Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 8840–8844, October 1991
Genetics

Functional heterogeneity of mutant rhodopsins responsible for autosomal dominant retinitis pigmentosa

CHING-HWA SUNG*, BARBARA G. SCHNEIDER†, NEERAJ AGARWAL†, DAVID S. PAPERMASTER†, AND JEREMY NATHANS*‡

*Howard Hughes Medical Institute, Departments of Molecular Biology and Genetics and of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205; and †Department of Pathology, University of Texas Health Science Center at San Antonio, San Antonio, TX 78284-7750

Communicated by Lubert Stryer, July 12, 1991 (received for review June 13, 1991)

ABSTRACT **Thirteen mutant rhodopsins responsible for autosomal dominant retinitis pigmentosa (ADRP) have been produced by transfection of cloned cDNA into tissue culture cells. Three mutants [class I: Phe-45 → Leu, Gln-344 → termination (deletion of C-terminal positions 344–348), and Pro-347 → Leu] resemble wild-type rhodopsin in yield, regenerability with 11-*cis*-retinal, and plasma membrane localization. Ten mutants [class II: Thr-17 → Met, Pro-23 → His, Thr-58 → Arg, Val-87 → Asp, Gly-89 → Asp, Gly-106 → Trp, Arg-135 → Leu, Arg-135 → Trp, Tyr-178 → Cys, and Asp-190 → Gly] accumulate to significantly lower levels, regenerate with 11-*cis*-retinal variably or not at all, and are transported inefficiently to the plasma membrane, remaining primarily in the endoplasmic reticulum. These data suggest that there are at least two distinct biochemical defects associated with different rhodopsin mutants in ADRP.**

Retinitis pigmentosa (RP) is a group of inherited disorders that cause a progressive loss of retinal function. The hallmarks of RP are decreased rod sensitivity, progressive loss of visual fields, a diminished electroretinographic response referable to photoreceptors, and characteristic pigmentary deposits in the retina (1).

Recently, some patients with autosomal dominant RP (ADRP) were found to carry mutations in the gene encoding rhodopsin, the visual pigment mediating rod vision (2–5). The mutations cosegregate with RP and are absent from control populations with normal vision. In a previous study of 161 unrelated families with ADRP, 39 were found to carry 1 of 13 different point mutations in the rhodopsin coding region (5). The goal of the present study is to define the biochemical differences between wild-type (wt) rhodopsin and the variants responsible for ADRP. For this purpose we have produced in tissue culture cells each of the 13 mutant human rhodopsins described above and determined their yield, regenerability with 11-*cis*-retinal, and subcellular localization.

MATERIALS AND METHODS

Tissue Culture Expression. A rhodopsin cDNA clone was isolated from a human retina cDNA library (6, 7), and a fragment containing the entire coding region was inserted into the expression plasmid pCIS (8). *In vitro* mutagenesis and production of opsin after transient or stable transfection of 293S cells were performed as described (9, 10).

Absorbance Spectra. Cells from 20 10-cm plates were collected 60 hr after transient transfection, and membranes were prepared as described (9) except that the final membrane pellet was solubilized in 0.3 ml of 0.1 M sodium phosphate, pH 6.5/1 mM EDTA/1% 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS; Sigma). The solubilized sample was regenerated with 11-*cis*-retinal, incubated with 50 mM hydroxylamine for 30 min, and the photobleaching difference spectrum was determined (9).

Immunoblotting. Membrane samples prepared from cells 60 hr after transfection were mixed with an equal volume of 2× Laemmli sample buffer (1× = 0.125 M Tris·HCl, pH 6.8/4% SDS/20% glycerol/10% 2-mercaptoethanol/0.012% bromophenol blue), resolved on a SDS/12.5% polyacrylamide gel, and electroblotted onto nitrocellulose (11). Immunoreactive protein was visualized by using a 1:1 mixture of monoclonal antibodies (mAbs) B6-30 and 1D4, which recognize, respectively, the amino- and carboxyl-terminal regions of rhodopsin (12, 13), and the enhanced chemiluminescence detection system (Amersham).

Immunohistochemistry. For light microscopy, 293S cells were grown on glass cover slips coated with 0.1% gelatin, transfected as described above, and processed 24 hr after transfection. For fluorescent staining, cells were fixed for 10 min at room temperature in 4% paraformaldehyde in phosphate-buffered saline (PBS), permeabilized for 3 min with ice-cold methanol, and incubated with mAb B6-30 or 1D4 (ascites diluted 1:5000), followed by fluorescein isothiocyanate-conjugated goat anti-mouse Ig. For avidin–biotin complex (ABC)–peroxidase staining, cells were rinsed with PBS, air-dried, fixed and permeabilized for 10 min with ice-cold methanol, and incubated with mAb B6-30 or 1D4 followed by biotinylated goat anti-mouse immunoglobulin.

For immuno-EM, cell lines were grown for 3–4 days on Aclar film prior to embedding in Lowicryl K4M as described (14, 15). Thin sections were labeled with 0.1 mg of mAb 1D4 per ml for detection of wt rhodopsin and the P23H§ mutant or with 0.025 mg of a polyclonal sheep anti-opsin antibody per ml, which primarily detects the amino termini of vertebrate rhodopsins (16) for detection of wt rhodopsin and mutants P347L and Q344ter (Gln-344 → termination—i.e., deletion of C-terminal positions 344–348). Bound antibodies were detected with rabbit anti-mouse or rabbit anti-sheep IgG followed by goat anti-rabbit IgG bound to 10-nm gold particles as described (17). As a control, a nonimmune mouse IgG or a preimmune sheep IgG was substituted for the anti-rhodopsin antibody.

Pulse–Chase Analysis and Immunoprecipitation. Stable cell lines growing in 10-cm-diameter dishes were washed with PBS several times, incubated with 10% dialyzed fetal bovine serum (FBS) in methionine-free Dulbecco's modified Eagle's medium (DMEM) for 30 min at 37°C and then for an addi-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: RP, retinitis pigmentosa; ADRP, autosomal dominant RP; mAb, monoclonal antibody; Q344ter, termination at Gln-344; endo F and endo H, endoglycosidases F and H; wt, wild type; ER, endoplasmic reticulum; PM, plasma membrane; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propane sulfonate.

‡To whom reprint requests should be addressed.

§Mutant names correspond to the amino acid change from the wt sequence in single letter code; thus, P23H means Pro-23 → His.

tional 30 min after addition of 0.2 mCi (1 Ci = 37 GBq) of [^{35}S]methionine. The cells were incubated in chase medium (10% FBS/DMEM containing 60 μg of unlabeled methionine and 10 μg of cycloheximide per ml) for the indicated time period and then collected, washed with cold PBS, and solubilized in lysis buffer (1% *n*-dodecyl-β-D-maltoside/50 mM Hepes, pH 7.0/150 mM NaCl/1 mM EDTA/1 mM phenylmethylsulfonyl fluoride) for 2 hr at 4°C. The cell extracts were microcentrifuged for 30 min, and the supernatants were preabsorbed with an irrelevant mAb bound to protein A-Sepharose beads and then incubated for 2 hr at 4°C with mAb 1D4 bound to protein A-Sepharose beads. After extensive washing with lysis buffer containing 0.1% bovine serum albumin, labeled antigen was eluted from the immunoprecipitate by boiling in Laemmli sample buffer and resolved on a SDS/12.5% polyacrylamide gel.

Endoglycosidase Treatment. Aliquots of immunoprecipitated protein in Laemmli buffer were mixed with an equal volume of 10% Nonidet P-40/150 mM glycine, pH 9.3/30 mM EDTA/1 mM phenylmethylsulfonyl fluoride/25 μg each of leupeptin, aprotinin, and benzylamidine per ml and incubated with 5 milliunits of endoglycosidase H (endo H) (Boehringer Mannheim) or 0.27 milliunits of endoglycosidase F (endo F) overnight at 37°C.

RESULTS

Production of Rhodopsin Mutants in Tissue Culture. Each of 13 mutant rhodopsin sequences previously described in patients with ADRP was introduced into a cloned human rhodopsin cDNA by site-directed mutagenesis (Fig. 1; ref. 5). In an initial set of experiments, wt and mutant rhodopsin sequences were transiently transfected into a human embryonic kidney cell line (293S), and the expressed proteins were analyzed by immunoblotting (Fig. 2). wt human opsin (the apoprotein of rhodopsin) expressed in 293S cells appears as a heterogeneous family of bands: the broad bands at 30–45 kDa and 60–75 kDa represent monomers and dimers, respectively, each of which is heterogeneously glycosylated as determined by endo F treatment (see below). Bovine opsin isolated from rod outer segments is known to aggregate upon denaturation; most likely the aggregation of expressed human opsin represents the same phenomenon.

The immunoblot patterns of the 13 human rhodopsin mutants fall into two groups. Mutants F45L, Q344ter, and P347L show electrophoretic patterns resembling that of wt and they accumulate to the wt level. The remaining 10 mutants appear predominantly as dimers or higher order multimers and accumulate to levels significantly lower than the wt.

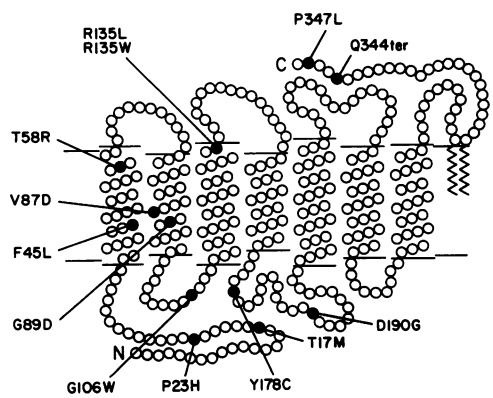

FIG. 1. Rhodopsin model showing the locations of 13 mutations responsible for ADRP (5). The amino- and carboxyl-termini face, respectively, the extracellular and intracellular sides of the membrane.

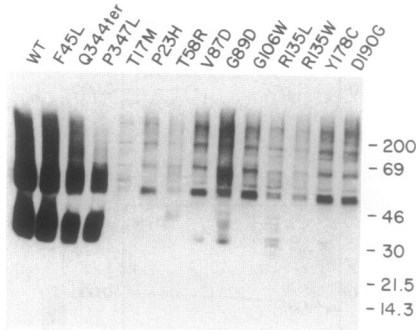

FIG. 2. Western blots of expressed rhodopsin mutants. Membrane proteins from transiently transfected cells were resolved on a SDS/12.5% polyacrylamide gel, and the opsin was visualized by immunoblotting. Samples of wt and mutants F45L, Q344ter, and P347L contain one-fourth as much total protein as the remaining 10 samples.

To determine whether or not the expressed proteins were correctly folded, we examined their ability to bind the chromophore 11-*cis*-retinal. Cell membranes from transiently transfected cells were solubilized in CHAPS and incubated with an excess of 11-*cis*-retinal for 2 hr in the dark. Absorbance spectra were recorded prior to and after photobleaching, and the difference spectra were calculated. In the difference spectra shown in Fig. 3, the positive peak in the visible region corresponds to rhodopsin and the negative peak in the UV region corresponds to released all-*trans*-retinal. wt human rhodopsin produced in tissue culture cells and rhodopsin isolated from human retinas (18) have absorbance spectra that are indistinguishable.

Mutants F45L, Q344ter, and P347L produce photolabile pigments with absorbance spectra that are indistinguishable from wt. Mutants V87D and G89D produce photolabile pigments at greatly reduced yield compared with wt, a characteristic observed for each in three independent experiments. The remaining eight rhodopsin mutants consistently fail to produce detectable photopigments upon incubation with 11-*cis*-retinal.

Subcellular Distribution of Rhodopsin Mutants. *Immunocytochemistry.* In the retina, opsin is synthesized on the rough endoplasmic reticulum (ER) and is transported via the Golgi apparatus to the plasma membrane (PM) (16, 19). To examine the possibility that some of the rhodopsin mutants may be defective in intracellular transport, we examined their subcellular distribution in 293S cells.

wt opsin expressed in 293S cells accumulates primarily in the plasma membrane (Fig. 4 *A* and *G* and Fig. 5*A*). After transient transfection, the subcellular distribution of each rhodopsin mutant was determined by both immunofluorescent and immunoperoxidase staining of fixed and permeabilized cells with mAbs 1D4 and B6-30. Table 1 summarizes the results of these experiments. Mutants F45L, Q344ter, and P347L accumulate primarily in the PM. These three mutants, as well as wt, also show a small and variable amount of immunoreactive material in a perinuclear distribution. Mutants T58R, V87D, G89D, G106W, R135L, and R135W accumulate in the PM and in the cell interior to similar levels. The interior staining appears as a fine reticular pattern with heaviest staining in the perinuclear region, a pattern consistent with protein accumulation in the ER. Mutants T17M, P23H, Y178C, and D190G show prominent intracellular staining in the ER pattern described above and a striking absence of cell surface staining (Fig. 4 *C*, *E*, and *H*). The presence or absence of cell surface staining was confirmed for each mutant by staining nonpermeabilized cells with mAb B6-30, which recognizes rhodopsin's extracellular amino-terminal domain (data not shown).

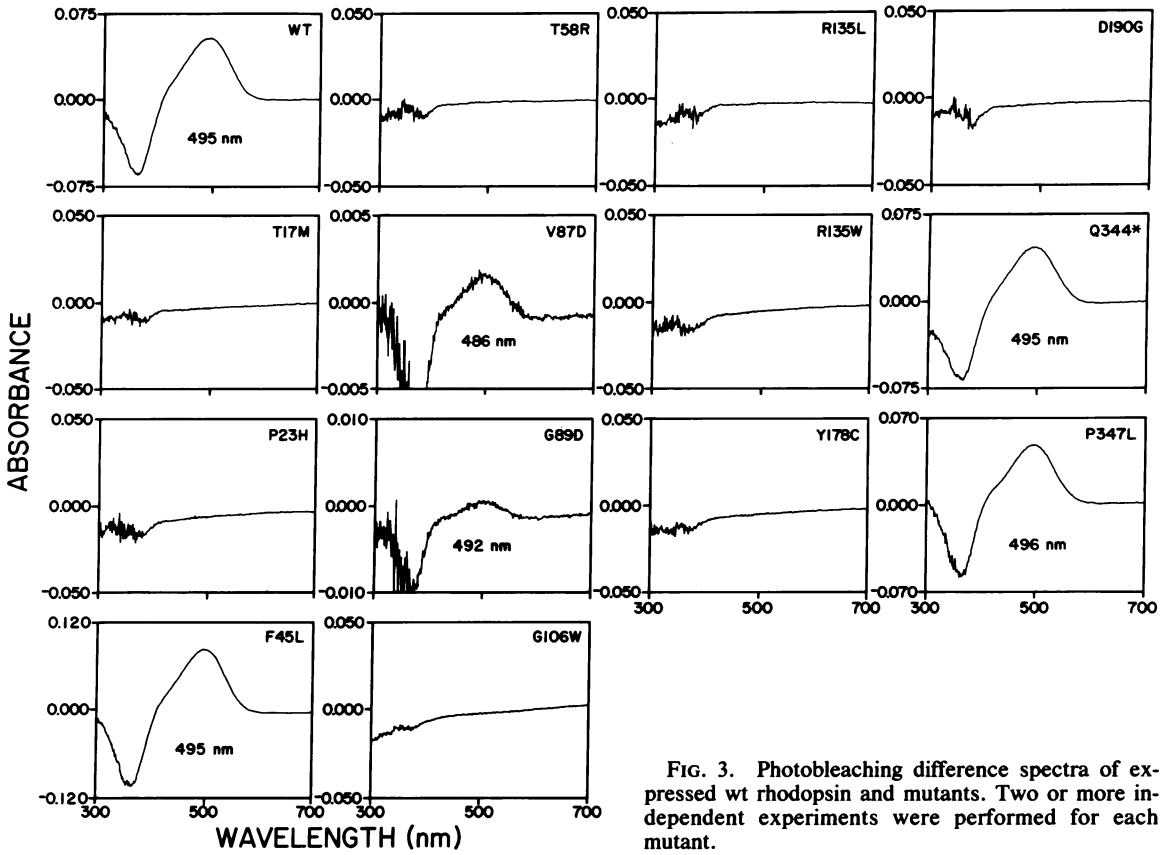

FIG. 3. Photobleaching difference spectra of expressed wt rhodopsin and mutants. Two or more independent experiments were performed for each mutant.

Post-embedding colloidal-gold immuno-EM revealed prominent PM labeling of cell lines expressing Q344ter and P347L (Fig. 5 *C* and *D*). Two cell lines expressing P23H showed no labeling with mAb 1D4 or sheep anti-opsin above the background level obtained by staining with normal mouse IgG or preimmune sheep IgG, respectively (Fig. 5*B*). Upon passaging, both P23H cell lines showed an increasing fraction of nonexpressing cells as determined by light microscopy after immunoperoxidase staining. However, this heterogeneity cannot account for the lack of detectable immunostaining observed by EM, as the sample size was sufficiently large to insure that it included expressing cells. Most likely, the low level of accumulated P23H protein distributed over the large interior area accounts for the low density of colloidal gold particles (below the background level for this technique) compared to that observed for wt at the PM.

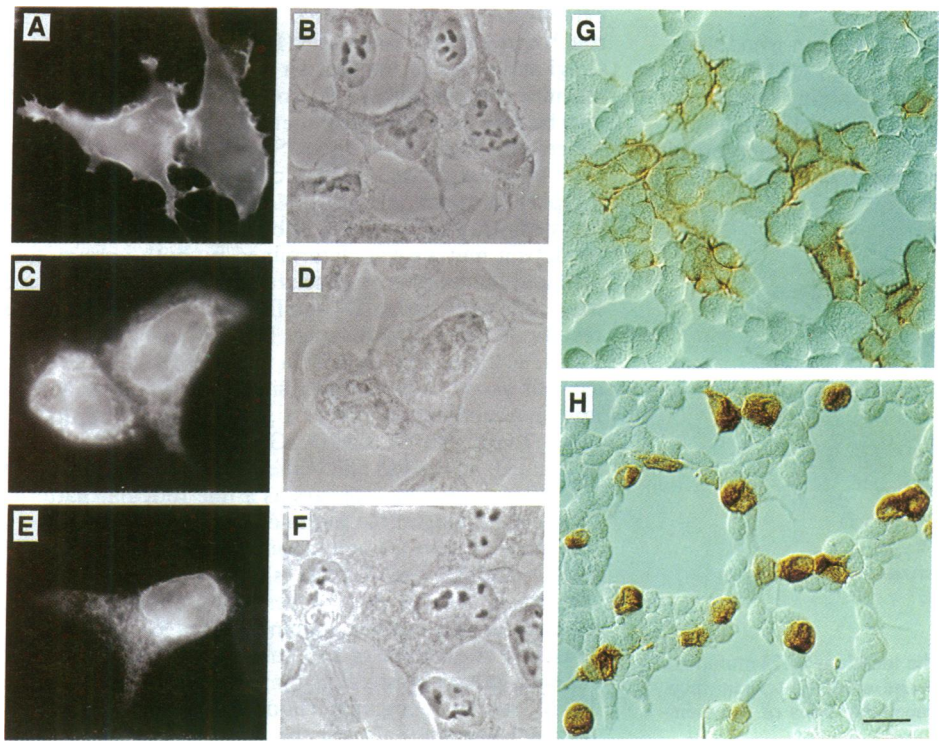

FIG. 4. Immunolocalization of wt opsin (*A*, *B*, and *G*) and mutants P23H (*C*, *D*, and *H*), and Y178C (*E* and *F*) in transiently transfected 293S cells. Cells were fixed 24 hr after transfection and stained with mAb 1D4 followed by a fluorescent second antibody (*A–F*) or horseradish peroxidase-coupled avidin–biotin complex (*G* and *H*). *B*, *D*, and *F* are the phase-contrast counterparts of *A*, *C*, and *E*, respectively. [Bar = 10 μm (*A–F*) and 25 μm (*G* and *H*)].

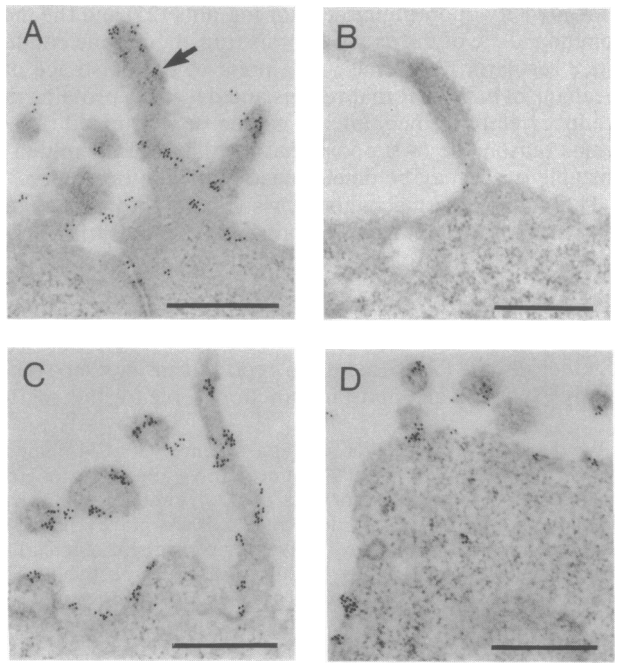

FIG. 5. Immuno-EM localization of opsin expressed in cell lines. (*A*) Cells expressing wt opsin show cell surface labeling (solid arrow) with mAb 1D4. Labeling density was variable from cell to cell and along the PM of individual cells. (*B*) Cells expressing mutant P23H show no surface labeling above background levels with mAb 1D4. Cells expressing mutant Q344ter (*C*) and P347L (*D*) show PM labeling with sheep anti-opsin antibody that is indistinguishable from wt. [×25,600 (*A* and *B*), ×28,000 (*C* and *D*); bar = 0.5 μm.]

Glycosylation Patterns. Bovine rhodopsin is N-glycosylated at Asn-2 and -15 (20), sites that are conserved in the human rhodopsin sequence (7). High-mannose core oligosaccharides are added to the N-glycosylation sites in the ER and are trimmed and elaborated in the Golgi apparatus (21). Therefore, we examined the time course of acquisition and modification of N-linked oligosaccharides as a means of following the transit of newly synthesized opsin through the ER–Golgi–PM pathway.

293S cell lines expressing either wt opsin or the P23H mutant were labeled for 30 min with [^{35}S]methionine, followed by a variable chase period ranging from 15 min to 6 hr.

Table 1. Characteristics of rhodopsin mutants responsible for ADRP

Opsin mutant	Immuno-blot	Absorbance spectrum	Subcellular localization	Glyco-sylation pattern	Class
wt	wt	wt	PM > ER	1	I
F45L	wt	wt	PM > ER	1	I
Q344ter	wt	wt	PM > ER	1	I
P347L	wt	wt	PM > ER	1	I
T17M	*	<1%	PM << ER	3	IIa
P23H	*	<1%	PM << ER	2	IIa
Y178C	*	<1%	PM << ER	2	IIa
D190G	*	<1%	PM << ER	2	IIa
T58R	*	<1%	PM ≈ ER	2	IIb
V87D	*	1–5%	PM ≈ ER	2	IIb
G89D	*	1–5%	PM ≈ ER	2	IIb
G106W	*	<1%	PM ≈ ER	2	IIb
R135L	*	<1%	PM ≈ ER	2	IIb
R135W	*	<1%	PM ≈ ER	2	IIb

Immunoblot and spectral absorbance data are derived from Figs. 2 and 3. Asterisks indicate aggregated protein with reduced yield. Subcellular localization was determined by light microscopy. The glycosylation patterns of rhodopsin mutants were determined by immunoprecipitation of opsin from transiently transfected 293S cells labeled with [^{35}S]methionine for 30 min followed by a 30-min chase or, in a second experiment, for 8 hr without a chase. Patterns: 1, prominent bands at 31 and 39 kDa and a heterogeneous family of bands at lower mobility; 2, prominent bands at 31, 35, and 39 kDa and variable levels of a heterogeneous family of bands at lower mobility; 3, similar to pattern 2 except for the absence of the 39-kDa band.

Radiolabeled opsin was purified by immunoprecipitation and analyzed by SDS/PAGE (Fig. 6*A*). Newly synthesized wt opsin migrated predominantly as a 39-kDa band. During the chase period, this band decreased in intensity, and there was a concomitant increase in the intensity of a heterogeneous family of bands between 43 and 68 kDa that correspond to highly glycosylated monomeric opsin. (Opsin acquired oligosaccharides of larger average mass in stable cell lines than it did after transient transfection; compare Figs. 2 and 6.) No significant degradation was seen over the 6-hr chase period. By contrast, newly synthesized P23H mutant appeared as a triplet of bands at 31, 35, and 39 kDa. Over the 6-hr chase period, these bands appeared to be degraded; no higher molecular mass species were observed.

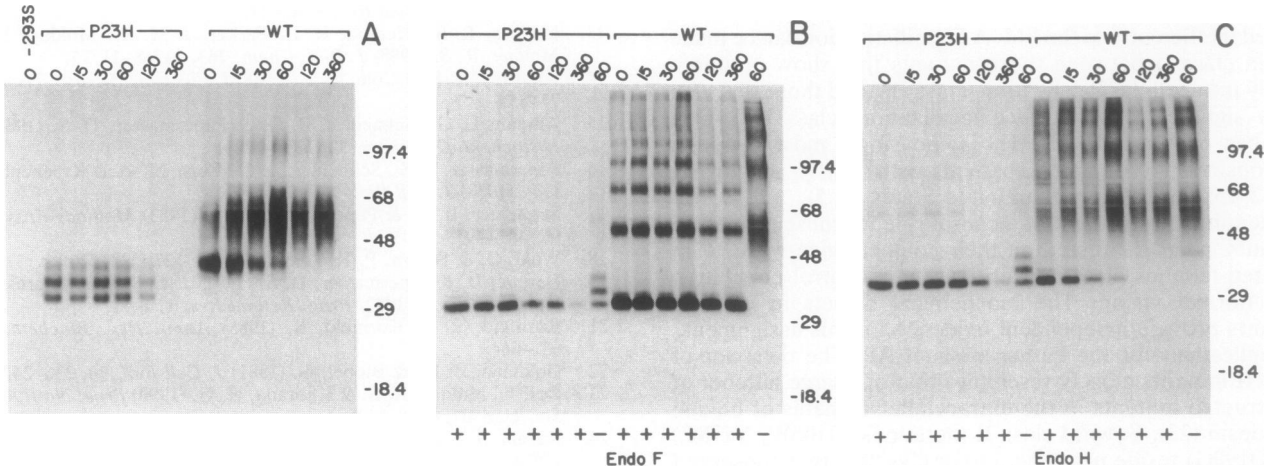

FIG. 6. Pulse–chase labeling of mutant P23H and wt opsin in 293S cell lines. Cells were labeled for 30 min with [^{35}S]methionine and incubated in unlabeled methionine for the indicated chase times in minutes above each lane. (*A*) Opsin was recovered by immunoprecipitation with mAb 1D4, resolved on a SDS/12.5% polyacrylamide gel, and visualized by autoradiography. No labeled material is precipitated from labeled 293S cell lysates. (*B* and *C*) Immunoprecipitated opsin was digested with (lanes +) or without (−) either endo F (*B*) or endo H (*C*) prior to electrophoresis.

To identify the oligosaccharide structures associated with the different molecular mass opsin species, each was cleaved with either endo F (Fig. 6*B*) or endo H (Fig. 6*C*). endo F cleaves from the protein all N-linked oligosaccharides; endo H cleaves the high-mannose form but not the complex Golgi forms. As expected, all of the higher molecular mass forms of opsin, both wt and mutant P23H, were cleaved by endo F to yield a species migrating at the position of the core polypeptide. In the wt sample, multimers were also present, presumably formed by aggregation of denatured opsin. The core polypeptide comigrated with the 31-kDa band, a mobility closely matching that reported for opsin synthesized *in vitro* (22). Treatment with endo H cleaves only the 35- and 39-kDa species, producing the expected 31-kDa core polypeptide. These data show that the 35- and 39-kDa species carry high-mannose core oligosaccharides, whereas the oligosaccharides carried by the heterogeneous family of bands between 43 and 68 kDa have been modified by passage through the Golgi apparatus. The 31-, 35-, and 39-kDa species appear to represent polypeptides carrying 0, 1, and 2 high-mannose oligosaccharides, respectively. Support for this assignment comes from the observation that mutant T17M, in which the Asn-Ala-Thr N-glycosylation site at positions 15–17 has been destroyed, produces only the 31-kDa and 35-kDa species. This biosynthetic analysis shows that wt opsin proceeds from ER to Golgi to PM with little turnover, whereas P23H does not proceed to the Golgi apparatus and is rapidly degraded.

After transient transfection of 293S cells, each of the mutant opsins has been biosynthetically labeled with [^{35}S]methionine both for 30 min followed by a 30-min chase and for 8 hr with no chase, immunoprecipitated, and analyzed by SDS/PAGE (data not shown). In all cases including wt, bands are seen at 31 and 39 kDa; however, some mutants, including P23H show a band at 35 kDa (Table 1). (The minor differences between glycosylation patterns in transiently and stably transfected cells may arise from heterogeneity after transient transfection in protein expressed per cell or in cell viability, or in both.)

DISCUSSION

Classification System for Rhodopsin Mutants. Table 1 summarizes the properties of wt and mutant rhodopsins described in this paper. Two major classes are evident: class I mutants resemble wt in yield, electrophoretic pattern, regenerability with 11-*cis*-retinal, and subcellular localization; class II mutants accumulate to significantly lower levels, appear predominantly as aggregates on immunoblots, regenerate with 11-*cis*-retinal to variable extents or not at all, and are transported inefficiently to the PM. A finer distinction can be made within class II between those mutants that show predominantly intracellular localization (class IIa) and those that also show significant cell surface localization (class IIb). One member of class IIa, P23H, is the single most common rhodopsin mutation among patients with ADRP, accounting for 12–15% of cases in the United States (2, 5).

The initial assignment of a causal role for these rhodopsin mutants in ADRP rested on their coinheritance with RP in affected families, and their absence in a control population with normal vision. The biochemical defects in class II mutants provide independent evidence for this assignment.

Implications for the Pathogenesis of RP. The behavior of class II mutants closely resembles that of a large number of constructed mutants in the extracellular domains of bovine rhodopsin (23). Several class II mutations (G106W, Y178C, and D190G) reside near Cys-110 or Cys-187, two conserved residues that form an essential disulfide bond in bovine rhodopsin (24). These mutations may destabilize the protein by interfering with disulfide bond formation. Class II rhodopsin mutants join a growing list of PM protein mutants that share the property of retention in the ER, including class 2 low density lipoprotein receptor mutants (25) and the most common allele of the cystic fibrosis transmembrane conductance regulator (26). These data point to the existence of a mechanism in the ER that retains and degrades proteins that are incorrectly or incompletely folded or assembled (27). It seems reasonable to suppose that synthesis of misfolded or unstable opsin may be deleterious to the photoreceptor.

The pathogenic mechanisms associated with class I mutants are not evident from the present set of experiments. It is possible that one of the class I mutants, F45L, represents a mild class II phenotype, as it is the most conservative of the amino acid substitutions studied. The other two class I mutants reside close to rhodopsin's carboxyl terminus and, together with a P347S mutation reported in another ADRP family (3), imply an important functional role for this region.

We thank Ms. P. Miller for skillful assistance with EM immunocytochemistry, Dr. L. Stryer (Stanford University) and the Hoffmann–La Roche Company for gifts of 11-*cis*-retinal, Dr. G. Hart (Johns Hopkins) for a gift of endo F, and Dr. P. Hargrave (University of Florida) and Dr. R. Molday (University of British Columbia) for making available hybridomas B6-30 and 1D4, respectively. This work was supported by the Howard Hughes Medical Institute, by a grant from the National Retinitis Pigmentosa Foundation to J.N., and by National Institutes of Health Grant EY-06892 to D.S.P.

1. Heckenlively, J. R. (1988) *Retinitis Pigmentosa* (Lippincott, Philadelphia).
2. Dryja, T. P., McGee, T. L., Reichel, E., Hahn, L. B., Cowley, G. S., Yandell, D. W., Sandberg, M. A. & Berson, E. L. (1990) *Nature (London)* **343**, 364–366.
3. Dryja, T. P., McGee, T. L., Hahn, L. B., Cowley, G. S., Olsson, J. E., Reichel, E., Sandberg, M. A. & Berson, E. L. (1990) *N. Engl. J. Med.* **323**, 1302–1307.
4. Inglehearn, C. F., Bashir, R., Lester, D. H., Jay, M., Bird, A. C. & Bhattacharya, S. (1991) *Am. J. Hum. Genet.* **48**, 26–30.
5. Sung, C.-H., Davenport, C. M., Hennessey, J. C., Maumenee, I. H., Jacobson, S. G., Heckenlively, J. R., Nowakowski, R., Fishman, G., Gouras, P. & Nathans, J. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 6481–6485.
6. Nathans, J., Thomas, D. & Hogness, D. S. (1986) *Science* **232**, 193–202.
7. Nathans, J. & Hogness, D. S. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 4851–4855.
8. Gorman, C., Gies, D. R. & McCray, G. (1990) *DNA Protein Eng. Techniques* **2**, 3–9.
9. Nathans, J. (1990) *Biochemistry* **29**, 937–942.
10. Nathans, J., Weitz, C. J., Agarwal, N., Nir, I. & Papermaster, D. S. (1989) *Vision Res.* **29**, 907–914.
11. Burnette, W. N. (1981) *Anal. Biochem.* **112**, 195–203.
12. Adamus, G., Arendt, A., Zam, Z. S., McDowell, J. H. & Hargrave, P. A. (1988) *Peptide Res.* **1**, 42–47.
13. Hodges, R. S., Heaton, R. J., Parker, J. M. R., Molday, L. & Molday, R. S. (1988) *J. Biol. Chem.* **263**, 11768–11775.
14. Kingsley, R. E. & Cole, N. L. (1988) *J. Electron Microsc. Tech.* **10**, 77–85.
15. Altman, L. G., Schneider, B. G. & Papermaster, D. S. (1984) *J. Histochem. Cytochem.* **32**, 1217–1223.
16. Papermaster, D. S., Schneider, B. G., Zorn, M. A. & Kraehenbuhl, J. P. (1978) *J. Cell Biol.* **77**, 196–210.
17. Schneider, B. G. & Papermaster, D. S. (1983) *Methods Enzymol.* **96**, 485–495.
18. Wald, G. & Brown, P. K. (1958) *Science* **127**, 222–226.
19. Deretic, D. & Papermaster, D. S. (1991) *J. Cell Biol.*, in press.
20. Hargrave, P. A. (1982) *Prog. Retinal Res.* **1**, 1–51.
21. Kornfeld, R. & Kornfeld, S. (1985) *Annu. Rev. Biochem.* **54**, 631–664.
22. Goldman, B. M. & Blobel, G. (1981) *J. Cell Biol.* **90**, 236–242.
23. Doi, T., Molday, R. S. & Khorana, H. G. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 4991–4995.
24. Karnik, S. S. & Khorana, H. G. (1990) *J. Biol. Chem.* **265**, 17520–17524.
25. Hobbs, H. H., Russell, D. W., Brown, M. S. & Goldstein, J. L. (1990) *Annu. Rev. Genet.* **24**, 133–170.
26. Cheng, S. H., Gregory, R. J., Marshall, J., Paul, S., Souza, D. W., White, G. A., O'Riordan, C. R. & Smith, A. E. (1990) *Cell* **63**, 827–834.
27. Klausner, R. D. & Sitia, R. (1990) *Cell* **62**, 611–614.