REFERENCES AND NOTES

1. R. Baserga, *The Biology of Cell Reproduction* (Harvard University Press, Cambridge, MA, 1985), pp. 71–75, 98.
2. R. T. Johnson and P. N. Rao, *Nature (London)* **226**, 717 (1970).
3. S. I. Matsui, H. Yoshida, H. Weinfeld, A. A. Sandberg, *J. Cell Biol.* **54**, 120 (1972).
4. T. Nishimoto, E. Eilen, C. Basilico, *Cell* **15**, 475 (1978).
5. T. Nishimoto *et al.*, *J. Cell. Physiol.* **109**, 299 (1981).
6. R. A. Walters, R. A. Tobey, C. E. Hildebrand, *Biochem. Biophys. Res. Commun.* **69**, 212 (1976).
7. J. H. Tjio and T. T. Puck, *J. Exp. Med.* **108**, 259 (1958).
8. P. N. Rao *et al.*, *J. Cell. Physiol.* **91**, 131 (1977).
9. F. M. Davis, T. Y. Tsao, S. K. Fowler, P. N. Rao, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 2926 (1983).
10. D. M. Prescott, in *Progress in Nucleic Acid Research and Molecular Biology*, J. N. Davidson and W. E. Cohn (Academic Press, New York, 1964), p. 33.
11. Very high doses of caffeine (25 to 50 m*M*) have been shown to cause clumping of interphase chromatin in exponentially growing cells [V. M. Borodina *et al.*, *Exp. Cell Res.* **122**, 391 (1979); S. Ghosh and I. Ghosh, *Naturwiss enschaften* **59**, 277 (1972)]. We believe our results to be unrelated to the cellular response these authors described since they saw no changes in the cytoplasmic morphology or the nuclear membrane and they did not examine chromosome morphology. Our finding that 5 m*M* caffeine did not induce PCC in exponentially growing BHK cells is consistent with this interpretation.
12. R. B. Painter, *J. Mol. Biol.* **143**, 289 (1980).
13. S. K. Das, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **44**, 1141 (1985).
14. R. Schlegel and A. B. Pardee, unpublished data.
15. G. M. Donnelly and J. E. Sisken, *Exp. Cell Res.* **46**, 93 (1967).
16. R. A. Tobey and H. A. Crissman, *ibid.* **75**, 460 (1972).
17. J. L. Hamlin and A. B. Pardee, *ibid.* **100**, 265 (1976).
18. This work was aided by NIH grant CA 22427 to A.B.P. We thank S. Norris for technical assistance and J. Rheinwald, D. Coppock, R. Craig, and T. Woodford for their critical reviews. A special thanks to Dr. Rheinwald for the use of his camera-equipped microscopes and to Dr. P. N. Rao for his generous gift of monoclonal antibody MPM-2.

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Visual Pigment Homologies Revealed by DNA Hybridization

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A bovine rhodopsin complementary DNA probe was used to detect homologous visual pigment genes in a variety of species. Under stringent DNA hybridization conditions, genomic DNA from most vertebrate species carried a single homologous fragment. Additional homologies were detected in some vertebrates by reducing the hybridization stringency. Homologous fragments were also detected in DNA isolated from invertebrate species, a unicellular alga, and an archaebacterium; many of these fragments were homologous to a *Drosophila* opsin probe. These results suggest that photosensory pigments in a wide variety of species arose from a common precursor.

VISUAL PIGMENTS ARE A CLASS OF receptor proteins that absorb light and trigger sensory signals. Rhodopsins, the visual pigments in vertebrate rod-type photoreceptor cells, consist of an intrinsic membrane protein (opsin) covalently linked to a vitamin A chromophore (*1*). Other classes of visual pigments, including cone-type photoreceptor pigments and invertebrate visual pigments, have been less well characterized biochemically because of their low abundance and instability. As an alternative to studying visual pigments at the protein level, the genes encoding these proteins can be identified, their sequences can be determined, and the comparative genetic information can be assessed. We explored the potential for using a bovine opsin complementary DNA (cDNA) probe to identify homologous genes in other species. Using genomic Southern blot hybridization (*2*), we probed the genomes of a variety of species in a manner analogous to that reported for other protein families (*3*). Our results demonstrate that bovine opsin has coding regions homologous with visual pigment genes of vertebrate, invertebrate, and phototactic unicellular species.

We surveyed a variety of vertebrate species for homologous visual pigment genes under stringent hybridization conditions that allow reannealing of the probe with only closely related sequences (*4*). Genomic DNA isolated (*5*) from human, mouse, cow, sheep, chicken, chameleon, gecko, frog, and goldfish tissues was prepared and hybridized by the method of Southern (*2*) with a radioactive cDNA probe (SP1116) containing the entire coding sequence for bovine opsin (Fig. 1a). Under these conditions, homologous DNA fragments were detected in most of the vertebrate DNA tested (Fig. 2a and Table 1). With one possible exception—goldfish—the homologous sequences were confined to a single restriction fragment in the pattern produced by at least one of the restriction endonucleases used. Such a hybridization pattern, combined with an assessment of the intensity of hybridization (lane 1 in Fig. 2a), suggests that the homologous sequences are present as single copies within their respective genomes. The hybridization patterns shown in Fig. 2a and Table 1 for the bovine and human DNA samples are entirely consistent with the restriction maps (*6*) for the corresponding opsin genes shown in Fig. 1, b and c. It is apparent that SP1116 hybridizes specifically to the rod visual pigment genes present as a single copy in these two species. We suggest that the homologies detected for the other vertebrate DNA under these stringent hybridization conditions also represent opsin genes with marked sequence conservation.

The two reptile species included in our survey, the gecko and chameleon, produced some unusual hybridization results. Hybridization of SP1116 to gecko DNA under stringent conditions gave no detectable signal, indicating a lack of strong homology between the gecko and bovine rod visual pigment genes (Table 1). The lack of hybridization to gecko DNA under these conditions cannot be attributed to a large genome size, as reptiles generally have genomes that are smaller than those of mammalian species (*7*). Although both the bovine and gecko retinas contain rod-type photoreceptor cells, gecko rods are unlike the rod cells of other species (*8*). It has been proposed that gecko rods evolved from cone-type photoreceptors by a process of transmutation, and there is corroborating physiological evidence that the gecko rod pigment is more like a cone pigment than an opsin (*8*). Such a hypothesis would explain our inability to detect opsin-like visual pigment genes in this species under stringent hybridization conditions. Conversely, the chameleon *Anolis carolinensis* is considered to lack rod-type photoreceptors altogether (*9*), yet our data show hybridization of SP1116 under stringent conditions (lane 7 in Fig. 2a). Thus we can detect the presence of a gene closely related to bovine opsin in an animal with an all-cone retina. Possible explanations are (i) that the gene encodes a cone visual pigment that is strongly homologous to the rod pigments of other animals, (ii) that the gene is not expressed in the retina, and (iii) that this animal does indeed possess a small population of rod photoreceptor cells that have escaped detection by other methods. A detailed molecular analysis should provide a clearer understanding of

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the nature of the reptilian visual pigment genes.

To determine whether other, more distantly related, sequences could be detected with the bovine opsin probe, some hybridization experiments were performed under reduced stringency conditions. Conditions of low stringency should allow reannealing of SP1116 with genomic fragments bearing as much as 35% base-pair mismatch with the probe sequence (*4*). Figure 2b illustrates that, in addition to the putative opsin genes identified under hybridization conditions of high stringency, a number of weakly hybridizing fragments can be detected in chicken, goldfish, and chameleon DNA with low-stringency conditions and prolonged autoradiographic exposures. Although the nature of these additional hybridizing fragments is unclear, they potentially represent genes that encode proteins related to opsin. Such proteins could include cone visual pigments or opsin-like pigments reported to be present in the iris and brain tissues in some species (*10*). A summary of the data for the various hybridization conditions is shown in Table 1. Interestingly, while it is possible to detect additional homologous fragments under conditions of reduced stringency for most species, the mouse, bovine, and sheep genomes do not demonstrate additional fragments.

After identifying homologies to SP1116 in vertebrate DNA samples, we extended our survey to several invertebrate species. Homologies between vertebrate and invertebrate visual pigments might be predicted on the basis of biochemical similarities between bovine and cephalopod rhodopsins and the fact that the latter can substitute for bovine rhodopsin in triggering activation of the G-protein cyclic guanosine monophosphate phosphodiesterase cascade (*11*). We probed the DNA of three commonly studied invertebrate species—the fruit fly, crayfish, and octopus. As shown in Fig. 3a (see also Table 1), homologous fragments could be detected under low-stringency hybridization conditions in all three species. The potential of SP1116 for identifying invertebrate visual pigment genes is verified by its recent use in the isolation of a *Drosophila melanogaster* opsin gene (*12*). A comparison of the SP1116 and *Drosophila* opsin sequences reveals that the hybridization is driven by short regions with 77 to 89% homology (*12*). The restriction map of the *Drosophila* opsin gene (Fig. 1d) accurately predicts the sizes of the strongest hybridizing fragments in the Southern blots (lanes 1 and 2 in Fig. 3a; see also Table 1). The additional hybridizing fragments in the *Drosophila* blots might represent additional visual pigment genes, as fly retinas are known to contain several different types. Likewise, the homologies detected with SP1116 in the crayfish and octopus DNA (lanes 3 to 5 in Fig. 3a) probably also represent visual pigment genes. Southern blots hybridized with a *Drosophila* opsin probe yielded an identical hybridization pattern (Table 1).

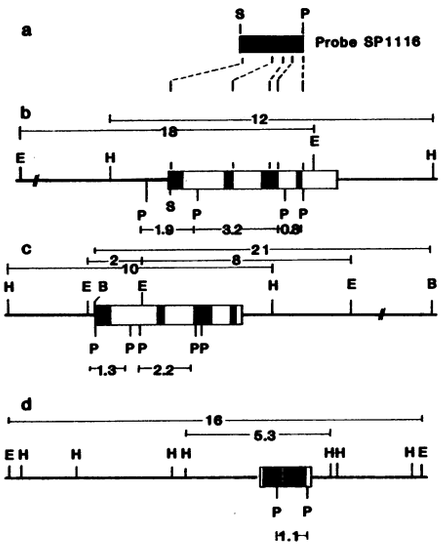
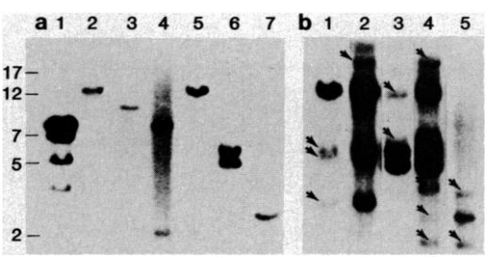


Fig. 1. Structure of the bovine opsin probe and restriction maps of the bovine, human, and *Drosophila* opsin genes. DNA fragment sizes are given in units of kilobase pairs. Untranslated sequences are shown as open boxes and coding regions as closed boxes. (a) The SP1116 probe was constructed from a 2.65-kilobase (kb) cDNA clone (cbr26) isolated in our laboratory (*18*). A Sma I–Pst I restriction fragment containing the full 1044–base pair (bp) coding region plus 40 and 30 bp of 5′ and 3′ flanking sequences, respectively, was joined by blunt-end ligation to Pst I linkers and subcloned. The isolated insert was gel-purified and used as the hybridization probe. (b to d) Restriction maps of the (b) bovine, (c) human, and (d) *Drosophila* opsin genes (*6, 12*). The predicted sizes of the restriction fragments carrying the opsin genes are in agreement with the Southern blot data shown in Figs. 2 and 3 and Table 1. Restriction sites are Eco RI (E), Bam HI (B), Hind III (H), Pst I (P), and Sma I (S).

The homologies between SP1116 and the invertebrate DNA samples, as well as the similarities between the bovine and *Drosophila* opsin genes (*12*), point to a common evolutionary origin for vertebrate and invertebrate visual pigments.

Because many present-day unicellular spe-



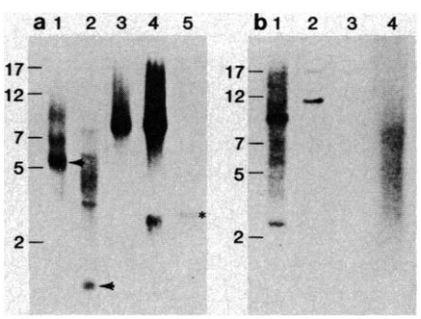


Fig. 2 (left). Southern blot hybridizations of restriction endonuclease–digested vertebrate animal genomic DNA. DNA (10 μg per well) was digested exhaustively with enzyme, subjected to electrophoresis in 0.5% agarose gels, and further processed (*2*). The salt and temperature conditions of the filter hybridization and washing steps are given elsewhere (*4*). The SP1116 probe was nick-translated (Amersham kit N5000) to a specific activity of $>1 \times 10^8$ dpm/μg, denatured, and added to the hybridization solution at a concentration of 25 ng/ml. After hybridization for 36 hours, filters were air-dried and autoradiographed for 1 day. (a) High-stringency hybridizations. Lane 1 is a hybridization control containing 8.2-, 6.8-, 5.4-, and 4.0-kb plasmid constructs of bovine opsin cDNA present at 10, 3, 1, and 0.3 mammalian genomic equivalents, respectively. A 2.7-kb nonrecombinant plasmid was included as a control to ensure that the SP1116 insert was free from possible plasmid contamination (three genomic equivalents). Lane 2, Hind III–digested bovine DNA; lane 3, Bam HI–digested mouse DNA; lane 4, Eco RI–digested human DNA; lane 5, Eco RI–digested chicken DNA; lane 6, Eco RI–digested goldfish DNA; and lane 7, Eco RI–digested chameleon DNA. (b) Low-stringency hybridizations. Arrows indicate weakly hybridizing fragments detectable only under reduced stringency. Lane 1, Eco RI–digested chicken DNA, 1-day exposure [compare with lane 5 in (a)]; lane 2, longer exposure (4 days) of the material represented in lane 1; lane 3, Eco RI–digested goldfish DNA, 1-day exposure [compare with lane 6 in (a)]; lane 4, longer exposure (4 days) of the material represented in lane 3; and lane 5, Eco RI–digested chameleon DNA, 1-day exposure [compare with lane 7 in (a)]. Fig. 3 (right). Low-stringency Southern blot hybridizations of restriction endonuclease–digested nonvertebrate genomic DNA. (a) Invertebrate DNA. Lane 1, Hind III–digested *Drosophila* DNA and lane 2, Pst I–digested *Drosophila* DNA. The major hybridizing fragments, indicated by the arrows, correspond to the published restriction map of the *Drosophila* opsin gene (*12*). The weaker hybridizing fragments might represent additional pigment genes, as fly retinas are known to contain several different visual pigment types. Lane 3, Sst I–digested crayfish DNA, 1-day exposure. Lane 4, a longer exposure (5 days) of the material represented in lane 3, demonstrating the existence of an additional weakly hybridizing fragment. Lane 5, Eco RI–digested octopus DNA. A weakly hybridizing fragment can be detected (asterisk) after 7 days of exposure. The same fragment is more easily detected with a *Drosophila* opsin DNA probe (Table 1). (b) Unicellular organism and plant DNA. Lane 1, Sst I–digested *Chlamydomonas* nuclear DNA; lane 2, Bam HI–digested *Halobacterium halobium* DNA; lane 3, Eco RI–digested *Blastocladiella* DNA; and lane 4, Eco RI–digested *Z. maize* DNA.

cies have photosensory pigments, it is possible that visual pigments first evolved in single-celled organisms. Accordingly, we tested for and observed hybridization of SP1116 with genomic DNA from two phototropic unicellular species, *Chlamydomonas reinhardtii* and *Halobacterium halobium* (lanes 1 and 2 in Fig. 3b). *Chlamydomonas* is a motile green alga with an eyespot and with a photosensitive action spectrum similar to that of animal rhodopsins. Recently, Foster *et al.* (*13*) showed that the bovine and *Chlamydomonas* pigments behave similarly when recombined with synthetic vitamin A analogs, implying that the two pigments are structurally related. Our hybridization experiments detected the presence of opsin-like genes in this organism, consistent with the idea that the *Chlamydomonas* visual pigment is related to higher animal rhodopsins.

We also identified homologies to the bovine opsin probe in genomic DNA from the archaebacterium *Halobacterium halobium*. This is an intriguing result since a vitamin A–based photopigment, bacteriorhodopsin, has already been identified and characterized in this organism (*14*). Bacteriorhodopsin, which functions as a light-driven proton pump rather than a sensory transducer, shares striking conformational similarities with animal opsins, yet bears no apparent amino acid sequence homology (*15*). However, *Halobacterium* also contains other photopigment molecules for which there is no sequence information. At least one of these additional pigments is a sensory rhodopsin that serves to direct the phototactic behavior of the cell (*16*). Spudich and Bogomolni (*16*) recently reported kinetic similarities in the behavior of certain animal visual pigments and *Halobacterium* sensory rhodopsin. Thus the *Halobacterium* sensory rhodopsin might be considered functionally analogous to the visual pigments of higher animals. It will be interesting to ascertain whether the opsin-like sequences we have detected in *Halobacterium* DNA represent sensory rhodopsin genes.

It seems unlikely that many of the fragments we detected with SP1116 are the result of homology to noncoding DNA sequences or genes unrelated to visual pigments. We have demonstrated by hybridization methods the ability of this probe to isolate opsin clones from a *Drosophila* genomic library. The Southern blot hybridization patterns we report are consistent with the known restriction fragment sizes for the bovine, human, and *Drosophila* opsin genes. We have searched the NIH GenBank repository (over 4000 sequences) for homologies to SP1116 using criteria that permit identification of the weak homologies between

Table 1. Compilation of Southern blot hybridization data generated with the SP1116 bovine opsin cDNA probe. Values are derived from two or more blots, with the exception of the frog data. Homologous fragment sizes are given in units of kilobase pairs (kb), as estimated by comparison to restriction digests of λ phage and pBR322 plasmid DNA standards. All fragments listed were detectable under conditions of reduced hybridization stringency. When high-stringency hybridization experiments were performed on identically prepared blots, those fragments that were detectable under both high and low stringency are boldface. Double asterisks indicate those samples in which identically prepared blots were hybridized with a *Drosophila* opsin cDNA probe restricted to 84% of the coding region (bases 284 through 1225). Those fragments that hybridized with both probes are likewise indicated by double asterisks.

Organism	Enzyme	Homologous fragment sizes (kb)
	Vertebrates	
Human* (*Homo sapiens*)	Eco RI	**8, 2**
	Bam HI	**21**, 12.3, 10, 4.4, 2.5, 2
	Hind III	**10**, 2.7, 2, 1.4
	Pst I	5, 4.4, **2.4**, **1.4**
Mouse† (*Mus musculus*, C57BL/6J)	Eco RI	5
	Bam HI	**11**
Bovine (*Bos bos*)	Eco RI	**18**
	Bam HI	**15**
	Hind III	**12.5**
	Pst I	**3.3**, **1.9**, **1**
	Sst I	**5.5**
	Xba I	**12**
Sheep (*Ovis familiaris*)	Eco RI	**18**
	Bam HI	**6**
Chicken (*Gallus domesticus*)	Eco RI	21, **12**, 6, 5.5, 3.2
	Bam HI	**4.4**, 3.2, 3, 2.5, 1.8
	Hind III	15, 10, 7.3, 5.5, 4.2, **4**, 2.5
	Pst I	17.4, 7.5, 3, **2**, 1.5
Chameleon (*Anolis carolinensis*)	Eco RI	4, **2.7**, 1.8
	Bam HI	6.8, 5, 2.7
	Hind III	8.5, 6.2, 4, 2.7
	Pst I	4.4, 3.3, 2.2
Gecko‡ (*Gekko gekko*)	Bam HI	8.5
	Hind III	4
	Vertebrates (continued)	
Frog‡ (*Rana pipiens*)	Eco RI	13, 9.5, 5, 4.5
	Bam HI	12.3, 7.3
	Hind III	10, 7.3, 1.2, 0.9
	Pst I	5, 3.2, 1.5, 1.2
Goldfish (*Carassius auratus*)	Eco RI	19, 11, 6, **5.7**, **5**, 4, 2.6, 2
	Bam HI	11, 7, 5.8, 3.8, 2.7
	Hind III	15, 12, **8.5**, **6.5**, **6**, 3.6, 3, **2**
	Pst I	6, 5.5, 3.8, 3.1, 2.7, 2, 1.1
	Nonvertebrates	
Fruit fly (*Drosophila melanogaster*, Oregon R)	Eco RI	16, 11, 7.3, 6, 4.4, 2.7, 2
	Bam HI	9, 5.2, 2.8, 1.8, 1
	Hind III**	9**, 7**, 5.3**, 3.1
	Pst I	7.3, 5.8, 5.2, 2.8, 1.1
Crayfish (*Procambarus blandingii*)	Sst I**	7.3**, 2.5**
Octopus (*Argonauta argo*)	Eco RI**	2.6**
Chlamydomonas (*Chlamydomonas reinhardtii*)	Pst I**	12, 4.4, 1.3**
	Sst I**	9.5**, 3
Halobacterium (*Halobacterium halobium*, strain S9)	Bam HI	16, 11
	Hind III	33, 28
	Pst I**	10, 5.5**, 3.2, 1.8**, 0.9
	Sma I**	5.4**, 3.7, 3, 2.7**, 1.7

*Because low-stringency hybridization conditions resulted in excessive background hybridization to the DNA lane, moderate hybridization conditions (*4*) were used when examining human genomic samples under reduced stringency. Only high-stringency hybridizations were performed for Eco RI–digested samples. For the human genome, underscored values indicated for Bam HI and Hind III are from (*6*); values for Pst I are based on the restriction sites determined from the complete gene sequence obtained from NIH GenBank. An additional Pst I fragment of >2.9 kb is also predicted. †Low-stringency hybridization experiment not performed for Bam HI. ‡High-stringency hybridization not observed for these species. Previous studies have demonstrated antigenic and peptide-mapping similarities between the bovine and frog opsin proteins (*19*). The lack of hybridization of SP1116 to frog DNA under stringent conditions is probably due to the large size of amphibian genomes (*7*)—in some cases up to 20 times the size of mammalian genomes—rather than a lack of homology between the bovine and frog opsin genes. In contrast, reptiles (gecko) generally have genomes that are smaller than those of mammalian species (*7*).

the bovine and *Drosophila* opsin sequences, and we have found no homologies other than to opsin genes. We did not detect SP1116 hybridization to DNA from the eubacteria *Klebsiella pneumoniae* and *Escherichia coli*, the fungus *Blastocladiella emersoni*, and the plant *Zea maize* (lanes 3 and 4 in Fig. 3b). Thus we are able to detect homologies only in organisms for which there is independent evidence for the existence of photosensory pigments. Finally, additional Southern blot results suggest that many of the genomic fragments that hybridize to SP1116 are also homologous to a *Drosophila* opsin DNA probe (Table 1).

On the basis of our observations that opsin-like genes can be identified in a wide variety of vertebrate, invertebrate, and unicellular species, we speculate that a primitive photopigment gene first evolved in unicellular organisms and was passed on, with some degree of sequence conservation, to a wide variety of present-day species. Because it is possible to identify more than one class of opsin-like genes in some animals, it seems likely that the progenitor gene was amplified during evolution, giving rise to a family of related products. Some of these products are probably themselves visual pigments, since many organisms have more than one type. Recently, Nathans and Hogness (*6*) found that the human cone pigment genes have weak sequence homology to the opsin gene and similar intron positions, supporting the idea that they arose from a common precursor (*17*). Perhaps in other cases a duplicated photopigment gene became adapted to a different function. This could have occurred during the evolution of *Halobacterium*, resulting, for example, in one class of photopigment that is involved in ion pumping and another that subserves a sensory function.

The bovine opsin probe has excellent potential for identifying genes encoding photosensory pigments in a wide variety of species. Comparisons of the structure of photosensory receptors should reveal the extent to which vertebrate, invertebrate, and unicellular organisms employ the same functional mechanisms for triggering a sensory response.

REFERENCES AND NOTES

1. G. Wald, *Nature (London)* **219**, 800 (1968); E. A. Dratz and P. A. Hargrave, *Trends Biochem. Sci.* **8**, 128 (1983).
2. E. Southern, *J. Mol. Biol.* **98**, 503 (1975).
3. D. W. Cleveland *et al.*, *Cell* **20**, 95 (1980); V. Wilson *et al.*, *J. Mol. Biol.* **166**, 457 (1983); W. McGinnis, R. L. Garber, J. Wirz, A. Kuroiwa, W. J. Gehring, *Cell* **37**, 403 (1984).
4. Hybridization conditions were varied to allow the probe to reanneal with genomic sequences containing different degrees of homology. Stringent hybridizations (allowing ~10% mismatch) were performed at 68°C and blots were washed in 0.2× standard saline citrate (SSC). Moderate (~20% mismatch) and low-stringency (~35% mismatch) hybridizations were performed at 58°C and washed in 0.2× or 2× SSC, respectively. The mismatch estimates assume that (i) a perfectly matched DNA duplex has a melting temperature (T_m) of about 87°C in 1× SSC, (ii) changing the salt concentration (N) of the hybridization solution influences T_m by the relation $\Delta T_m = 16.6 \log \Delta N$, and (iii) the T_m of a DNA duplex decreases by about 1°C for every 1% base-pair mismatch. In addition, the degree of hybridization to a fixed amount of DNA depends on the relative genome size of the organism. For a more detailed discussion, see T. Maniatis, E. F. Fritsch, J. Sambrook, Eds., *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982), pp. 324 and 388; G. A. Beltz, K. A. Jacobs, T. H. Eickbush, P. T. Cherbas, F. C. Kafatos, *Methods Enzymol.* **100**, 266 (1983); J. Meinkoth and G. Wahl, *Anal. Biochem.* **138**, 267 (1984).
5. Genomic DNA was isolated by a modification of the method of N. Blin and D. W. Stafford [*Nucleic Acids Res.* **3**, 2303 (1976)]. The DNA was further purified by CsCl–ethidium bromide density gradient centrifugation. An aliquot was compared electrophoretically with λ DNA to ensure that fragment sizes exceeded 50 kilobase pairs.
6. J. Nathans and D. S. Hogness, *Cell* **34**, 807 (1983); *Proc. Natl. Acad. Sci. U.S.A.* **81**, 4851 (1984).
7. H. Rees and R. N. Jones, *Int. Rev. Cytol.* **32**, 53 (1972).
8. G. L. Walls, *Am. J. Ophthalmol.* **17**, 892 (1934); F. Crescitelli, in *Biochemical Evolution*, H. Gutfreund, Ed. (Cambridge Univ. Press, Cambridge, 1981), p. 301; in *Handbook of Sensory Physiology*, F. Crescitelli, Ed. (Springer-Verlag, Berlin, 1977), vol. VII/5, p. 391.
9. F. Crescitelli, in *Handbook of Sensory Physiology*, H. J. A. Dartnall, Ed. (Springer-Verlag, Berlin, 1972), vol. VII/1, p. 245; L. Yu and R. S. Fager, *Invest. Ophthalmol. Visual Sci.* **22** (suppl.), 43 (1982).
10. A. Oksche, *Ophthalmic Res.* **16**, 88 (1984); H. W. Korf and I. Vigh-Teichmann, *ibid.*, p. 96; R. G. Foster, B. K. Follett, J. N. Lythgoe, *Nature (London)* **313**, 50 (1985); L. Z. Bito and D. G. Turansky, *Comp. Biochem. Physiol.* **50A**, 407 (1975); J. N. Lythgoe, *Vis. Res.* **24**, 1539 (1984).
11. T. Ebrey, M. Tsuda, G. Sassenrath, J. L. West, W. H. Waddell, *FEBS Lett.* **116**, 217 (1980); H. R. Saibil and M. Michel-Villaz, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 5111 (1984).
12. W. Baehr *et al.*, *Invest. Ophthalmol. Visual Sci.* **26** (suppl.), 43 (1985); J. E. O'Tousa *et al.*, *Cell* **40**, 839 (1985); C. S. Zuker, A. F. Cowman, G. M. Rubin, *ibid.*, p. 851.
13. K. W. Foster *et al.*, *Nature (London)* **311**, 756 (1984).
14. R. Henderson, *Annu. Rev. Biophys. Bioeng.* **6**, 87 (1977).
15. Y. A. Ovchinnikov, *FEBS Lett.* **148**, 179 (1982).
16. J. L. Spudich and R. A. Bogomolni, *Nature (London)* **312**, 509 (1984).
17. L. Stryer, *ibid.*, p. 498.
18. W. Baehr and M. Applebury, *Biophys. J.* **41**, 340a (1983); W. Baehr, R. Martin, M. L. Applebury, *Invest. Ophthalmol. Visual Sci.* **25** (suppl.), 155 (1984).
19. D. S. Papermaster, B. G. Schneider, M. A. Zorn, J. P. Kraehenbuhl, *J. Cell Biol.* **77**, 196 (1978); S. A. H. Al-Mahdawi, C. Converse, A. Cooper, *Biochem. Soc. Trans.* **9**, 319 (1981).

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Technical Comments

Human Brain Receptor Distribution

D. F. Wong *et al.* (*1*) describe the use of ^{11}C-labeled 3-*N*-methylspiperone and positron emission tomography for the measurement of regional alterations in dopamine and serotonin receptors in the living human brain and demonstrate the feasibility of routine imaging of receptor ligand distributions by emission tomographic methods. The ultimate goal of this and earlier studies (*2*) is the development of a quantitative procedure for measurement of regional neurotransmitter receptor density in vivo in the human brain. Such methodology would then allow direct testing of hypotheses implicating receptor alterations in a variety of neurologic and psychiatric disorders. While the experiments presented by Wong and his colleagues are encouraging with respect to eventual achievement of this goal, we have reservations about their data analysis and interpretation.

Wong *et al.* present a three-compartment model describing tracer distribution in brain tissue similar to that in use in a number of laboratories, including ours (*3, 4*):

$$C_p \underset{K_2}{\overset{K_1}{\rightleftharpoons}} C_e \underset{K_4}{\overset{K_3}{\rightleftharpoons}} C_r \qquad (1)$$

where C_p is the content of tracer in the plasma, C_e is the content of exchangeable (free plus nonspecifically bound) tracer in tissue, and C_r is the content of receptor-bound tracer in tissue. The descriptions of the intercompartmental transfer coefficients in the model above require clarification. First, K_1 and K_2, describing diffusion of ligand between capillary plasma (C_p) and extracellular space (C_e) require redefinition in order to account for potential influences of cerebral blood flow on ligand delivery to the brain.

$$C_a \underset{K_2^*}{\overset{K_1^*}{\rightleftharpoons}} C_e \underset{K_4}{\overset{K_3}{\rightleftharpoons}} C_r \qquad (2)$$

Introduction of C_a, the arterial tracer concentration, replacing C_p, allows direct quantitative comparison of different brain regions from the same animal. K_1^* and K_2^* are then functions of both the blood-brain barrier permeability surface area product (K_1) and cerebral blood flow (F), as described previously (*5*).

$$K_1^* = F(1 - e^{-K_1/F}) \qquad (3)$$

$$K_2^* = K_2 K_1^*/K_1 \qquad (4)$$