Expression of Transducin in Retinal Rod Photoreceptor Outer Segments

A guanine nucleotide-binding (G) protein "translocation" mechanism has been proposed (1) to explain long-term adaptive physiology in the rod photoreceptor. Large, prolonged changes in immunoreactivity of the α subunit of transducin (αT) were detected within the rod outer segment (ROS) at both light onset and light offset (1). Concurrent and opposite changes in αT immunoreactivity within the rod inner segment suggested that the amount of transducin within the photoreceptor is altered daily by massive movement of transducin subunits from one cellular locale to another.

To independently check the putative transducin translocation event upon which the model is based, we quantified the amount of the α and β subunits (βT) of transducin within the ROS at several times during the day and night. We also checked the influence of protein denaturation on transducin antibody binding in light and dark. Tissue sections were prepared and stained as described in (1). Before antibody staining, sections were either fixed by immersion in 4% formaldehyde in saline for 20 min or in cold (-20°C) acetone for 10 min. The primary antibody was a previously characterized antiserum specific for αT (2), Gl-2, at 1:100 dilution and the secondary antibody was goat antiserum to rabbit immunoglobulin G labeled with fluorescein isothiocyanate.

We prepared ROS (3) from retinas of 45-day-old Long-Evans or Sprague-Dawley rats raised on a 12-hour light:12-hour dark cycle. At each of six times evenly spaced throughout the light:dark cycle, ROS were isolated from 4 to 12 retinas. The recovery of ROS from the retinas was greater than 50% as determined from absorbance at 500 nm. Purity and intactness of the ROS were estimated from the absorbance ratio 280/500 nm (4), the appearance of the ROS with light microscopy, and SDS-polyacrylamide gel profiles. The α and β subunits of transducin were identified in these preparations by three criteria. Transducin subunits are the second largest polypeptide components of outer segment membranes and show a characteristic mobility on SDS-polyacrylamide gels with a low ratio of N,N'-methylenebisacrylamide to acrylamide (5). In addition, transducin is characterized eluted from bleached ROS disk membranes at guanosine triphosphate (GTP) concentrations greater than 40 μM under low salt concentrations (5, 6). A previously characterized antiserum to transducin (7) was used to confirm the localization of α and β subunits on immunoblots of ROS polypeptides.

Peak heights from densitometric profiles of Coomassie blue-stained SDS-polyacrylamide gels (Ultroscan 2202 laser densitometer, LKB Instruments) were used to quantify αT and βT in each ROS preparation. The transducin peak heights were normalized to the height of the rhodopsin peak in each gel lane. The resulting ratio of transducin to rhodopsin allows comparison of the relative transducin concentrations from different ROS samples with variable amounts of ROS polypeptides (8).

A "soluble" pool of transducin was also recovered to check the possibility that transducin was preferentially lost into the ROS supernatant during preparation of dark-adapted membranes. Transducin subunits were recovered from both dark- and light-adapted ROS supernatants with DE52 column chromatography (3, 5, 6).

When prepared as above, the ROS were free from major contamination by non-ROS proteins. An absorbance ratio (280/500 nm) of 2.6 indicated ROS of relatively high purity and, although inner segments and cell debris were visible in light micrographs, these contaminants were present in low amounts. Further, SDS-polyacrylamide gel profiles of rat ROS showed all major polypeptides present in purified bovine, and frog ROS (9) (rhodopsin, transducin, phosphodiesterase, and 48K) with no additional contaminants.

Fig. 1. Coomassie blue-stained gel of purified bovine transducin (a) and dark-adapted ROS isolated at 9:30 p.m., 1:30 a.m., and 5:30 a.m. (b, c, and d) and light-adapted ROS isolated at 9:30 a.m., 1:30 p.m., and 5:30 p.m. (e, f, and g). The light-dark cycle is indicated schematically below the gel. Densitometry scans of lanes f (light-adapted ROS) and c (dark-adapted ROS) are shown at right (8).

Fig. 2. Localization of αT immunoreactivity in frozen sections of eyes from dark-adapted (A and C; 9:30 p.m.) and light-adapted (B and D, 5:30 p.m.) rats. Section were pretreated with cold acetone (A and B) or 4% formaldehyde in saline (C and D) and stained with a polyclonal antibody specific for αT (2) and FITC-labeled secondary antibody. Nomarski micrographs of sections in (A) and (C) are on the left. Scale bar, 22 μm. Abbreviations: OS, outer segments; IS, inner segments; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer.
Table 1. The amounts of αT, βT, and rhodopsin (Rh) measured by laser densitometer scans of Coomassie blue-stained gels. Values are expressed as the mean ± SEM (n = 6).

<table>
<thead>
<tr>
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<th>αT/Rh</th>
<th>βT/Rh</th>
<th>αT/βT + βT</th>
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<tbody>
<tr>
<td>Light</td>
<td>0.43 ± 0.04</td>
<td>0.50 ± 0.05</td>
<td>0.46 ± 0.02</td>
</tr>
<tr>
<td>Dark</td>
<td>0.45 ± 0.04</td>
<td>0.56 ± 0.06</td>
<td>0.44 ± 0.02</td>
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</table>

Response: Roof and Heth report a disagreement between subcellular fractionation and immunocytochemical data concerning the amount of the alpha subunit of transducin (αT) within rod outer segments (ROS). As we previously reported (1), they observe that, under certain fixation conditions, the αT immunoreactivity of ROS is less during the day than at night. On the other hand, when they isolated ROS and measure αT content by scanning SDS-polyacrylamide gels, αT levels appear constant. On the basis of this disagreement, the authors interpret the data as showing that the amounts of αT do not change and that light induces a masking of antigenic sites on αT. I believe this conclusion is unlikely to be correct. First, the observed changes in αT immunoreactivity are difficult to explain in terms of antigen masking. Second, the presented measurements of αT levels in isolated ROS are confounded by technical limitations. These limitations are reinforced by the recent publication of a report by Philip et al. (2) which demonstrates that the αT levels in isolated ROS change as predicted by immunocytochemical data.

Epitope masking is an unlikely explanation of the immunocytochemical data because at least three spatially separated epitopes would have to be involved. The antigen to αT used in our study recognize two epitopes, one in the NH2-terminal region of the protein and another within a central segment (3). Further, the immunocytochemical observations were confirmed with a second antibody that is directed to the COOH-terminus of αT. Even more difficult to explain with such a model is the simultaneous and reciprocal changes in immunoreactivity in the rod inner segments (RIS). That is, even if light were able to simultaneously block three antigenic sites on αT in the ROS, what is the mechanism of reciprocal changes in immunoreactivity in the RIS? In support of their epitope-masking hypothesis, Roof and Heth present a comparison of immunocytochemical data in which two fixation procedures were used. When tissue was fixed with acetone, a less marked change in immunoreactivity was observed than
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