and picric acid (3%) in phosphate buffer. Transplants were dissected and sectioned on a cryostat. Sections were washed in phosphate-buffered saline (PBS) and incubated with antibody to rat IgG for 1 hour, washed, and incubated again with a monoclonal antibody against ChAT (Boeringer Mannheim; 1:10 in PBS with 0.2% Triton X-100) for 48 hours. They were washed and incubated with antibody to rat IgG conjugated with fluorescein (1:10 in PBS with 0.2% Triton X-100). Sections were washed and mounted in glycerin-PBS (9:1) and studied with an epi-illumination Nikon fluorescence microscope. With the use of a nonparametric measure of cholinergic neuron density (0 to 4) and ratings by a blinded observer, cholinergic neuron density in the conjugate group was determined to be 3.65 ± 0.71. There were no significant differences in the control groups; the average cholinergic neuron density was 1.14 ± 0.22.

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24. Consideration of the role of transport of NGF from the circulation to the graft via the vasculature of the ciliary epithelium, with subsequent diffusion through the aqueous humor, is very relevant because the ciliary epithelium has been shown to possess several properties of brain blood vessels [S. I. Harik *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 4261 (1990); G. Raviola, *Exp. Eye Res.* (suppl.) **25**, 27 (1977); G. Raviola and J. Butler, *Invest. Opthalmol. Visual Sci.* **25**, 827 (1984); M. Kupersmith and M. Shakib, in *Implications of the Blood-Brain Barrier and its Manipulation*, E. A. Neuwelt, Ed. (Plenum, New York, 1989), pp. 369–390], although the existence of transferrin receptors on ciliary vessels has not been examined. Several considerations suggest this mechanism is less likely than direct delivery through the brain graft vasculature. First, as shown by direct measurement [M. Kottler *et al.*, *Invest. Ophthalmol.* **9**, 758 (1970); F. J. Macri and J. O'Rourke, *Arch. Ophthalmol.* **83**, 741 (1970)], the aqueous humor turns over rapidly, with a half-life of substantially less than 1 hour. Thus, this route would be an inefficient means to deliver materials to the brain transplant, considering the rapid loss of the conjugate from the bloodstream. Second, as shown by direct histological examination, the transplants also develop a pia mater and glia limitans surface covering like intact brain. These membranes would further constitute diffusional barriers from the aqueous humor to the transplant. Third, in order to achieve trophic effects on the survival of medial septal brain transplants (*10*) direct intraocular administration of approximately 1 μM doses of NGF were required. In our experiments, the NGF conjugate concentration in the blood was in the range of only 10 to 100 nM, and our direct measurements of NGF brain levels after systemic administration of the conjugate only approximate a 20 to 40 pM level. Thus, greater than 1000 times more NGF was required for comparable effects when placed directly into the anterior chamber, which further supports the conclusion that within the anterior chamber a diffusional barrier exists for large molecules to pass into the transplant and that specific transport of the NGF conjugate across the BBB via the transferrin receptor is a much more efficient process. Still higher doses of NGF were required for medial septal neuron rescue in vivo after intracerebroventricular administration (*4, 5*).
25. We thank S. Putney, R. Pops, and R. Starzyk for helpful discussions and critical reading of this manuscript. Supported in part by a Small Business Innovation Research (SBIR) grant from NIH (NS29601-01).

24 August 1992; accepted 10 November 1992

Retinal Degeneration in Choroideremia: Deficiency of Rab Geranylgeranyl Transferase

Miguel C. Seabra, Michael S. Brown, Joseph L. Goldstein

Rab geranylgeranyl transferase (GG transferase) is a two-component enzyme that attaches 20-carbon isoprenoid groups to cysteine residues in Rab proteins, a family of guanosine triphosphate–binding proteins that regulate vesicular traffic. The mutant gene in human choroideremia, an X-linked form of retinal degeneration, encodes a protein that resembles component A of rat Rab GG transferase. Lymphoblasts from choroideremia subjects showed a marked deficiency in the activity of component A, but not component B, of Rab GG transferase. The deficiency was more pronounced when the substrate was Rab3A, a synaptic vesicle protein, than it was when the substrate was Rab1A, a protein of the endoplasmic reticulum. The data imply the existence of multiple component A proteins, one of which is missing in choroideremia.

Choroideremia (CHM) is an X-linked form of retinal degeneration that is subsumed under the broad classification of retinitis pigmentosa (*1*). Affected males experience night blindness in their teens, which usually progresses to tunnel vision or complete blindness by middle age. Histologically, there is degeneration of the retinal pigment epithelium and its two adjacent cell layers, the choroid which contains the blood vessels, and the retinal photoreceptor cells. Which of these three layers is the primary site of the disease is not known. Carrier females are generally asymptomatic, but they have patchy pigmentation and degeneration of the pigment epithelium and choroid consistent with the presence of clonal areas of disease attributable to random X-inactivation.

The defective gene in CHM was localized initially by linkage analysis and identified by positional cloning on the basis of information on deletions and translocations surrounding the locus at the chromosome Xq21 band (*2, 3*). Analysis of a partial cDNA encoded by the CHM gene isolated from a retinal cDNA library (*2, 3*) revealed that the CHM mRNA is expressed in cell types other than those of the retina, including Epstein-Barr virus–immortalized B lymphoblasts. The CHM 5.4-kb mRNA encodes a protein of more than 395 amino acids (*2, 3*) whose function has heretofore been unknown. The sequence of the CHM protein from the human and the mouse resembles that of the bovine protein designated *smg*p25/Rab3A GDI (*4, 5*), which was identified and purified (*6*) on the basis of its ability to inhibit the exchange of guanosine triphosphate (GTP) for guanosine diphosphate (GDP) bound to Rab3A, a low molecular weight guanine nucleotide-binding protein present in synaptic vesicles (*7*).

The Rab family includes more than 20 sequence-related proteins (~25 kD) that are bound to the cytoplasmic surfaces of specific membranous organelles in all eukaryotic cells (*7*). By analogy to other guanine nucleotide-binding proteins, the Rab proteins are considered inactive in their GDP-bound form (*7*). When activated, the GDP is exchanged for GTP, and the Rab proteins then participate in the process by which membranous vesicles fuse with each other in a highly selective and unidirectional fashion. The Rab proteins become inactivated when they hydrolyze the bound GTP to GDP, allowing the cycle to be repeated (*7*).

In order for Rab proteins to bind to membranes, they must contain a covalently bound hydrophobic prenyl group, generally the 20-carbon isoprenoid geranylgeranyl (GG) (*8, 9*). Shortly after Rab proteins are translated, one or more GG groups are attached in thioether linkage to cysteine residues at or near the COOH-terminus (*8*). Rab3A, which terminates in Cys-Ala-Cys, contains GG groups on each of these cysteines (*8*). Rab1A, a protein of the endoplasmic reticulum, contains at least one GG group on its COOH-terminal Cys-Cys sequence (*8*).

Prenylation of Rab3A and Rab1A is catalyzed by a two-component enzyme called Rab geranylgeranyl transferase (GG transferase) that has been purified from rat brain (*10, 11*). Component B of this enzyme consists of two (60 and 38 kD) (*10*) tightly associated polypeptides whose amino acid sequences resemble those of the α and β subunits, respectively, of $p21^{ras}$ farnesyltransferase, another prenyltransferase (*12*). Component A of Rab GG transferase, which has no counterpart in the farnesyltransferase, is a 95-kD polypeptide (*11*). Component B has little, if any GG transferase activity in the absence of component A (*10, 11*).

Although the sequences of six peptides

Department of Molecular Genetics, University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Boulevard, Dallas, TX 75235.

from purified component A of the rat brain Rab GG transferase resemble those of the human CHM gene product, they resemble those of the mouse even more (71 out of 78 residues identical) (*11*). This comparison suggests that the CHM gene encodes the human counterpart of component A of rat Rab GG transferase (*11*). We now describe our test of this hypothesis.

Cytosolic extracts of lymphoblasts (*13*) from two control subjects (*14*) transferred [^{3}H]GG from [^{3}H]geranylgeranyl pyrophosphate ([^{3}H]GGPP) to Rab3A as assayed by precipitation with ethanol and HCl (Fig. 1A). The activity rose (nonlinear) as the concentration of lymphoblast extract was increased. This sigmoidal pattern is to be expected if the extract contained two loosely associated components, both of which are required for Rab GG transferase activity. Lymphoblast extracts from four subjects with CHM (*14*) showed less Rab GG transferase activity than the normal extracts (Fig. 1A), and we were able to estimate that the CHM extracts had approximately 25% of the normal amount of Rab GG transferase activity. The experiment was repeated, and another control subject was included (Fig. 1B). The residual activity in the CHM cells, although reduced, was still dependent on the Rab3A substrate (Fig. 1B, inset).

To determine whether the low Rab GG transferase activity in the CHM cell extracts is attributable to a deficiency of component A, we made a semiquantitative assay of the activity of component A in the presence of increasing concentrations of exogenously added purified component B. To standardize this assay, we used purified components A and B from rat brain as well as a Rab3A substrate (Fig. 2, A and B) and a Rab1A substrate (Figs. 2, C and D). With Rab3A, component B had no GG transferase activity in the absence of component A (Fig. 2A). With increasing amounts of component A, the concentration curves for

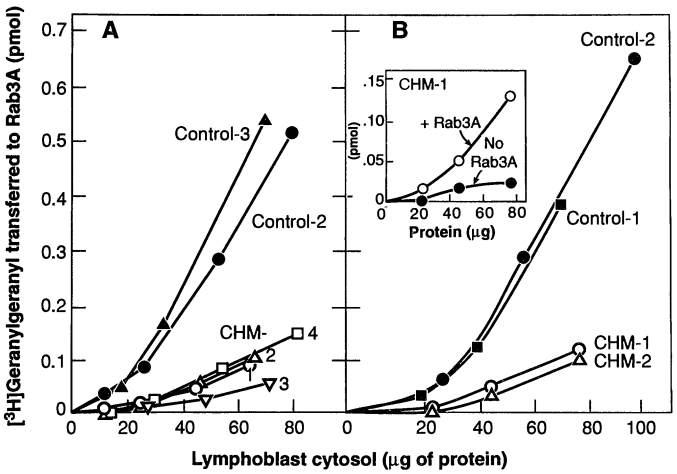

Fig. 1. Geranylgeranylation of Rab3A by lymphoblast extracts from three normal subjects (Controls 1–3) and four subjects with choroideremia (CHM 1–4). Lymphoblast extracts were prepared (*13, 14*), and Rab GG transferase activity was determined by measuring the amount of [^{3}H]geranylgeranyl transferred from [^{3}H]GGPP (American Radiolabeled Co.), to Rab3A (*10*). Unless otherwise indicated, the standard reaction mixture contained the following concentrations of components in a final volume of 50 μl: 50 mM sodium Hepes (pH 7.2), 5 mM $MgCl_2$, 0.3 mM Nonidet P40, 3 mM dithiothreitol, 2 μM recombinant bovine Rab3A (*10*), 0.5 μM [^{3}H]GGPP (33,000 dpm/pmol), and varying concentrations of 10^5g cytosolic extract (*13*) prepared from permanent lymphoblast cell lines from the indicated subject (*14*). After incubation for 30 min at 37°C, the reaction mixtures were precipitated by the addition of 0.5 ml of ethanol and HCl (9:1, v/v), followed by incubation for at least 15 min at room temperature (to hydrolyze unreactive [^{3}H]GGPP) (*21*) and filtration on a 2.4-cm glass fiber filter (Fisher). Each assay tube was rinsed once with 2 ml of 100 percent ethanol, and each filter was washed three times with ethanol and counted in a scintillation counter. Background values measured in parallel reactions without cell extract—0.023 (**A**) and 0.025 pmol (**B**)—were subtracted. Each value represents the average of duplicate incubations. The inset shows assays of CHM-1 extracts that were carried out in the absence (●) or presence (○) of Rab3A. The protein contents of Rab3A and lymphoblast extracts were measured by the Lowry method (*22*).

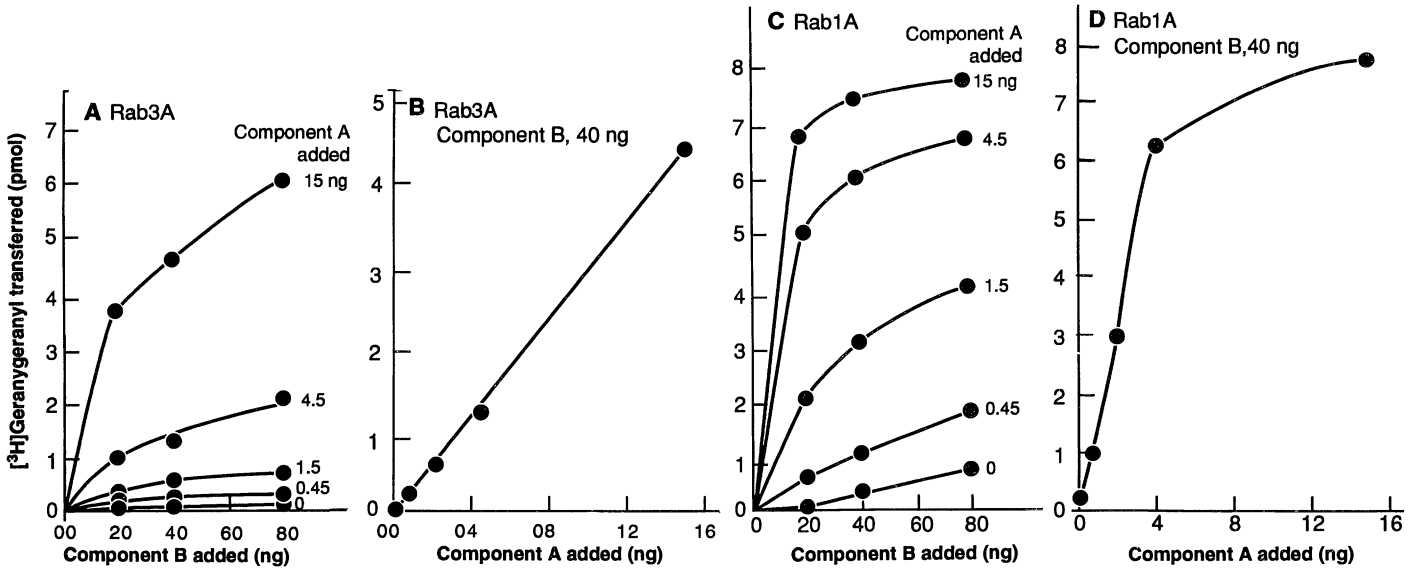

Fig. 2. Geranylgeranylation of Rab3A (**A**) and Rab1A (**C**) by purified rat brain Rab GG transferase: Dependence on concentrations of components A and B. Each reaction mixture contained either 2 μM recombinant bovine Rab3A or canine Rab1A (*11*) as indicated, 0.5 μM [^{3}H]GGPP (33,000 dpm/pmol), and the indicated concentration of purified components A and B of rat Rab GG transferase. Duplicate reactions were carried out for 30 min at 37°C, after which the radioactivity precipitable by ethanol was measured as in Fig. 1. A background value determined in parallel reactions without components A and B (0.03 pmol) was subtracted. Components A and B of Rab GG transferase were purified from rat brain, and their protein contents were estimated as previously described (*10, 11*). (**B**) and (**D**) show a plot of the amount of [^{3}H]GG transfer compared to the amount of component A added to a fixed amount (40 ng) of component B.

component B became progressively more steep (Fig. 2A). These data were then plotted as a function of the concentration of component A at one standardized concentration of component B (40 ng) (Fig. 2B). This plot indicated a linear relation between the amount of component A and the amount of [^{3}H]geranylgeranyl transferred, thus allowing an estimation of the amount of component A in an unknown sample. Similar data were obtained with Rab1A as a substrate (Fig. 2C) except that with this substrate component B had a low intrinsic activity, and the rate of the reaction increased steeply as the concentration of component A increased (Fig. 2D). These data indicate that our preparation of component B may have been contaminated with a trace amount of component A. It is not clear why Rab1A is much more sensitive to low levels of component A than is Rab3A since the affinity of the holoenzyme toward both substrates is similar (*10, 11*).

This semiquantitative assay was applied to extracts of lymphoblasts from one control subject and one subject with CHM (Fig. 3). With Rab3A as substrate and increasing concentrations of purified component A, the activity of the CHM and control cell extracts was similar (Fig. 3A), indicating that both extracts had similar amounts of component B. When the concentration of component B was varied, the activity of the control cells was 16 times greater, whereas that of the CHM cells rose only slightly, indicating a functional deficiency of component A (Fig. 3B). With Rab1A as a substrate, the CHM cells also showed normal activity in the presence of added component A and deficient activity with added component B (Fig. 3, C and D), but the deficiency was not nearly so pronounced as observed with Rab3A as substrate.

We next conducted quantitative assays for the activities of component A and B with Rab3A as substrate using varying amounts of extracts from lymphoblasts of all four CHM patients and three control subjects supplemented with either component A or B (Fig. 4). In contrast to the sigmoid concentration curves with unsupplemented extracts (Fig. 1), the activities of the supplemented extracts increased linearly as the amount of extract increased, indicating that the sigmoidicity was indeed attributable to the necessity of the extracts to supply both components A and B. As an additional control for the specificity of the defect in CHM, we studied lymphoblasts from two patients with Usher syndrome type II, an autosomal recessive form of retinal degeneration that resembles choroideremia but is associated with sensory nerve deafness and is caused by a mutation that maps to the 1q31-qter region of chromosome 1 (*15*). Lymphoblast extracts from all three groups of subjects responded similarly in the presence of added component A, indicating that they all had similar amounts of component B activity (Fig. 4, experiments A). Lymphoblast extracts from all four CHM subjects showed reduced Rab GG transferase activity in the presence of added component B (Fig. 4, experiments B), indicating a functional deficiency of

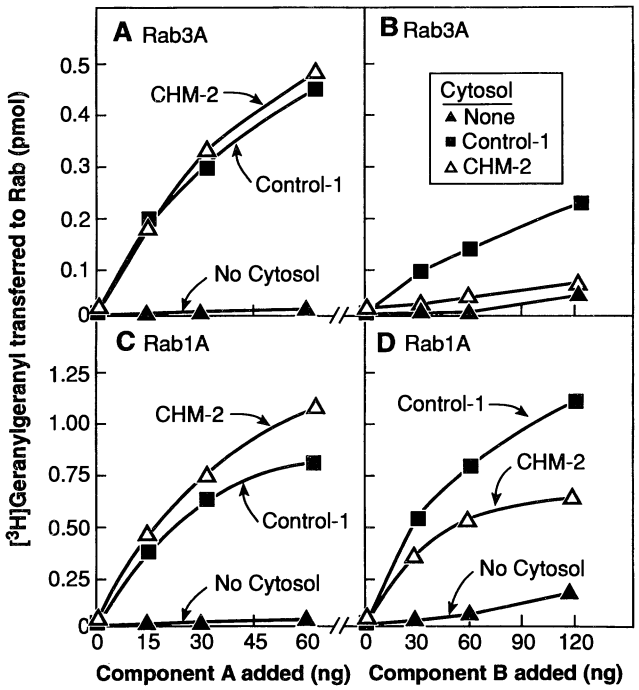

Fig. 3 (left). Rab GG transferase activity in lymphoblast extracts: Stimulation by components A and B. Each reaction mixture contained either 2 μM recombinant Rab 3A (**A** and **B**) or Rab1A (**C** and **D**) as indicated, 0.5 μM [^{3}H]GGPP (33,000 dpm/pmol), 7 μg of protein (*13*) from the indicated lymphoblast cell line (*14*), and the indicated concentration of either purified component A (A and C) or component B (B and D). Duplicate reactions were carried out for 30 min at 37°C, after which the radioactivity precipitable by ethanol was measured as in Fig. 1. Background values measured in parallel reactions without extract orcomponents A and B—0.021 pmol in (A and B) and 0.034 in (C andD)—were subtracted.

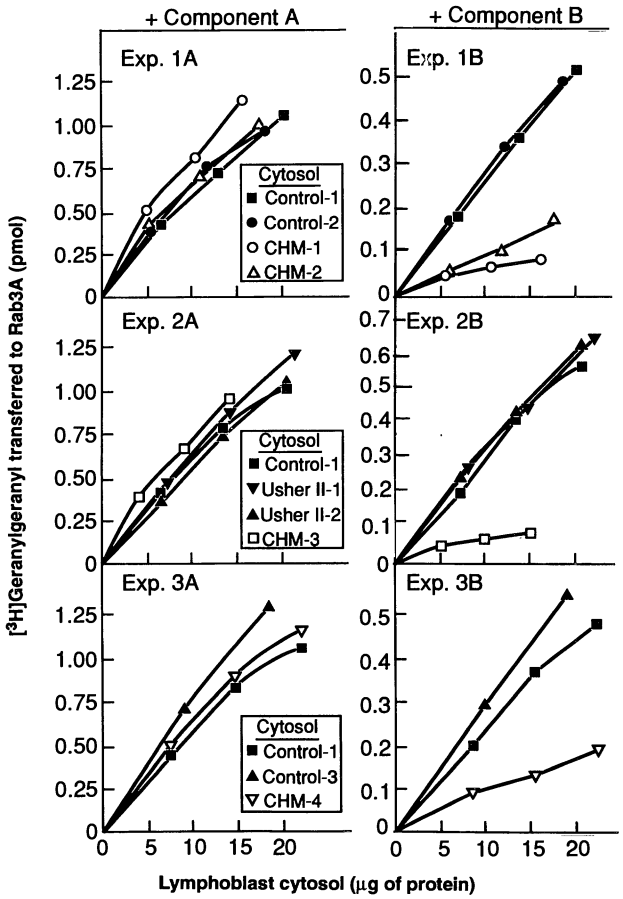

Fig. 4 (right). Deficiency of component A of Rab3A GG transferase in lymphoblast extracts from choroideremia subjects. Each reaction mixture contained 2 μM recombinant Rab3A, 0.5 μM [^{3}H]GGPP (33,000 dpm/pmol), the indicated concentration of protein (*13*) from the indicated lymphoblast cell line (*14*), and either 30 ng of component A (experiments 1A, 2A, and 3A) or 40 ng of component B (experiments 1B, 2B, and 3B) as indicated. Duplicate reactions were carried out for 30 min at 37°C, after which the radioactivity precipitable by ethanol was measured as in Fig. 1. Background values measured in parallel reactions containing component A (0.017 to 0.031 pmol in experiments 1A, 2A, and 3A) or component B (0.048 to 0.059 in experiments 1B, 2B, and 3B) in the absence of cytosol were subtracted.

component A. Lymphoblast extracts from the two individuals with Usher syndrome type II showed normal activity of component A (Fig. 4, experiment 2B).

Using the slopes of the lines from Fig. 4 and the standard curve for component A activity shown in Fig. 2B, we obtained a rough estimate of the amount of component A activity in the control lymphoblast extracts. We calculated that each 20 μg of crude lymphoblast extract protein transferred approximately 0.5 pmol of [^{3}H]GG per 30 min to Rab3A in the presence of 40 ng of purified component B. This is equivalent to the activity obtained with approximately 1 ng of purified component A (Fig. 2B). Thus, component A accounts for ~1/20,000th of the protein in the cell extract, which is similar to the calculated amount of component A in rat brain (a purification of 30,000-fold was required) (*11*). When we use the data with Rab1A as a substrate (Figs. 2D and 3D), we reach a similar conclusion, that is, 20 μg of lymphoblast extract protein contained about 1 ng of component A activity. In the CHM lymphoblasts with Rab3A as a substrate, the amount of component A activity was reduced by 75 to 80 percent (Fig. 4). With Rab1A as a substrate, the reduction in component A activity was only about 25 percent (Fig. 3D). Although results with only one CHM patient are shown in Fig. 3, we observed a similar 20 to 30 percent deficiency of component A activity with Rab1A as substrate when all four CHM cell lines were studied.

To further confirm the specificity of the defect in component A in CHM, we assayed lymphoblast extracts for protein farnesyltransferase activity using $p21^{H\text{-}ras}$ as a substrate (*16*). The activity in the four CHM cell lines (from 40 to 94 pmol of [^{3}H]farnesyl transferred from [^{3}H]farnesyl pyrophosphate in 30 min per milligram of protein) did not differ from those in the three control lymphoblast lines (35 to 67 pmol per 30 min per milligram of protein) or the two Usher syndrome type II lines (34 and 56 pmol per 30 min per milligram of protein).

Our data confirm a functional deficiency of component A of Rab GG transferase in lymphoblasts from patients with CHM and support the notion that the CHM gene encodes at least one form of component A. Three of the four CHM lymphoblast lines in our study were obtained from individuals with large deletions of their CHM gene and no detectable expression of CHM mRNA in lymphoblasts (*14*). The finding that these mutant lymphoblasts showed residual component A activity indicates that lymphoblasts must have at least one additional protein with component A activity that is encoded by a gene distinct from the CHM locus. Furthermore, the observation that the defect in the CHM cells is more severe with Rab3A than with Rab1A supports the possibility that there is a family of component A proteins, each having different activity with respect to different Rab proteins. Indeed, Cremers *et al.* (5) recently identified a gene on chromosome 1 that encodes a 656–amino acid protein that shows 76 percent sequence identity with the X chromosome–encoded partial CHM gene product of 395 amino acids. This gene, designated CHML (choroideremia-like), maps to the 1q31-qter region of chromosome 1, which is also the map location of the Usher syndrome type II (*15*). It is not yet known whether this disease is caused by mutations in the CHML gene nor whether this gene encodes a protein with component A activity.

The presence of residual component A activity may also explain why CHM subjects have a disease that is restricted in its clinical manifestations to the retina and choroid. Total loss of all Rab GG transferase activity from all cell types would likely be fatal in utero. The confinement of the symptomatic disease to the retina and choroid suggests that these tissues have a special requirement for the form of component A that is encoded by the CHM gene.

At this stage in our knowledge, at least four models for the pathogenesis of CHM can be advanced.

1) The retinal photoreceptors, pigment epithelium, or choroid (or any combination thereof) may have a high concentration of a particular Rab substrate, for which the CHM version of component A has the highest affinity. Indeed, the component A deficiency in the CHM cells is more complete for Rab3A, a neural-specific protein, than it is for Rab1A, which is expressed in multiple cell types. If Rab3A or a related Rab performs a crucial function in retinal pigment epithelial or photoreceptor cells (or both), then CHM may be attributable to a failure to adequately geranylgeranylate this protein.

2) The retinal photoreceptor and pigment epithelial cells with their extremely high rates of membrane turnover (*17*) may have large requirements for multiple Rab proteins and may be unusually sensitive to a deficiency of any one of several component A–like proteins.

3) The failure to geranylgeranylate one or more Rab proteins in a normal fashion may lead to the accumulation of unprenylated Rab proteins, one of which may block the function of other normally prenylated Rab proteins, perhaps by competing for Rab binding proteins.

4) Component A may be required for the geranylgeranylation of a non–Rab protein that is crucial for long-term survival of the retina and choroid. The Rab GG transferase prefers substrates that terminate in Cys-Cys or Cys-X-Cys sequences, where X may be any amino acid. The enzyme also requires certain as-yet-undefined upstream sequences in Rab proteins (*11*). It is possible that proteins other than Rab proteins share these recognition sequences and are thus substrates for Rab GG transferase, requiring the action of component A.

The defect in component A of Rab GG transferase is one of several causes of retinitis pigmentosa to be defined biochemically. The most common defects consist of substitutions or small in-frame deletions in the rhodopsin gene, producing an autosomal dominant form of the disease (*18*). Another type of dominant mutation inactivates the gene encoding a protein called peripherin or RDS (for retinal degeneration slow), which is located at the periphery of the rod discs (*19*). These two forms of retinitis pigmentosa differ from choroideremia predominantly by lesser involvement of the choroid. In an autosomal recessive form of atypical retinitis pigmentosa called gyrate atrophy, the mutation inactivates an enzyme, ornithinine aminotransferase (*20*). Gyrate atrophy, like choroideremia, is associated with early degeneration of the choroid.

In a more general sense, the apparent multiplicity and functional redundancy of component A genes creates a situation in which defects in any of them might not cause a lethal phenotype, but rather a degenerative disease of the organ in which that particular form of component A is most essential. It will be of interest to isolate the genes encoding the other component A proteins and to determine whether mutations in these genes underlie degenerative diseases of the nervous system or other organs.

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13. Lymphoblasts (*14*) were maintained in suspension culture at 37°C in a 5% CO_2 incubator in RPMI 1640 medium supplemented with 15% (v/v) heat-inactivated fetal calf serum, 1 mM sodium pyruvate, penicillin G at 100 unit/ml, and streptomycin at 100 μg/ml. On day 0, 1 × 10^7 cells were suspended in a 75-cm^2 flask containing 20 ml of the above medium with 10% fetal calf serum. On day 2, each flask received 20 ml of additional fresh medium of identical composition. On day 3, the cells in each flask were collected by centrifugation (2000 rpm, 10 min, 4°C), washed with 8 ml of ice-cold Dulbecco's phosphate-buffered saline (without Ca^{2+} and Mg^{2+}), and centrifuged as above. The cell pellet was frozen at −70°C and used within 2 weeks. To prepare a cell extract, each thawed pellet was resuspended in 0.5 to 1.5 ml of ice-cold buffer containing 50 mM sodium Hepes (pH 7.2), 0.3 mM Nonidet-P40, 1 mM dithiothreitol, and 10 mM NaCl. The suspension was passed ten times through a 21-gauge needle and then centrifuged at $10^5 g$ for 1 hour at 4°C. The supernatant was used for all enzyme assays.
14. Permanent lymphoblast cell lines were established by Epstein-Barr virus transformation of blood lymphocytes. Cell lines from normal subjects were established in Dallas and designated as follows: Control-1 (male, age 26; culture designation L430); control-2 (female, age 24; L429); control-3 (male, age 38; L389). Cell lines from CHM subjects were established by R. Nussbaum in Philadelphia or by F. Cremers in Nijmegen, the Netherlands, and are designated as follows: CHM-1 [male, age 26; patient 45-06 in (*3*); large deletion of CHM gene with no detectable mRNA; culture L659]; CHM-2 [male, age 45; patient 95-02 in (*3*); no rearranged CHM gene by Southern gel analysis, but reduced mRNA; culture L660]; CHM-3 (male, age 54; patient 7.6 in (*2*); large deletion with no detectable mRNA; culture L665); CHM-4 [male, age 35; patient 25.6 in (*2*); large deletion with no detectable mRNA; culture L669]. Cell lines from Usher syndrome type II were established by S. D. van der Velde-Visser, A. van Aarem, and C. Cremers in Nijmegen and designated as follows: Usher II-1 (male, age 59; culture L663); and Usher II-2 (male, age 52; culture L664).
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10 September 1992; accepted 10 November 1992

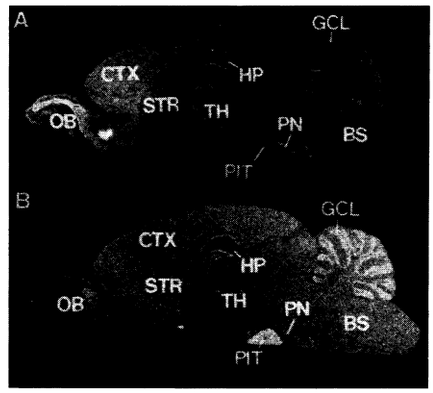

Fig. 1. In situ hybridization of (**A**) heme oxygenase-2 and (**B**) cytochrome P-450 reductase mRNA in brain sagittal sections. Ribonuclease (RNase) pretreatment of sections (10 μg ml^{-1} for 1 hour at 37°C) and 100-fold excess of unlabeled probe each abolished labeling, which indicates specificity. A high stringency wash (70°C) did not reduce labeling. Abbreviations: OB, olfactory bulb; CTX, cortex; STR, striatum; TH, thalamus; HP, hippocampus; PN, pontine nucleus; PIT, pituitary; GCL, granule cell layer; and BS, brain stem.

Carbon Monoxide: A Putative Neural Messenger

Ajay Verma, David J. Hirsch, Charles E. Glatt, Gabriele V. Ronnett, Solomon H. Snyder*

Carbon monoxide, an activator of guanylyl cyclase, is formed by the action of the enzyme heme oxygenase. By in situ hybridization in brain slices, discrete neuronal localization of messenger RNA for the constitutive form of heme oxygenase throughout the brain has been demonstrated. This localization is essentially the same as that for soluble guanylyl cyclase messenger RNA. In primary cultures of olfactory neurons, zinc protoporphyrin-9, a potent selective inhibitor of heme oxygenase, depletes endogenous guanosine 3′,5′-monophosphate (cGMP). Thus, carbon monoxide, like nitric oxide, may be a physiologic regulator of cGMP. These findings, together with the neuronal localizations of heme oxygenase, suggest that carbon monoxide may function as a neurotransmitter.

Abundant evidence indicates that nitric oxide (NO) is a physiologic messenger molecule that mediates the regulation of blood vessel tone (it is also known as endothelial-derived relaxing factor). NO also regulates the tumoricidal and bactericidal actions of macrophages and serves as a putative neurotransmitter in the central and peripheral nervous systems (*1*). Molecular cloning reveals that distinct proteins account for neuronal (*2*), macrophage (*3*), and endothelial (*4*) NO synthase (NOS). NO is a short-lived free radical gas that can activate soluble guanylyl cyclase by binding tightly to the heme moiety of the enzyme (*1*). If a normally noxious gas such as NO is a physiologic messenger, perhaps other gases serve similar functions. Our clone of brain NOS revealed close homology with only one other mammalian protein, cytochrome P-450 reductase (*2*). Cytochrome P-450 reductase is best known as the source of electrons for the P-450 class of enzymes, although it also serves as the electron donor for heme oxygenase, which degrades heme to biliverdin and releases carbon monoxide (CO) in the process (*5–7*). Like NO, CO is a noxious gas that activates guanylyl cyclase (*8*). In this study, we used in situ hybridization to examine the localization of mRNA for heme oxygenase-2, the constitutive form of heme oxygenase in the brain, and the ability of heme oxygenase in neuronal cultures to regulate cGMP.

Two forms of heme oxygenase have been characterized (*9*). Heme oxygenase-1 is induced by heme and is expressed at high concentrations in the spleen and liver, where it is responsible for destruction of heme from red blood cells. Heme oxygenase-2 is not inducible and is widely distributed throughout the body with high concentrations in the brain (*9*). Our initial in situ hybridization studies failed to detect heme oxygenase-1 in rat brain, which agrees with previous findings that this protein is not normally evident in the brain [although after heat shock some heme oxygenase-1 is expressed in glia and certain neurons (*10*)]. By contrast, we observed heme oxygenase-2 mRNA in discrete sites

A. Verma, Department of Neurology, Walter Reed Army Medical Center, Washington, DC 20307.
D. J. Hirsch, C. E. Glatt, S. H. Snyder, Departments of Neuroscience, Pharmacology and Molecular Sciences, and Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD 21205.
G. V. Ronnett, Departments of Neurology and Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

*To whom correspondence should be addressed.