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Structure and function in rhodopsin: Expression of functional mammalian opsin in *Saccharomyces cerevisiae*

(G-protein-coupled receptor/11-*cis*-retinal/protein folding/immunoaffinity/transducin)

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ABSTRACT **The yeast *Saccharomyces cerevisiae* has been investigated for expression of mammalian opsin as an alternative to the currently used expression in COS-1 mammalian cells. The synthetic opsin gene was placed under the control of the inducible promoter GAL1 in the multicopy yeast/*Escherichia coli* shuttle vector YEpRF1. Transformation of a GAL$^+$ *S. cerevisiae* strain with the vector and growth of galactose-induced cultures to saturation showed the production of 2.0 ± 0.5 mg of opsin from about 10^{10} cells by ELISA. The addition of 11-*cis*-retinal to either cell spheroplasts or lysed cells showed that a fraction (2–4%) of the total expressed opsin reconstituted to rhodopsin. This fraction was purified to homogeneity and was shown to be fully functional and indistinguishable from bovine rhodopsin by the following criteria: (*i*) UV-visible absorption spectra, (*ii*) the formation of metarhodopsin II and its rate of decay, and (*iii*) initial rate of transducin activation as measured by the formation of a complex between transducin (α subunit) and guanosine 5′-[γ-[^{35}S]thio]triphosphate. The purified fraction was homogeneously glycosylated. However, glycosylation was distinct from that of bovine rhodopsin as judged by mobility on SDS/PAGE and endoglycosidase H sensitivity.**

Structure–function studies of rhodopsin depend a great deal on the availability of a variety of mutants in suitable amounts. Expression by transient transfection of COS-1 cells (1) and other mammalian cell lines (2) has been used in most of the reported work, but there are severe limits on the amounts of the pure mutant proteins obtained by these methods. Therefore, examination of alternative expression systems is desirable for studies where larger amounts of rhodopsin mutants are required. We now report on an investigation of the yeast *Saccharomyces cerevisiae* for this purpose. The yeast system has proved to be useful in the expression of a number of both soluble and integral membrane proteins (for example, see refs. 3–7). Our results reported herein show that bovine opsin is expressed in relatively large amounts in *S. cerevisiae* (2.0 ± 0.5 mg per about 10^{10} cells). However, only 2–4% of this is able to reconstitute to rhodopsin with 11-*cis*-retinal under the conditions used. The reconstituted rhodopsin fraction has been purified to homogeneity and characterized to be fully functional in a variety of ways.§

MATERIALS AND METHODS

Materials. Dodecyl β-D-maltoside (DM) was from Anatrace (Maumee, OH). D-Raffinose, D-galactose, D-glucose, and Triton X-100 were from Sigma. Protease inhibitors were purchased from Boehringer Mannheim, except benzamidine and phenylmethylsulfonyl fluoride, which were from Sigma. Casamino acids, yeast extract, and peptone were obtained from Difco. The source of glass beads (425–600 μm) was Biospec Products (Bartlesville, OK). The BA-85 nitrocellulose filters were from Schleicher & Schuell. Centricon-10 filter units were from Amicon. Peptide N-glycanase F (PNGase-F) and endoglycosidase H (endo-H) were from New England Biolabs. Sequenase (version 2.0) was obtained from United States Biochemical. The Geneclean II kit was obtained from Bio 101, and SeaPlaque agarose was from FMC. All radioactive materials were obtained from NEN. The Enhanced Chemiluminescence detection kit and horseradish peroxidase-conjugated sheep anti-mouse IgG were from Amersham. The Silver Stain Plus kit was from Bio-Rad, and CNBr-activated Sepharose 2B was from Pharmacia.

Bovine retinas were purchased from the J. A. Lawson Corp. (Lincoln, NB). 11-*cis*-Retinal was a generous gift of Rosalie Crouch (Medical University of South Carolina and the National Eye Institute). The cell line for production of the anti-rhodopsin antibody rho1D4 (9) was generously provided by Robert S. Molday of the University of British Columbia. The purification of rho1D4 from hybridoma supernatant was by protein A-Sepharose (Repligen). The coupling of rho1D4 to Sepharose was performed as recommended (Pharmacia). The nonapeptide corresponding to the C-terminal sequence of bovine opsin was from the Biopolymer Laboratories of the Cancer Center Research Institute at Massachusetts Institute of Technology. The column used for immunopurification was from Pierce.

Buffers. Buffer A is PBS [0.01 M phosphate (pH 6.3) containing 0.15 M saline]. Buffer B is buffer A containing 0.7 M sorbitol. Buffer C is buffer A containing 1% DM. Buffer D is buffer A containing 0.1% DM. Buffer E is 2 mM sodium phosphate (pH 6.0) containing 0.05% DM. Buffers A–D also contained the protease inhibitor mixture (1 mM phenylmethylsulfonyl fluoride/1 mM benzamidine/1 mM aprotinin/1 mM pepstatin A/1 mM leupeptin).

Construction of the Expression Vector (YEpRF1). The synthetic opsin gene from pMT4 (10) was cloned into pSP6 (Promega) to create more convenient restriction sites. The vector pFD30 was then derived by substituting the synthetic duplex shown below between the *Bgl*II and Asp-718 sites.

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   BglII  BamHI                  ⟶ Asp-718
5′-GATCTGGATCCAAAAAAAATGAACG-3′
      ACCTAGGTTTTTTTTACTTGCCATG
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The 1.06-kb *Bam*HI fragment from pFD30 corresponding to the synthetic opsin gene was cloned into the unique *Bam*HI site

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Abbreviations: DM, dodecyl β-D-maltoside; PNGase-F, peptide N-glycanase F; endo H, endoglycosidase H; ROS, rod outer segment; UV-vis, UV-visible.

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of a derivative of the 2-μm URA3 vector YEp352 (11). This derivative contains the GAL1 and GAL10 gene promoter sequences and is designated herein as YEp352-GAL. The correct orientation of the opsin gene in YEpRF1 (Fig. 1), along with the integrity of the inserted DNA duplex and the ligation sites, were verified by sequencing.

Yeast Transformation, Growth, Galactose Induction, and Spheroplast Preparation. CKY96 yeast strain (*ura*3-52, *leu*2-3, *his*4-619, GAL$^+$) was transformed with YEpRF1, or YEp352-GAL, by treatment with lithium acetate (12). The cells were cultured in a medium containing yeast nitrogen base, 2% raffinose, 3% glycerol, and 0.2% Casamino acids. Growth was in sealed flasks at 30°C with vigorous aeration at 220 rpm. To induce rhodopsin expression, galactose was added to a final concentration of 2% at the beginning of the exponential growth phase (OD_{600} = 1 to 2). The cells were either harvested at OD_{600} = 3.0 (for spheroplast preparation) or were allowed to reach stationery phase ($OD_{600} \approx 8.0$). For spheroplast formation, the harvested nonstationary cells were first treated at a concentration of <50 OD_{600} units/ml in 0.2 M Tris·HCl, pH 9.0/20 mM EDTA/0.1 M 2-mercaptoethanol at room temperature for 10 min. The cells were then centrifuged, washed, and treated with β-1,3-glucanase (13), and the extent of spheroplast formation was estimated by observing the drop in absorbance at λ = 600 nm in 1% Triton X-100.

Opsin Determination by ELISA. *Antibody titers.* To determine whether the ELISA response is affected by detergent type, rho1D4 hybridoma supernatant was titered in the absence of any detergent and in the presence of 0.5% DM, 2.5% CHAPS, or 2.5% Tween 80 against rod outer segment (ROS) rhodopsin previously immobilized on Nunc microtiter plate wells (14). Serial dilutions of rho1D4 hybridoma supernatant were made with PBS (pH 7.0) containing 1% BSA with no detergent, or the above detergents at the appropriate final concentrations. Binding of primary and secondary antibodies was allowed to proceed for 1 h at room temperature. Detection of primary antibody binding was by the reaction of horseradish peroxidase-conjugated secondary antibody [sheep anti-mouse $F(ab')_2$] with 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) in 0.01% H_2O_2/100 mM citrate/122 mM sodium phosphate, pH 4.0, followed by measurement of light absorbance at 405 nm.

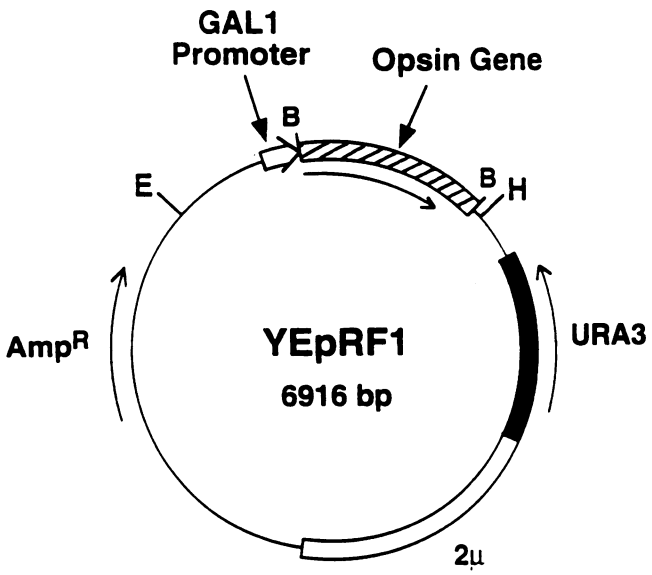

FIG. 1. A yeast–*Escherichia coli* shuttle vector (YEpRF1) for expression of the opsin gene in *S. cerevisiae*. The indicated restriction sites are as follows: E, *Eco*RI; H, *Hin*dIII; B, *Bam*HI. The sites for cloning the GAL1 promoter and the opsin gene are shown.

ELISAs. Competition ELISAs were performed essentially as described (14) except that the colorimetric assay described above was used for detection. Serial dilutions of rhodopsin standard and test samples were made in PBS containing 1% BSA, 0.25% fetal calf serum, 0.25% NaN_3, and 1% DM or 2.5% CHAPS in vinyl microtiter plates. Standard solutions of bovine rhodopsin were made from solubilized ROS in 1% DM or 2.5% CHAPS that had been centrifuged for 1 h at 100,000 × *g* to remove particulates. Rhodopsin was quantitated by absorbance at 500 nm, using an ε_{500} of 42,700 $M^{-1}\cdot cm^{-1}$ (15). Aliquots of each serial dilution of standards and samples were added to an equal volume of rho1D4 diluted in PBS/1% BSA/0.25% FCS/0.025% NaN_3. The mixtures were incubated in parallel for 1 h at room temperature or overnight at 4°C. Aliquots of each mixture were then transferred to pretreated Nunc plates containing immobilized rhodopsin. After binding for 1 h at room temperature, binding mixtures were rinsed out and the extent of rho1D4 binding to immobilized rhodopsin in the plates was quantitated as described above. Total proteins in cell extracts were quantitated (16).

Rhodopsin Chromophore Formation with 11-*cis*-Retinal and Immunoaffinity Purification. Typically, several liters of the galactose-induced yeast culture in the exponential phase of growth (OD_{600} = 3.0; 4 × 10^9 cells per liter) were used. The results described herein are for a preparation in which cells from a 3.7-liter culture at OD_{600} = 3.0 were harvested. The wet cell pellet (21 g) was converted to spheroplasts. The latter were washed with buffer B and resuspended in buffer B. All subsequent manipulations were under dim red light (Kodak no. 2 safelight filters). 11-*cis*-Retinal (96 μl from a 10 mM stock solution in ethanol) was added to the suspension over a period of 2 h at 4°C. The spheroplasts were centrifuged down and then resuspended in 50 ml of buffer C to allow solubilization in DM for 2 h at 4°C. The unlysed spheroplasts were then collected by centrifugation and subjected to vigorous agitation with ice-cold acid-washed glass beads in buffer A at 4°C until cell lysis was complete as verified by microscopic inspection. The resulting membranes were collected (100,000 × *g* at 4°C for 30 min) and were reextracted for 90 min by addition of the primary DM suspension that had been centrifuged at 100,000 × *g* at 4°C for 1 h. The material thus solubilized was centrifuged as above. The supernatant fraction (50 ml) was applied to a column (8 mm × 5 mm) of rho1D4 Sepharose beads pre-equilibrated with buffer C (binding capacity, 0.8 mg/ml; bed-volume, close to 0.25 ml), at a flow rate of 10–12 drops per min. The column was washed with 7.5 ml of buffer D followed by 5 ml of buffer E. Elution was then carried out at a flow rate of 2–3 drops per min with 5 ml of buffer E containing 30 μM nonapeptide followed by 5 ml of the same eluant containing 150 mM NaCl. Eluant fractions (0.5 ml) were monitored for absorbance at 280 nm and at 500 nm.

Before the above final procedure, experiments were carried out in which the cell cultures were grown to saturation and 11-*cis*-retinal was added to lysed cells instead of spheroplasts. DM solubilization of membranes and rho1D4 purification of solubilized material was subsequently carried out.

Characterization of Yeast Rhodopsin. *SDS/PAGE and immunoblot analysis.* SDS/PAGE was performed in 10% gels (17), and visualization of proteins was by silver staining and by immunoblot analysis using rho1D4 or rho4D2. Glycosylation was characterized by treatment with the PNGase-F and endo-H enzymes, according to manufacturer's instructions.

UV-visible (UV-vis) spectroscopy. This was as described (18). The formation of metarhodopsin II (λ_{max} = 380 nm) was measured after illumination with light (λ > 495 nm), and the rate of retinal release on its decay was measured in 0.1% DM/10 mM sodium phosphate, pH 6.0, by the described fluorescence assay (19). Acid denaturation of rhodopsin was performed in the dark by addition of sulfuric acid (0.02 M) to pH 1.9.

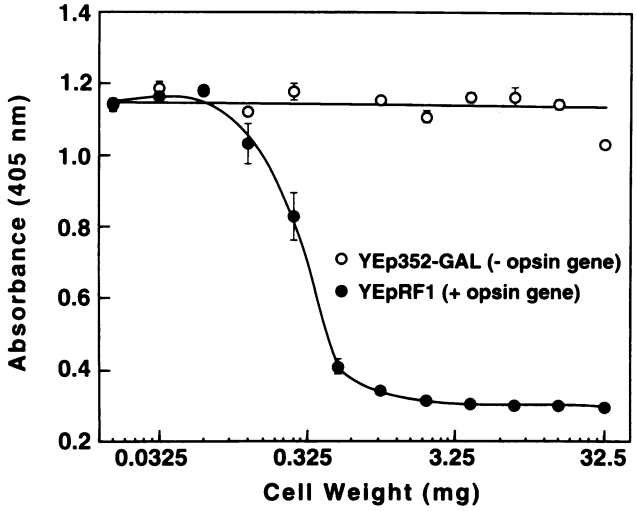

FIG. 2. An ELISA for quantitation of the opsin expressed in YEpRF1-transformed *S. cerevisiae* cells using CHAPS as detergent. The preparation of cell extracts was described in the text.

Transducin activation: Initial rate. This was measured at room temperature as described (18).

RESULTS

Assay of Expressed Opsin in Yeast Extracts. Cells transformed with the vector YEpRF1 (Fig. 1), or the corresponding vector lacking the opsin gene (YEp352-GAL), were induced with galactose and grown to saturation. After complete lysis of the cells with glass beads, the opsin in the extracts was measured by ELISA, using rho1D4 as the primary antibody. ROS rhodopsin was assayed in parallel using serial dilutions. Using ROS rhodopsin as a standard, opsin concentrations of 0.25–30 μM could be measured accurately by ELISA (SD < 10%). Since the antibody titers were found to be independent of detergent type, the quantitation of yeast opsin by competition ELISAs was performed using either CHAPS or DM as

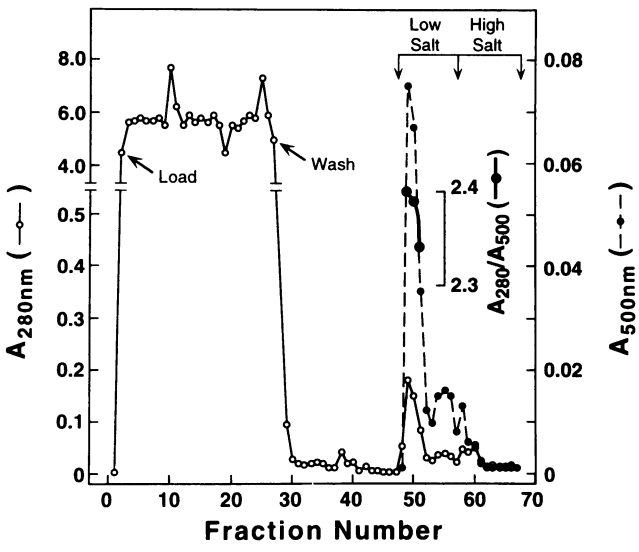

FIG. 3. Immunoaffinity purification of reconstituted (with 11-*cis*-retinal) yeast rhodopsin from total DM-solubilized proteins of YEpRF1-transformed *S. cerevisiae* cells. The conditions for loading the DM-solubilized material onto rho1D4-Sepharose column, washing, and subsequent elution at low salt followed by high salt are in the text. The rhodopsin fractions were monitored for A_{500} and the A_{280}/A_{500} absorption ratios.

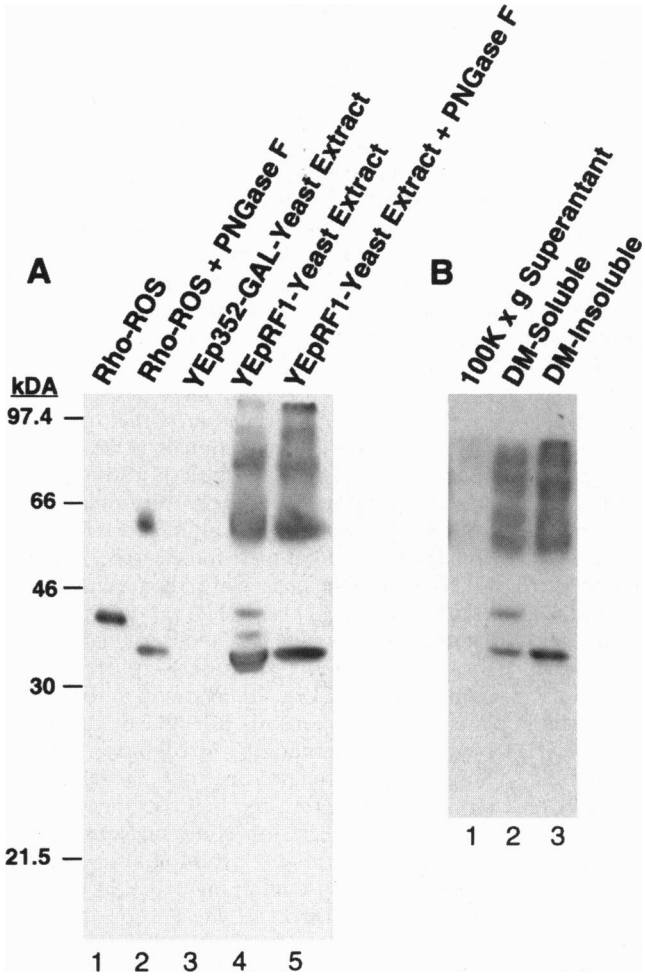

FIG. 4. Analysis of opsin in yeast extracts and during detergent solubilization by immunodetection with rho1D4 antibody. (*A*) Immunoblot of the YEpRF1 and YEp352-GAL yeast extracts (lanes 3 and 4) and PNGase-F sensitivity of the opsin species in the YEpRF1 extract (lane 5). (*B*) Immunoblot of fractions collected during the DM solubilization of the YEpRF1 extract (lanes 1–3).

detergent. Fig. 2 shows the typical results obtained. These results were obtained using CHAPS as detergent, and similar results were obtained with DM. The amount of expressed opsin was thus determined to be 2.0 ± 0.5 mg/liter of the cell culture containing approximately 10^{10} cells. This represents about 0.4% of the total protein in the cell extracts.

Purification of 11-*cis*-Retinal-Reconstituted Rhodopsin. After treatment of spheroplasts with 11-*cis*-retinal, the cells were completely lysed, and the membrane fraction was subjected to DM solubilization. The DM-soluble material was applied to a rho1D4-Sepharose column for rhodopsin immunopurification. The column profile is shown in Fig. 3. After washes for removing the non-opsin yeast proteins, elution at pH 6.0 in low salt gave mainly pure rhodopsin with A_{280}/A_{500} ratio between 2.2 and 2.4. A repeat of the immunoabsorption procedure decreased this ratio to between 1.6 and 1.8. Subsequent elution of the column in high salt would be expected to elute the non-retinal-binding opsin. However, as seen in Fig. 3, and in experiments where a large excess of 1D4-Sepharose beads was used, very little material was eluted at this stage. This result is consistent with the observation that the bulk of non-retinal-binding opsin was DM-insoluble (see below). The total amount of rhodopsin recovered in this experiment was 134 mg from about 1.5×10^{10} cells.

Characterization of Yeast Rhodopsin. *Identification of expressed opsins by immunoreactivity.* Fig. 4*A* shows an analysis of

the total yeast extract by immunoreactivity against rho1D4. The cells harboring YEpRF1 gave a positive immunoresponse after induction with galactose whereas YEp352-GAL extract did not, indicating that the immunoreactive bands are due to opsin species (lanes 3 and 4; also see Fig. 2). Three well-defined opsin bands and a number of higher molecular weight forms, presumably oligomers of the monomeric faster-migrating species, were observed (lane 4). Of the three opsin bands with apparent molecular masses of 42, 39, and 37 kDa (lane 4), the slowest band traveled slightly above ROS rhodopsin (lane 1), whereas the fastest band comigrated with deglycosylated ROS rhodopsin (lane 2). Deglycosylation of the total YEpRF1 extract with PNGase-F gave only the fastest migrating species (lane 5). Thus, the slow band (apparent molecular mass, about 42 kDa) is the mature form of opsin, whereas the intermediate band may be a partially glycosylated or misfolded opsin. Fig. 4*B* shows the corresponding immunoanalysis of fractions collected during the DM solubilization of the YEpRF1 extract. Thus, the supernatant fraction from the initial 100,000 × *g* centrifugation of the extract to recover membranes for solubilization contained no opsin (lane 1), indicating association of the latter with the membrane pellet. Extraction with the DM-containing buffer (buffer C) gave all of the mature opsin and a part of the unglycosylated opsin in the supernant fraction (lane 2). A substantial portion of the fast migrating species, which does not contribute to chromophore formation, remained in the DM-insoluble pellet (lane 3).

Comparison with ROS rhodopsin and behavior toward endoglycosidases. Fig. 5 compares the mobility of purified yeast rhodopsin with that of ROS rhodopsin (lanes 2 and 3) (SDS/PAGE, silver staining). The lower mobility of yeast rhodopsin reflects differences in nature/size of the N-glycosylating oligosaccharide relative to ROS rhodopsin. In agreement with the results shown in Fig. 4*A*, the yeast and ROS rhodopsins showed similar sensitivity to PNGase-F (lanes 4 and 5). However, while treatment of the samples with endo-H led to complete deglycosylation of yeast rhodopsin, only partial deglycosylation of purified ROS rhodopsin was observed (lanes 6 and 7). This finding is expected given that elongation of core sugars in *S. cerevisiae* takes place only by the addition of mannose residues (20).

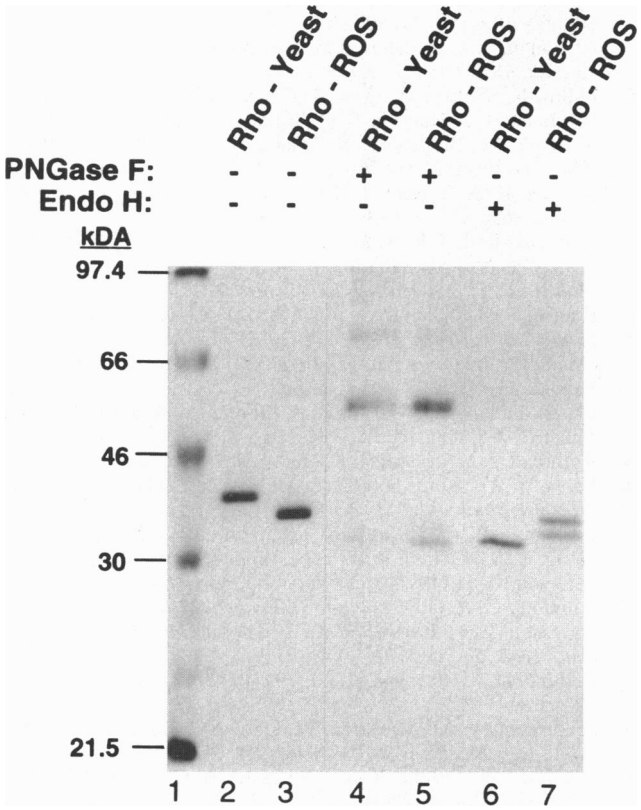

FIG. 5. Comparison of the gel mobility and glycosylation properties of purified yeast and ROS rhodopsins by SDS/PAGE. Visualization was by silver staining. For glycosylation analysis, the rhodopsin samples were treated separately with PNGase-F (lanes 4 and 5) and endo H (lanes 6 and 7).

Characterization by UV-vis spectral properties. Fig. 6 compares the UV-vis absorption spectra of purified ROS rhodopsin and those of purified yeast rhodopsin, both in dark and after illumination. Thus, the λ_{max} for both rhodopsins in the dark and those of the metarhodopsin II species formed on illumination were identical. Furthermore, acid denaturation of yeast rhodopsin in the dark yielded the characteristic protonated Schiff base species (λ_{max} = 439 ± 2 nm) (21). From the spectral intensities before and after acid denaturation, the ε_{500} value for yeast rhodopsin in 0.05% DM was within experimental error of that of bovine ROS rhodopsin under the same conditions.

The decay rate of yeast metarhodopsin II was measured by the intrinsic fluorescence, and the half-life was determined to be 14.4 min, which is within experimental error of that of ROS rhodopsin under the same conditions (data not shown).

Initial rate of transducin activation as measured by formation of a complex between $G_{T(\alpha)}$ and guanosine 5'-[γ-[^{35}S]thio]-

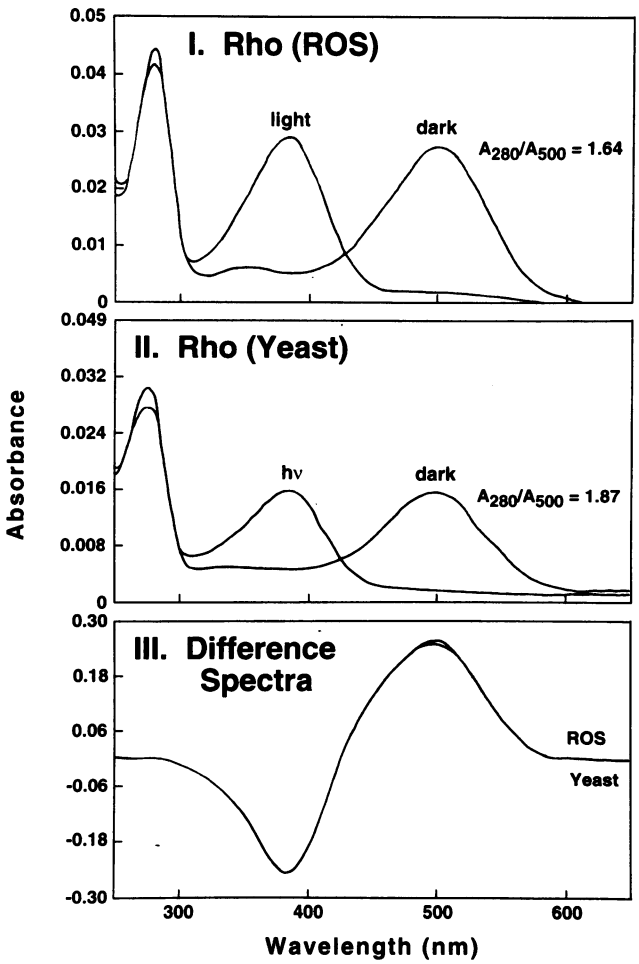

FIG. 6. UV-vis absorption spectra of purified yeast and ROS rhodopsins, with the corresponding difference spectra (superimposed). Prior to obtaining the difference spectra of *III* (spectra in light substracted from those in dark), the dark and light spectra for yeast rhodopsin were each normalized with respect to the corresponding ROS spectra shown in *I*.

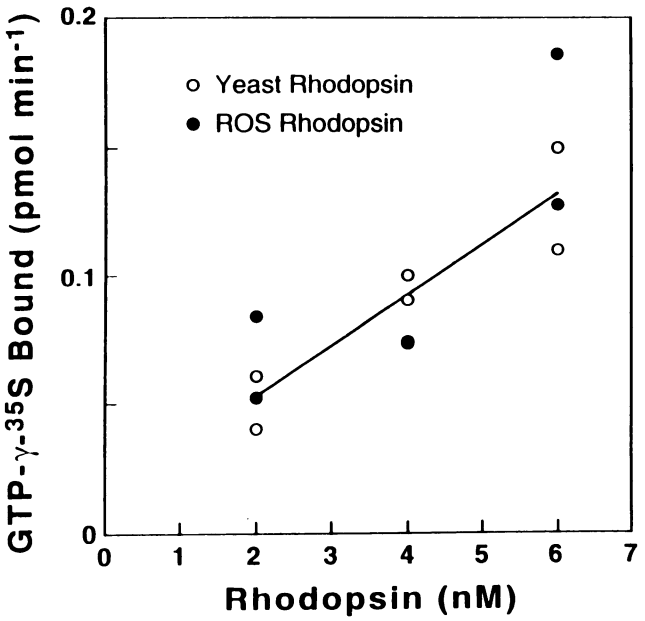

FIG. 7. Comparison of the initial rates of transducin (G_T) activation as measured by complex formation between G_T (α subunit) and guanosine 5′-[γ-[^{35}S]thio]triphosphate (GTP-γ-[^{35}S]) at 2, 4, and 6 nM purified yeast and ROS rhodopsins. The results are from duplicate measurements for each concentration. The linear regression analysis for the yeast data is shown.

triphosphate. As shown in Fig. 7, purified yeast rhodopsin gave the same initial rates of transducin activation as purified bovine ROS rhodopsin, when assayed at 2, 4, and 6 nM rhodopsin concentrations. This demonstrates that the metarhodopsin II formed from yeast rhodopsin is functionally equivalent to that from the ROS rhodopsin.

DISCUSSION

This report has demonstrated the expression of a mammalian opsin in yeast and subsequent preparation of rhodopsin from the expressed material. The rhodopsin fraction, reconstituted with 11-*cis*-retinal, was purified to homogeneity by immunoaffinity chromatography. The purified yeast rhodopsin is indistinguishable from ROS rhodopsin by several functional criteria. Moreover, the purified fraction is homogeneously glycosylated, although it has slightly slower electrophoretic mobility than ROS rhodopsin. This difference is due to specific differences in glycosylation, as the deglycosylated forms of ROS and yeast rhodopsins have identical electrophoretic mobility. The homogeneity of glycosylation and the level of endo-H sensitivity suggest that the mature yeast rhodopsin has the typical mannose glycosylation pattern that is observed in *S. cerevisiae* (20).

Only a small fraction of the total expressed opsin was reconstituted to rhodopsin under the conditions used for induction of expression and subsequent chromophore recovery. Several directions can be investigated for favoring the correct folding of the expressed opsin, such as the fine-tuning of the induction parameters by changes in growth conditions or use of alternative promoters. Overloading of the protein post-translational processing machinery may be occurring under the current induction conditions, as suggested by the presence of partially glycosylated and nonglycosylated yeast opsin species that do not form chromophore. Investigation of other species of yeast that have been successfully used for expression of heterologous proteins might also prove useful. For instance, bacteriorhodopsin, another seven-helical integral membrane proteins, which binds all-*trans*-retinal, has been expressed in properly folded form in *Schizosaccharomyces pombe* (22).

The total amount of chromophore recovered in this study was considerable (134 mg from about 1.5×10^{10} cells), despite the low percentage of reconstitution of the expressed opsin. The yeast system also offers the important compensation of simplicity and convenient scale-up of cell culture. Additionally, the availability of *S. cerevisiae* mutant strains with specific defects in the folding machinery makes this system appealing for *in vivo* folding studies of rhodopsin. Thus, the yeast system has the promise of providing an effective tool for structural and functional studies of rhodopsin.

Note. Recently, N. G. Abdulaev, M. P. Popp, W. C. Smith, and K. D. Ridge (personal communication) have investigated expression of bovine opsin in the methylotrophic yeast *Pichia pastoris* with results similar to those reported herein.

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1. Farrens, D. L. Altenbach, C., Yang, K., Hubbell, W. L. & Khorana, H. G. (1996) *Science*, in press.
2. Oprian, D. D., Molday, R. S., Kaufman, R. J. & Khorana, H. G. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 8874–8878.
3. Nathan, J., Weitz, C. J., Agarwal, N., Nir, I. & Papermaster, D. S. (1989) *Vision Res.* **29,** 907–914.
4. Hitzeman, R. A., Hagie, F. E., Levine, H. L., Goeddel, D. V., Amerer, G. & Hall, B. D. (1981) *Nature (London)* **293,** 717–727.
5. Fujita, N., Nelson, N., Fox, T. D., Glaudio, T., Lindstrom, J., Riezman, H. & Hess, G. P. (1986) *Science* **231,** 1284–1287.
6. Sentenac, H., Bonneaud, N., Minet, M., Lacroute, F., Salmon, J-M., Gaymrad, F. & Grignon, C. (1992) *Science* **256,** 663–665.
7. Kuchler, K. & Thorner, J. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 2303–2306.
8. Fridovich-Keil, J. L. & Jinks-Robertson, S. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 398–402.
9. Molday, R. S. (1989) *Prog. Retinal Res.* **8,** 173–209.
10. Franke, R. R., Sakmar, T. P., Oprian, D. D. & Khorana, H. G. (1988) *J. Biol. Chem.* **263,** 2119–2122.
11. Hill, J. E., Myers, A. M., Koerner, T. J. & Tzagoloff, A. (1986) *Yeast* **2,** 163–167.
12. Ito, H., Fukuda, Y., Murata, K. & Kimura, A. (1983) *J. Bacteriol.* **153,** 163–168.
13. Rothblatt, J. & Schekman, R. (1989) *Methods Cell Biol.* **32,** 3–36.
14. Laird, D. W., Wong, S. Y. C. & Molday, R. S. (1987) *Membrane Proteins: Proceedings of the Membrane Protein Symposium*, ed. Goheen, S. C. (Bio-Rad Lab., Richmond, CA), pp. 45–70.
15. Hong, K. & Hubbell, W. L. (1973) *Biochemistry* **12,** 4517–4523.
16. Peterson, G. L. (1977) *Anal. Biochem.* **83,** 346–356.
17. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
18. Davidson, F. F., Loewen, P. C. & Khorana, H. G. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 4029–4033.
19. Farrens, D. & Khorana, H. G. (1995) *J. Biol. Chem.* **270,** 5073–5076.
20. Kukuruzinska, M. A., Bergh, M. L. E. & Jackson, B. J. (1987) *Annu. Rev. Biochem.* **56,** 915–944.
21. Sakmar, T. P., Franke, R. R. & Khorana, H. G. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 8309–8313.
22. Hildebrandt, V., Polakowski, F. & Buldt, G. (1991) *Photochem. Photobiol.* **54,** 1009–1016.