SUPPLEMENTARY MATERIAL

In addition to ROS-GC1, P1 neuronal layer expresses ROS-GC2.

A highly specific polyclonal antibody was raised against ROS-GC2. Western analyses of P1 and retinal membrane fractions were carried out with the ROS-GC2 antibody according to standard protocols and the results are presented in figure 1. The single immunoreactive band, visible in the “P1” as well as the “Retina” lanes, is indicated by an arrow. The mobility is in agreement with that expected for ROS-GC2. Unlike ROS-GC1, which is now known to be expressed in several neurons outside of photoreceptors, this is the first such demonstration for ROS-GC2.

Figure 1. Western analysis with membrane fractions of retina and P1 using an anti-ROS-GC2 antibody.

The above result adds ROS-GC2 to ROS-GC1 and S100β to the list of ROS-GC signaling components present in the P1 neuronal layer. The presence of GCAP1 in this layer has already been shown through immunohistochemical analyses (Frins et al., 1996; Howes et al., 1998; Kachi et al., 1999). The significance of multiple ROS-GC components being expressed in the synaptic layer remains to be investigated.
**ROS-GC1 interacts directly with S100β: analysis by cross-linking.**

ROS membranes (480 µg rhodopsin) were incubated with 12 µg of S100β and 1 mM CaCl₂. Cross-linking was performed with the noncleavable, water-soluble cross-linker BS₃ (Pierce) at a final concentration of 1 mM similar as described previously (Duda et al. 2000). Samples were analyzed by SDS-PAGE (7.5-17.5 % gradient gel) and subsequent western blotting. Blots were probed with antibodies against ROS-GC1 and S100β (Fig. 2). Both antibodies react with a band that corresponds to ROS-GC1 dimer : S100β complex (indicated by arrows). The appearance of multiple bands with S100β antibody after cross-linking is consistent with the fact S100β interacts with a wide variety of proteins and indicates that such interactions occur in the P1 neuronal layer.

![Figure 2. Cross-linking of ROS-GC1 and S100β](image)

**Figure 2. Cross-linking of ROS-GC1 and S100β.** The monomer of ROS-GC1 at 112 kDa is marked on the left blot. Both antibodies recognize a common band at 220-240 kDa (indicated by arrows) which corresponds to a ROS-GC1 dimer : S100β complex.
The 966R-972S region requires flanking regions to bind to S100\(\beta\).

Since the 966R-972S region is indispensable for binding of S100\(\beta\) activation of ROS-GC1 (Fig.8), it was important to determine if this region was sufficient for S100\(\beta\) binding. Two independent approaches were used to accomplish this goal. First, direct binding of 966R-972S peptide to S100\(\beta\) was assessed through SPR experiments. No binding signal was observed with the tested range of S100\(\beta\) concentrations (data not shown). Second, the ability of the 966R-972S peptide to compete with peptide #2 (aa962-981) for binding with S100\(\beta\) was tested. Peptide #2 was immobilized on a sensor chip and S100\(\beta\) was supplied in the mobile phase. Binding of S100\(\beta\) was recorded as an increase in resonance units (RU) (Fig. 3). SPR sensorgrams were then recorded in the presence of 1\(\mu\)M (A) or 10 \(\mu\)M (B) peptide #2 or peptide 966R-972S. The results clearly demonstrate that free peptide #2 can compete with immobilized peptide #2 for binding of S100\(\beta\), whereas peptide 966R-972S cannot (compare the inhibition at 1 \(\mu\)M and 10 \(\mu\)M by peptide #2 Vs. peptide 966R-972S). Thus, the 966R-972S region of ROS-GC1 requires the flanking regions present in peptide #2 to bind to S100\(\beta\).

![Figure 3. SPR analysis of S100\(\beta\) binding to peptide #2 and peptide 966R-972S. S100\(\beta\) (500 nM) was supplied in the mobile phase. Peptide #2 was immobilized. Free peptides were added at the indicated concentrations.](image-url)
In an independent approach, the ability of peptide $^{966}_{\text{R}}\text{R}^{972}_{\text{S}}$ to inhibit S100β-dependent stimulation of ROS-GC1 was investigated. The results presented in figure 4 show that there is a very marginal effect of the peptide at higher concentrations. Thus, for both binding and mediating activation of ROS-GC1, the consensus motif $^{966}_{\text{R}}\text{R}^{972}_{\text{S}}$ requires flanking regions present in the aa962-981 region.

**Figure 4.** Inhibition of ROS-GC1 activity by peptide #2 and $^{966}_{\text{R}}\text{R}^{972}_{\text{S}}$. Membranes containing ROS-GC1 (▼- native ROS membranes; ▲- COS cells expressing ROS-GC1) were incubated with indicated concentrations of the peptide $^{966}_{\text{R}}\text{R}^{972}_{\text{S}}$ in the presence of 4 µM S100β and 100 µM Ca$^{2+}$. Result with membranes of COS cells expressing ROS-GC1 exposed to peptide #2 (●) under identical conditions is presented as control. Experiments were done in triplicate and repeated at least two times for reproducibility. The data provided is from a representative experiment. Error bars are within the size of the symbols.
References

