sions by injection of 5,6- or 5,7-dihydroxytryptamine [which appear to selectively destroy serotonergic neurons (*15*)] also selectively inhibited high-affinity uptake of serotonin (*16*). Although such studies certainly suggest a more intense uptake or storage in nerve endings, they cannot rule out a contribution of high-affinity glial uptake associated mainly with subsequent metabolic degradation by monoamine oxidase, since in some studies monoamine oxidase inhibitors were not added and light microscopy does not clearly distinguish between neuronal and glial processes in the neuropil. It is also uncertain whether the lesion studies were completely specific for neurons. Thus destruction of serotonergic neurons may have indirect effects on astroglia surrounding such nerve endings. Also, prolonged exposure of cultures to 5,7-dihydroxytryptamine inhibits their high-affinity uptake of [^{3}H]serotonin (*17*).

In conclusion, our results show high-affinity uptake of [^{3}H]serotonin by immunocytochemically identified astrocytes in primary culture. If our results with normal astrocytes in culture apply to astrocytes in situ, then uptake by such cells is likely to be a significant route for the termination of action of serotonin and thus a possible site of action for drugs such as the antidepressants. Uptake into astroglia presumably occurs in addition to reuptake by nerve endings. The relative contributions of neurons and astroglia to uptake could be assessed if selective inhibitors of astroglial and neuronal uptake were found, and screening for such compounds could conveniently be done in astroglial and neuronal cultures and highly purified synaptosomal preparations. It is also possible that astrocytic uptake plays a role in the etiology of psychiatric diseases involving serotonin (*18*).

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Retinal S Antigen Identified as the 48K Protein Regulating Light-Dependent Phosphodiesterase in Rods

Abstract. *Retinal S antigen chromatographically purified from whole retina, induces experimental autoimmune uveoretinitis in laboratory animals. The 48K protein, a soluble protein found in rod outer segments, is purified through its specific binding to photoexcited rhodopsin and is involved in the quenching of light-induced guanosine 3′,5′-monophosphate–phosphodiesterase activity. Biochemical, immunological, functional, and pathological tests showed that retinal S antigen and the 48K protein are identical.*

Certain inflammatory diseases of the retina and uvea are thought to involve autoimmune mechanisms, either as primary mechanisms or secondary to a local injury (*1*). Retinal S antigen, a soluble protein of about 50 kilodaltons (kD) found in abundance in the photoreceptor cells of the retina, has a role in the autoimmune response (*1, 2*). This protein has been isolated and purified from the retinas of different mammals (*2, 3*). Immunization of laboratory animals with a few micrograms of purified S antigen in adjuvants induces experimental autoimmune uveoretinitis, an ocular inflammatory disease (*2, 4, 5*).

We report that the S antigen is identical with the so-called 48K protein characterized in rod outer segments (ROS) by its light-dependent binding to the disk membrane (*6*). The 48K protein binds specifically to photoexcited and phosphorylated rhodopsin (R*-P) (*7*) and quenches the activity of the light-dependent guanosine 3′,5′-monophosphate (cyclic GMP)–phosphodiesterase (*8*). This suggests that the 48K protein has a regulatory role in the light-induced amplifying cascade that controls cyclic GMP hydrolysis in ROS—a major step in the phototransduction process (*9*). This protein is highly soluble in dark-adapted ROS and represents 2 to 7 percent of the total protein content of ROS (*10*).

We applied biochemical, functional, immunological, and pathological tests to compare S antigen, prepared from total retina by a standard procedure (*3*), with the 48K protein, purified from ROS by a procedure based on its binding to illuminated disk membranes (*11*).

Both proteins migrate in sodium dode-

cyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) as polypeptides of about 50 kD (lanes 1 and 2 in Fig. 1). In the native form, they are eluted from molecular sieve columns as proteins of about 50 kD (*2, 3, 6, 9*) and are therefore monomeric. Both proteins bind to illuminated disk membranes (lanes 4 to 7 in Fig. 1) and more specifically to R*-P (lanes 8 to 11 in Fig. 1).

The immunological comparison is based on the use of monoclonal and polyclonal antibodies specific for S antigen (*12*). These antibodies specifically recognize the 48K protein in various extracts of ROS (*13*) (Fig. 2a). The immunoreactivity of purified 48K protein, quantified by a liquid phase radioim-

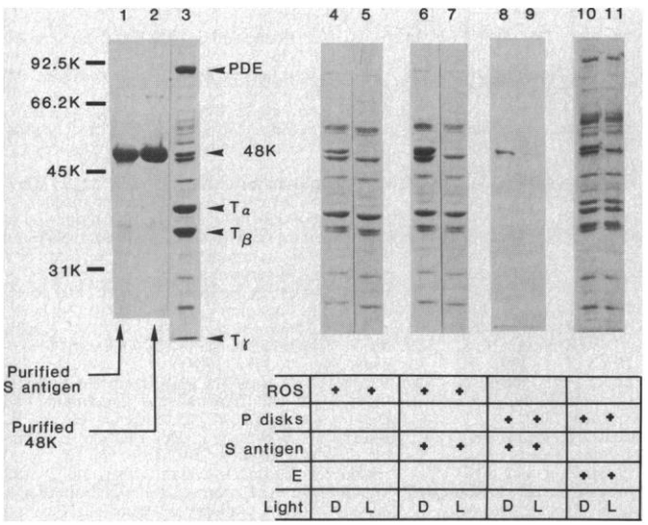
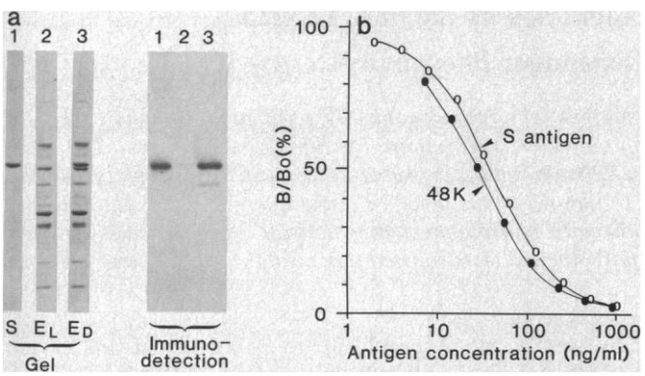


Fig. 1. The biochemical and functional identity of S antigen and the 48K protein of ROS, analyzed by SDS-PAGE (*17*). (Lanes 1 and 2) Overloaded gels of purified 48K protein (*11*) and S antigen (*3*), respectively. The major polypeptide (>95 percent pure) in both preparations has an apparent molecular mass of about 50 kD. The contaminants are essentially at about 70 kD (and 90 kD) for the 48K protein and at 38 kD (and 32 and 46 kD) for S antigen. Lane 3 shows an extract from dark-kept bovine ROS, containing the proteins that are soluble at low ionic strength [5 m*M* Hepes and 1 m*M* dithiothreitol (DTT) *p*H 7.0]. The major bands are the transducin subunits (T_α, T_β, and T_γ) and cyclic GMP-phosphodiesterase (PDE), which are directly involved in the enzymatic cascade activated by R*. At more physiological ionic strength (120 m*M* KCl, 20 m*M* Hepes, 1 m*M* $MgCl_2$, and 1 m*M* DTT), transducin and PDE are membrane associated and are therefore essentially absent from the supernatant (lanes 4 to 7, 10, and 11). The 48K protein migrates as the upper band of the doublet near 50 kD. (Lanes 4 to 11) Light-induced binding of both 48K protein and S antigen to disk membranes, shown by comparing pairs of supernatants obtained at physiological ionic strength from dark-kept (D) or illuminated (L) membrane suspensions (*6, 11*). Compositions of the suspensions before centrifugation are given below the gels. Lanes 4 to 7 contained ROS (rhodopsin, 6 mg/ml) supplemented with 2 m*M* adenosine triphosphate (ATP), and lanes 8 to 11 contained previously phosphorylated, regenerated, and washed disk membranes [P disks (*7*), rhodopsin 1 mg/ml] to which soluble proteins of ROS were added (extract E, containing the soluble proteins from ROS at physiological ionic strength). L-suspensions were illuminated for 3 minutes with strong orange light at 20°C for ROS (nearly 100 percent bleaching), and for 1 minute with weak orange light for P disks (about 10 percent bleaching). The 48K protein was present in D-supernatants (see lanes 4 and 10) but essentially absent from L-supernatants (lanes 5 and 11) since it sediments with the bleached disk membranes (*6, 11*). Purified S antigen, added either to ROS (lanes 6 and 7) or to P disks (lanes 8 and 9), migrates with the 48K protein (the 48K protein band is intensified in lane 6), and binds to illuminated disk membranes exactly as 48K protein does.



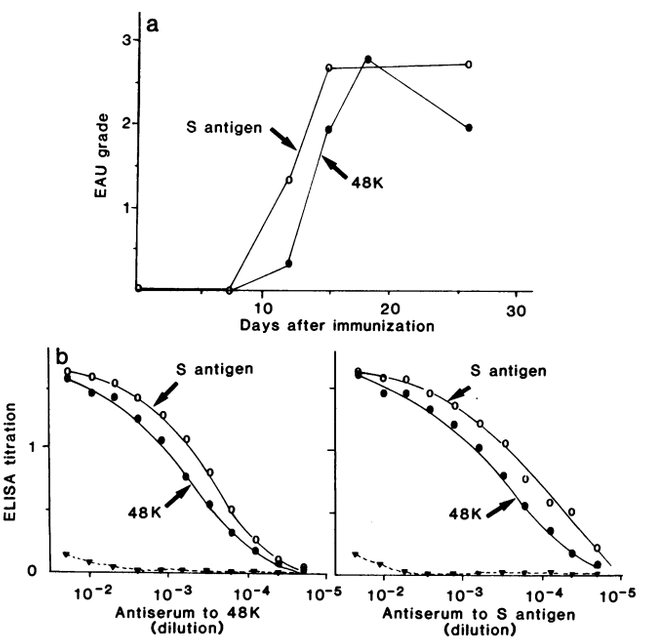


Fig. 2 (left). Immunological characterization of the 48K protein by antibodies to S antigen. (a) Recognition of the 48K protein transferred from an SDS gel to nitrocellulose sheet [Western blotting technique (*18*)]. The Coomassie blue-stained gel corresponds to the immunodetection blot. (Lane 1) Purified S antigen. (Lane 2) Soluble proteins extracted, at physiological ionic strength, from a ROS suspension after illumination in the presence of 2 m*M* ATP [light-extract (E_L) devoid of 48K protein; see lane 5 in Fig. 1]. (Lane 3) Soluble proteins extracted from ROS as in lane 2 but without illumination [dark-extract (E_D) containing 48K protein; see lane 4 in Fig. 1]. Four different monoclonal antibodies to S antigen were tested and gave identical results. No protein was recognized in E_L. The major protein recognized in E_D corresponds to the 48K protein, with intensity similar to that for S antigen (*19*). (b) Radioimmunoassay of 48K protein and S antigen. Different concentrations of either S antigen (○) or 48K protein (●) competed with a constant ^{125}I-labeled S antigen for a constant amount of rabbit antibody to bovine S antigen. The vertical scale (B/Bo) shows the percentage of ^{125}I bound to the antibody. Protein determination was carried out by the method of Bradford (*20*), with bovine gamma globulin used as a standard. Fig. 3 (right). (a) Ocular pathological response and (b) serum antibody levels in rats immunized with either 48K protein or S antigen. Five Lewis rats received a single immunization with 30 μg of the protein in both mycobacterial and *Bordetella pertussis* adjuvants (*5*). (a) The clinical course of experimental autoimmune uveitis (EAU) was followed by repeated examination of the eye with a slit lamp. The severity of the ocular inflammation was graded from 0 to 3 (*21*). Dots represent the mean value of the EAU grade for ten eyes. After immunization with either 48K or S antigen, all eyes had severe EAU from day 15 to day 18. (b) Enzyme-linked immunosorbent assay (ELISA) titration of a serum antibody to 48K protein or antibody to S antigen on plates coated with either 48K protein (●), S antigen (○), or bovine serum albumin (▼). Coating concentration, 0.5 μg/ml in carbonate buffer. The vertical scale shows absorbance at 490 nm.

munoassay (Fig. 2b), is shown by a titration curve practically identical with the standard curve for S antigen. Moreover, purified 48K protein induces the development of the characteristic pathology of experimental autoimmune uveitis with about the same efficiency as that observed with S antigen (Fig. 3a). The antibody levels in rats immunized with either protein are also similar (Fig. 3b).

These quantitative comparisons exclude the possibility that the pathological effects could be caused by a contaminant in one or both preparations. Because of the different purification procedures, there is little chance of a common contaminant, as confirmed in lanes 1 and 2 of Fig. 1. The 48K preparation contained as the major contaminant a few percent of a polypeptide of about 70 kD, most probably rhodopsin kinase [which copurifies with the 48K protein since it also binds reversibly to bleached disk membranes (*6*)]. In contrast, the S antigen preparation contained neither the 70-kD polypeptide, nor rhodopsin kinase activity (*14*). We therefore conclude that, in both preparations, the 50-kD polypeptide is responsible for the immunopathogenic properties of S antigen (Figs. 2 and 3). Zigler *et al.* (*15*) reported that extensively purified S antigen, containing only the 50-kD polypeptide, as shown by silver staining on gels, induces experimental autoimmune uveitis.

Immunofluorescent labeling of retinal sections has shown that S antigen is located exclusively in photoreceptor cells—not only in the outer segment but also in the inner segment and the synaptic terminal region (*2, 12*). This diffuse pattern may be related to intracytoplasmic diffusion of this highly soluble protein from its site of biosynthesis, the inner segment. Because the protein is absent from all other cells in the retina, in brain, and in other tissues (except the pinealocytes (*2*), which are phylogenetically related to photoreceptors (*16*), it is likely that its function is essentially restricted to phototransduction.

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17. The gel system of Laemmli [*Nature (London)* **227**, 680 (1970)] with 10 percent acrylamide was used. Gels were stained with Coomassie brilliant blue.
18. A 10 percent acrylamide-SDS Laemmli gel was run as in Fig. 1. The proteins were transferred to a nitrocellulose sheet by contact during 5 days at 4°C. Corresponding lanes on the same gel were subjected to Coomassie blue staining. The blot was saturated with bovine serum albumin, then incubated successively with the monoclonal antibody (overnight at 20°C) and finally with goat antibody to mouse immunoglobulin G coupled to peroxidase. The peroxidase activity was detected by aminobenzidine in the presence of Ni^{2+} and Co^{2+} salts [A. L. de Blas and H. M. Cherwincki, *Anal. Biochem.* **133**, 214 (1983)].
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Cassette of Eight Exons Shared by Genes for LDL Receptor and EGF Precursor

Abstract. *The amino acid sequences of the human low-density lipoprotein (LDL) receptor and the human precursor for epidermal growth factor (EGF) show 33 percent identity over a stretch of 400 residues. This region of homology is encoded by eight contiguous exons in each respective gene. Of the nine introns that separate these exons, five are located in identical positions in the two protein sequences. This finding suggests that the homologous region may have resulted from a duplication of an ancestral gene and that the two genes evolved further by recruitment of exons from other genes, which provided the specific functional domains of the LDL receptor and the EGF precursor.*

The low-density lipoprotein (LDL) receptor is a protein that carries a nutrient molecule, cholesterol, into cells by receptor-mediated endocytosis in clathrin-coated pits (*1*). Epidermal growth factor (EGF) is a 53-amino-acid peptide hormone that stimulates cells to divide (*2*); it is synthesized as a precursor of 1217 amino acids from which it is liberated by proteolysis (*3, 4*). Like the LDL receptor, the EGF precursor may exist as a membrane-bound molecule (*5–7*). The nucleotide sequences of cloned complementary DNA's (cDNA) for the EGF precursor and the LDL receptor revealed that the amino acid sequence of a