquid selective medium, lysed with glass beads in SDS–PAGE buffer containing 1 mg ml^{-1} Pefabloc SC and 4 mM benzamidine, and immunoblotted with crude rabbit antisera raised to a synthetic peptide overlapping the GH3 motif of G0S8 (amino acids 185–199). Specific detection of G0S8 protein (25 kD) was performed with anti-rabbit horseradish peroxidase secondary antibody and enhanced chemiluminescence; detection of the non-specific protein band of higher molecular weight serves as control for lane loading.

(ORF) encoded by the human fetal brain cDNA library clone S194, and within hypothetical ORFs from the genome of *C. elegans* (C05B5.7, F16H9.1, C29H12.3). The fungal GH proteins FlbA (*E. nidulans*) and Sst2p (*S. cerevisiae*) were previously reported to be 30 % identical, and both are involved in the control of asexual reproduction. FlbA is required for the initiation of conidiophore development in *Aspergillus* [6]. Sst2p expression is induced in yeast by pheromone signalling and acts to promote recovery from G1 arrest and reversion to asexual reproduction [7].

To determine the functional significance of the sequence homology shared by the G0S8/Sst2p family, *G0S8* was conditionally expressed under the control of the heterologous *GAL10* promoter (inducible by galactose) in haploid yeast responsive to α factor mating pheromone. As observed previously [7], haploid yeast lacking *SST2* were unable to recover from G1 arrest induced by 0.01 μM α factor, whereas wild-type yeast were at least 100-fold less sensitive to pheromone (Fig. 2; lethal arrest at 1 μM α factor). However, upon galactose induction of *G0S8* expression, both wild-type and pheromone-supersensitive yeast strains continued to grow in the presence of up to 10 μM α factor. The expression of a *G0S8* deletion mutant lacking most of the GH domain (deletion of amino-acids 105–193) did not decrease the sensitivity to pheromone (data not shown).

The ability of human *G0S8* to affect pheromone sensitivity in yeast underscores the functional relevance of the sequence homology shared by the G0S8/Sst2p family. The GH domain is not only present in G0S8 and Sst2p, but also in proteins which directly interact with activated receptor complexes, so the observed negative regulation of pheromone signalling by G0S8 and Sst2p may be mediated by their direct association with the activated pheromone receptor complex. Indeed, recent work characterizing the epistatic relationships between genes of the G-protein-mediated pheromone signalling pathway and gain-of-function *SST2* mutations points to Gpa1p (G protein α subunit) as the direct target of Sst2p action [8]. De Vries *et al.* [9] have recently provided evidence for such an interaction: they described the identification of human GAIP (Gα-interacting protein), which contains a GH domain and was shown to interact specifically with $G\alpha_{i3}$.

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