however, caused his performance to drop to zero in 15 seconds.

The ability to acquire arbitrary associations between pairs of words, which may be considered as conceptually equivalent to naming proper names, was finally tested. The patient was given the paired-associate learning task from the Wechsler Memory Scale and the Bisiach, Cappa and Vallar[7] battery. He quickly learned the 'easy' pairs (in which the two words have a semantic relation to each other, such as 'north'-'south'), but he showed a chance performance over several attempts in retrieving the second member of the pair, given the first, when the relation between the two was totally arbitrary (such as 'clock'-'pear'). By contrast, he had no difficulty in a word-learning task. Specifically his score on a ten-word free-recall task (three trials) was within the normal range[7]. At work he also had great difficulty in learning numerical labels consistently when applied to certain items of hardware.

Our evidence indicates a common denominator underlying L.S.'s performances: the inability to deal (at the retrieval level) with purely referential nondescriptive semantic relations. Indeed, proper names are believed by some philosophers of language[8,9] to be pure referring expressions. According to Frege's[10] distinction, proper names just carry 'reference', that is they denote the individuals or the entities that are called by them, but have no 'sense', that is, they do not describe any property or imply any attribute. They are the opposite of 'descriptions', which have sense and which encompass all common nouns. Meaningless labels, which is what pure referring expressions are, could indeed need an independent system to take care of them; in L.S. this system would be disrupted. Psychologically, this makes more sense than the alternative explanation that L.S.'s inabilities arose because of a difficulty purely in retrieving proper names; in this case one would be left with the problem of specifying what particular quality makes it useful for the brain to implement proper names independently from common ones. The need of an independent system may still be there even if proper names were not meaningless labels but 'shorthand descriptions', as some authors[11] prefer to the pure reference theory. A descriptive value, varying from none (or very little) to presumably a lot, would be in any case an important dimension in the organization of semantic memory. It would be orthogonal with other dimensions such as the one defined by the prevalence of structural or functional attributes, which also has been suggested to determine category effects in aphasia[12].

There are at present only a few highly speculative models of the actual wiring in the nervous system of such organization: those involving modularly distinct subsystems[12,13] or regions of relative specialization within a distributed net[2,5] are the more accredited ones.

Note added in proof. Note also that a substantially similar argument comes from modern linguistics. In Jackendoff's theory of natural language semantics, an important distinction in conceptual structure is the binary feature Type/Token[14]. What one learns and stores in memory can be linked either with Token (if one is remembering an individual) or with Type (if one has learned a category). Proper names appear typically to denote individuals. □

Received 4 July; accepted 17 October 1989.

1. Semenza, C. & Denes, G. *Aphasiology* **2,** 405–410 (1988).
2. McCarthy, R. A. & Warrington, E. K. *Nature* **334,** 428–430 (1988).
3. McKenna, P. & Warrington, E. K. *J. Neurol. Neurosurg. Psychiat.* **43,** 781–788 (1978).
4. Semenza, C. & Zettin M. *Cognitive Neuropsychol.* **5,** 711–721 (1988).
5. Warrington, E. K. McCarthy, R. A. *Brain* **110,** 1273–1296 (1987).
6. Goodglass, H. & Kaplan, E. *The Assessment of Aphasia and Related Disorders.* (Lea & Febiger, Philadelphia, 1972).
7. Bisiach, E., Cappa, S. & Vallar G. *Guida all'esame neuropsicologico* (Cortina, Milan, 1983).
8. Mill, J. S. *A System of Logic* (Longman, London, 1843).
9. Kripke, S. *Naming and Necessity* (Blackwell, Oxford, 1980).
10. Frege, G. in *G. Frege, Funktion, Begriff, Bedeuntung* (ed. Patzig, G.) 40–65 (Vandenhoeck und Ruprecht, Gottingen, 1982).
11. Searle, J. R. in *Semantics, an Interdisciplinary Reader in Philosophy, Linguistics and Psychology* (eds Steinberg, D. D. & Jakobovitz, L. A.) 134–141 (Cambridge University Press, 1971).
12. Warrington, E. K. & Shallice, T. *Brain* **107,** 829–853 (1984).
13. Warrington, E. K. & McCarthy, R. A. *Brain* **106,** 859–878 (1983).
14. Jackendoff, R., *Semantic and Cognition* (MIT Press, Cambridge, Massachusetts, 1983).

ACKNOWLEDGEMENTS. We are grateful to Professor Roberto Rago of the Clinica Ausiliatrice, Torino, for providing facilities to carry out this study.

Analysis of fusion gene and encoded photopigment of colour-blind humans

Jay Neitz, Maureen Neitz & Gerald H. Jacobs

Department of Psychology and Neuroscience Research Institute, University of California, Santa Barbara, California 93106, USA

In humans, long-wavelength-sensitive and middle-wavelength-sensitive cone pigments are encoded by genes lying in a head-to-tail tandem array on the X chromosome. Deficiencies in red–green colour vision seem to arise from unequal recombination of these normal X-linked genes[1,2]. In some dichromats this recombination is believed to yield a fusion gene encoding a product with an absorption spectrum similar to that of one or the other of the normal photopigments[2]. Until now, however, such a relationship between the structure of a pigment gene and the spectral properties of its encoded pigment has not been directly shown. We have now sequenced a fusion gene isolated from a red-green colour-blind human and determined the spectral properties of the pigment that it encodes. The absorption spectrum of the photopigment was very similar to that of normal middle-wavelength-sensitive photopigment, even though about half of its DNA coding sequence seems to be derived from a gene encoding normal long-wavelength-sensitive pigment. These results indicate the regions of the X-encoded photopigment apoproteins that are responsible for differences in their spectral tuning, and imply that the striking variations in colour vision among anomalous trichromats of a particular type are not attributable to anomalous pigments with differing spectral peaks.

We established the colour vision of a 31-year-old male by psychophysical tests in which the appearances of mixtures of red and green primary lights were compared with the appearance of a standard yellow light (the Rayleigh match). He accepted

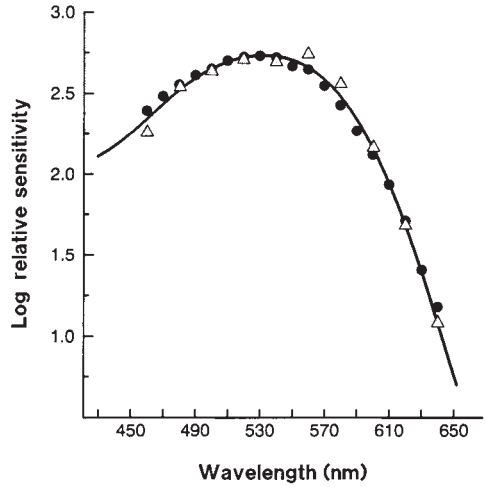


FIG. 1 Comparison of the spectral sensitivity of the MWS cone pigment from a protanope with the MWS cone pigment from a colour-normal human. ●, Protanope ERG sensitivity values corrected for pre-retinal absorption by the lens[12]. △, Spectral sensitivity of MWS cone pigment from a normal trichromat[6]. The wavelength of peak sensitivity for each was determined by translating a standard visual pigment absorption curve[13] on a log-wavenumber axis to obtain the best fit. The two pigment spectra have the same best-fitting curve (solid line; $\lambda_{max} = 530$ nm).

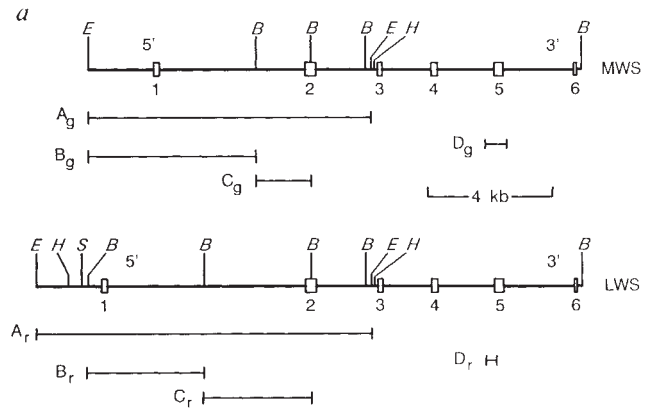
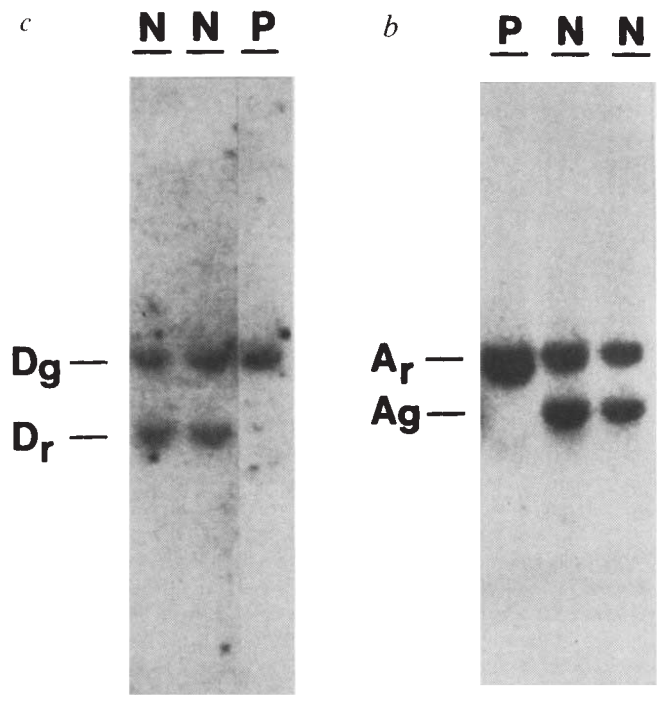


FIG. 2 *a*, Restriction maps and four restriction length polymorphisms that have been used to distinguish human X-linked cone pigment genes (from ref. 1). *B*, *Bam*HI; *E*, *Eco*RI; *H*, *Hin*dIII, *S*, *Sal*I. *b*, *c*, Two Southern blots of genomic DNA from the protanope (P) compared with identical digests of DNA from two colour-normal humans (N). A_r and A_g are *Eco*RI fragments visualized with a complementary DNA probe encompassing exon 1 and the 5′ half of exon 2. D_g and D_r are *Rsa*I fragments visualized with a probe from the 3′ end of intron 4.



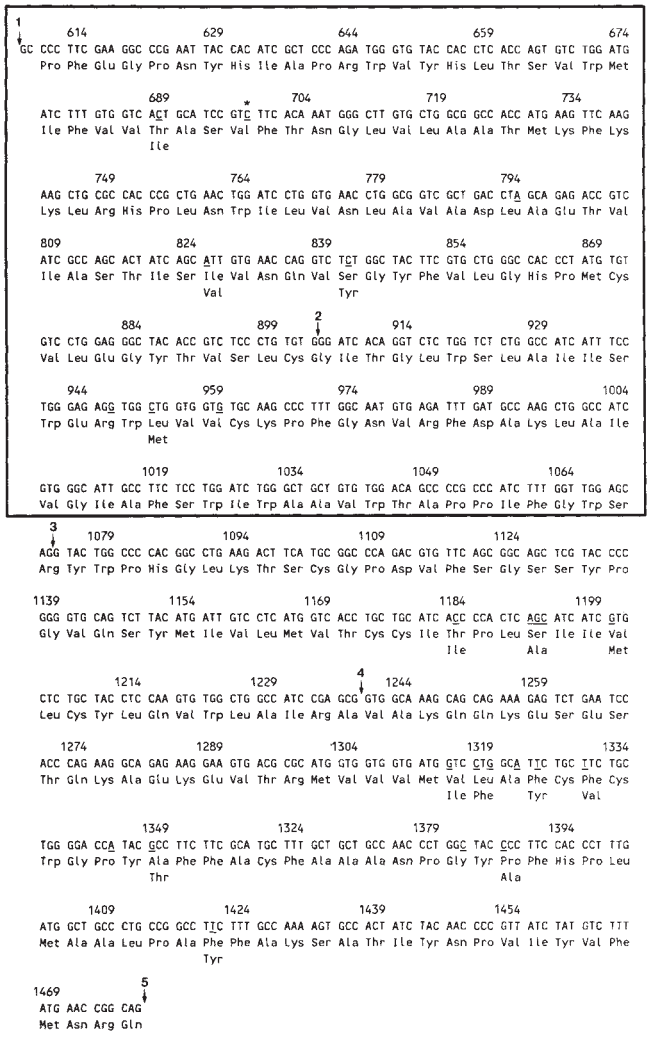


FIG. 3 Sequence of exons 2, 3, 4, and 5 of the MWS pigment gene from a protanope. The locations of the five introns[1] are indicated by arrows. The numbering convention is that of Nathans *et al.*[1]. For the region outside the box (exons 4 and 5), the protanope gene sequence is identical to that of an identified normal MWS pigment gene fragment (gJHN21; ref. 1). For these two exons the underlined nucleotides in the protanope gene sequence are those that differ from corresponding nucleotides in a genomic LWS pigment gene fragment (gJHN33; ref. 1), and the lower amino-acid sequence is that of the identified normal LWS pigment. Exons 2 and 3 (enclosed in the box) of the protanope gene are identical to those of the identified LWS pigment genes in all of the nucleotide substitutions that distinguish the identified normal MWS from the LWS pigment genes[1]. But exons 2 and 3 of the protanope gene differ slightly from those of each of the three genes (one derived from a genomic library and two derived from a human retina cDNA library, no two of which were identical) that Nathans *et al.*[1] identified as encoding normal LWS pigment. For example, exons 2 and 3 of the protanope gene differ from those of the genomic LWS pigment gene fragment (gJHN53; ref. 1) by three nucleotides, but for two of these substitutions the protanope gene is identical to the other two LWS pigment genes, whereas the third is a silent nucleotide substitution (indicated by the asterisk) in which the protanope gene sequence differs from both MWS and LWS sequences. For exons 2 and 3 the nucleotides of the protanope gene that differ from those of a MWS gene fragment (gJHN21; ref. 1) are underlined, and the lower amino-acid sequence is that deduced from the MWS pigment gene (identical amino-acid residues are left blank). A genomic library derived from germ line DNA from the protanope was constructed[14] in the cosmid vector plasmid pWE15 (ref. 15). A fragment containing the protanope MWS pigment gene was identified by its hybridization with a cDNA clone probe. Sequences (both strands) were determined by the dideoxy chain termination method[16].

the red and green mixture of all proportions as matching the yellow light in both small-field (2 degrees) and large-field (11 degrees) versions of the test. The subject was thus a true dichromat[3,4]—a protanope, having only a single X-encoded cone pigment absorbing light of middle to long wavelengths.

We determined the absorption spectrum of this X-encoded cone pigment by analysing a retinal gross potential—the electroretinogram (ERG). To measure relative sensitivity, we adjusted the quantal intensity of a rapidly flickering monochromatic test light to produce a response that matched in amplitude the response to an interleaved flickering reference light[5]. Repetition of this procedure for many test wavelengths yields a curve that accurately characterizes absorption spectra of middle-wavelength-sensitive (MWS) and long-wavelength-sensitive (LWS) cone pigments in dichromatic subjects[5]. The derived sensitivity of the dichromat curve (Fig. 1, ●) thus gave the spectral sensitivity of this MWS pigment. Schnapf *et al.*[6] have measured the spectral sensitivity of normal human cones. The absorption spectra for the protanope MWS cone and the normal MWS cone were very similar (Fig. 1) and the spectral peaks were identical ($\lambda_{max} = 530$ nm).

Southern blots for two different restriction-enzyme digests of genomic DNA from the protanopic subject are shown in Fig. 2. For humans with normal colour vision, similar analysis reveals two hybridizing bands corresponding to two classes of X-linked pigment genes[1]. For the protanopic subject, we observed only a single hybridizing band for each digest, indicating the presence of a single X-linked pigment gene. The earlier identification of the normal pigment genes[1,2] (Fig. 2*a*) enabled us to identify this pigment gene as a fusion gene which had derived its 5′ end from a normal LWS pigment gene and its 3′ end from a normal MWS pigment gene.

We obtained a DNA fragment containing the entire X-linked photopigment gene of the protanope from a library constructed using total genomic DNA, and determined the sequences of exons 2, 3, 4 and 5. Comparison with the normal X-linked pigment genes[1] indicated that this fusion gene had derived its exons 2 and 3 from a normal LWS pigment gene and its exons 4 and 5 from a normal MWS pigment gene (Fig. 3). The fact that the absorption spectrum of the pigment encoded by the fusion gene does not differ from that of a normal MWS pigment indicates that the amino-acid substitutions encoded in exons 2 and 3 have little or no effect on the absorption spectrum of the X-linked photopigments. Nathans *et al.*[1] found no differences between exons 1 or between exons 6 of the MWS and LWS pigment genes. Thus the spectral differences between MWS and LWS pigments seem to be governed entirely by the amino-acid substitutions encoded in exons 4 and 5.

Except for several homologous substitutions[7] (which are not expected to alter the absorption spectrum of the chromophore) all the amino-acid differences between MWS and LWS photopigments that occur in the transmembrane segments encoded by exons 4 and 5 are substitutions of nonpolar groups for hydroxyl-bearing groups[1]. Studies of bacteriorhodopsin demonstrate that similar substitutions of nonpolar for polar groups (for example, Phe for Trp) can have dramatic effects on the absorption spectrum of the chromophore[8]. Depending on their position relative to the chromophore, hydroxyl groups have been proposed to preferentially stabilize or destabilize the ground state relative to the excited state and thus shift the absorption spectrum to the shorter or longer wavelengths[9]. Figure 4 shows the location of all the nucleotide differences responsible for non-homologous amino-acid substitutions that are located in the transmembrane segments encoded by exons 4 and 5. There are two such substitutions in exon 4 and three in exon 5. The most 5′ proximal of these nucleotide substitutions in exon 5 is marked by the restriction site polymorphism that distinguishes fragment D_r from D_g.

The locations and small number of nucleotide substitutions encoding the amino acids that are candidates for producing the spectral difference between X-linked pigments (Fig. 4) limits the number of different pigments with intermediate spectral peaks that can result from genetic recombination. These limitations have implications for understanding the genetic mechanisms underlying the most common forms of defective colour vision—the anomalous trichromacies. Nathans *et al.*[2] propose that the anomalous pigments result from recombination of the identified normal pigment genes. Because all protanomalous individuals that have been studied using Southern blot analysis have fusion genes containing fragment D_g, and all deuteranomalous subjects have fusion genes containing fragment D_r (refs 2, 10), both types of anomalous pigment genes must be produced by crossovers upstream of the D_g–D_r marker. The nucleotide substitutions that give rise to the two hydroxyl-bearing amino-acid substitutions in the MWS exon 4 are separated by only seven nucleotides, so recombination events in the region between them are expected to occur only rarely. These observations suggest that one or both of the substitutions encoded in exon 4 are responsible for the small spectral shift differentiating the X-linked pigments in anomalous trichromats, whereas one or more of the substitutions encoded in exon 5 are

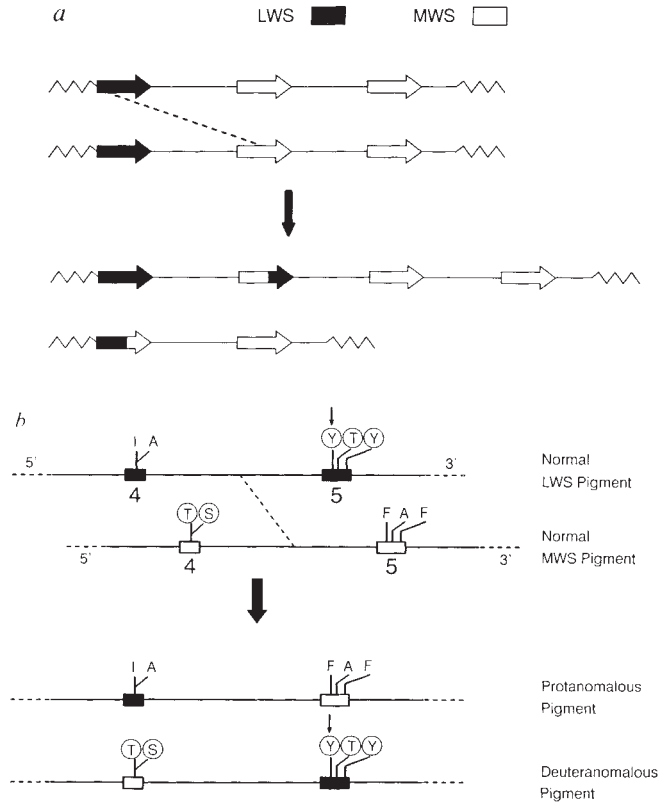


FIG. 4 *a*, Scheme showing how unequal intragenic recombination between normal MWS and LWS pigment genes can produce fusion genes. Each arrow represents a single gene (from ref. 2). *b*, Detail of *a*, showing only exons 4 and 5 of the pigment genes. The locations of nucleotide differences encoding amino-acid substitutions that are candidates for producing the spectral shifts differentiating the products of X-linked pigment genes are labelled according to the amino acid produced. The hydroxyl-bearing amino acids are circled (Y, tyrosine; T, threonine; S, serine), and their nonpolar counterparts are not circled (I, isoleucine; A, alanine; F, phenylalanine). The location of the *Rsa*I site that differentiates restriction fragment D_r from D_g (Fig. 1) is indicated by a small arrow. Cross-overs upstream of the two substitutions in exon 4 produce fusion genes that encode pigments with spectra unchanged from those of products encoded by the original genes. Exchanges in the region between the substitutions in exon 4 and those in exon 5 (such as the one illustrated) are proposed to produce two fusion genes, one that encodes a pigment that is slightly 'red-shifted' from the normal MWS pigment (protanomalous pigment) and a second that is slightly 'green-shifted' from the normal LWS pigment (deuteranomalous pigment).

responsible for the larger spectral shift that differentiates MWS from LWS pigments. It seems that, except for a crossover between the two exon 4 substitutions (Fig. 4), only a single spectrally intermediate protanomalous hybrid pigment and a single spectrally intermediate deuteranomalous pigment could be produced by exchanges upstream of the D_g-D_r marker.

Southern-blot analysis of pigment genes from colour-anomalous subjects does not seem to distinguish between individuals with mild and more extreme forms of anomalous trichromacy. Nathans *et al.*[2] suggest that different locations of crossing over can produce a variety of anomalous pigments with differing spectral peaks, and that this spectral variation accounts for the differences in colour-discrimination ability among anomalous trichromats. The results presented here indicate otherwise—that both mild and extreme forms of protanomalous colour vision are probably based on the same anomalous photopigment and that, similarly, a second anomalous pigment underlies both mild and extreme forms of deuteranomalous colour vision. This conclusion (1) is consistent with recent results from colour matching[3], which show that under conditions designed to maximize colour discrimination, both mild and extreme anomalous subjects make the same colour match and thus seem to have the same anomalous pigment; and (2) reinforces the conclusion reached from psychophysical studies[3,4,11] that the differences in colour-discrimination ability within each of the two classes of anomalous trichromats cannot be explained by differences in the absorption spectra of the underlying photopigments. □

Received 31 July; accepted 20 October 1989.

1. Nathans, J., Thomas, D. & Hogness, D. S. *Science* **232,** 193–202 (1986).
2. Nathans, J., Piantanida, T. P., Eddy, R. L., Shows, T. B. & Hogness, D. S. *Science* **232,** 203–210 (1986).
3. Nagy, A. L. *J. opt. Soc. Amer.* **72,** 571–577 (1982).
4. Pokorny, J. & Smith, V. C. *Color Res. Appl.* **7,** 159–164 (1982).
5. Neitz, J. & Jacobs, G. H. *J. opt. Soc. Amer.* **A1,** 1175–1180 (1984).
6. Schnapf, J. L., Kraft, T. W. & Baylor, D. A. *Nature* **325,** 439–441 (1987).
7. Lehninger, A. L. *Biochemistry* (Worth, New York, 1975).
8. Hackett, N. R., Stern, L. J., Chao, B. H., Kronis, K. A. & Khorana, H. G. *J. biol. Chem.* **262,** 9277–9284 (1987).
9. Kosower, E. M. *Proc. natn. Acad. Sci. U.S.A.* **85,** 1076–1080 (1988).
10. Drummond-Borg, M., Deeb, S. & Motulsky, A. G. *Am. J. hum. Genet.* **43,** 675–683 (1988).
11. Hurvich, L. M. & Jameson, D. *Mod. Probl. Opthalmol.* **13,** 200–209 (1974).
12. Wyszecki, G. & Stiles, W. S. *Color Science* (Wiley, New York, 1982).
13. Dawis, S. M. *Vision Res.* **21,** 1427–1430 (1981).
14. DiLella, A. G. & Woo, S. L. C. *Meth. Enzym.* **152,** 199–212 (1987).
15. Wahl, G. M. *et al. Proc. natn. Acad. Sci. U.S.A.* **84,** 2160–2164 (1987).
16. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74,** 5463–5467 (1977).

ACKNOWLEDGEMENTS. We thank D. F. Peters for being a subject in these experiments, K. E. Neitz for her cooperation, and S. K. Fisher for his support. Probes for the X-linked photopigment genes were provided by J. Nathans. This work was supported by the NIH.

Characterization of a naturally processed MHC class II-restricted T-cell determinant of hen egg lysozyme

S. Demotz, H. M. Grey, E. Appella* & A. Sette

Cytel, 11099 North Torrey Pines Road, La Jolla, California 92037, USA
* National Cancer Institute, National Institutes of Health, Building 37, Room 1B04, Bethesda, Maryland 20892, USA

COMPELLING evidence indicates that T cells recognize complexes formed by major histocompatibility complex-encoded molecules and antigenic peptide fragments[1–3]. This is based largely on the ability of small synthetic peptides to substitute for naturally processed antigen in stimulating T cells. Naturally processed fragments of exogenous antigen are thought to arise by limited proteolytic degradation of native antigen inside acidic compartments of antigen-presenting cells[3–6], but until now no physiologically processed antigen has been directly analysed. Here we report the characterization of physiologically processed antigen eluted from mouse class II major histocompatibility complex I-E^d molecules. The antigenic material corresponds to a previously described antigenic determinant of hen egg lysozyme (HEL 107–116) and has a relative molecular mass M_r of about 2,000. HPLC analysis identified at least two or three separate molecular species, suggesting limited, albeit significant, heterogeneity of naturally processed peptides. Finally, under our experimental conditions, it was calculated that a substantial proportion (10–40%) of I-E^d molecules were occupied by these HEL-derived antigenic determinants.

Taking advantage of the high stability of the complexes formed between major histocompatibility complex (MHC) molecules and antigenic peptides, we decided to attempt the isolation and characterization of naturally processed HEL peptides from HEL-pulsed A20-1.11 B lymphoma cells (A20 cells). As a first step, we determined whether complexes containing antigenic determinants could be identified in a preparation of affinity-purified class II MHC molecules. For this purpose, I-E^d molecules were purified from a lysate of HEL-pulsed A20 cells (HEL/I-E^d), and inserted into planar membranes[8,9]. These structures strongly stimulated interleukin-2(IL-2) production by the I-E^d-restricted T cell hybridoma 1 H-11.3, for which the sequence HEL 107–116 has previously been identified as the minimal antigenic determinant[7]. Thus, this I-E^d preparation carried naturally processed material corresponding to the HEL 107–116 determinant (Table 1, first row).

TABLE 1 Isolation of naturally processed antigenic material from antigen-pulsed B lymphoma cells

Source of I-E^d	Antigen added to purified I-E	IL-2 (U ml)$^{-1}$ produced by 1 H-11.3 T cells
HEL-pulsed A20	Nil	1,280
Unpulsed A20	Synthetic HEL 107–116 (20 ng)	20
Unpulsed A20	Synthetic HEL 107–116 (200 ng)	640
Unpulsed A20	Acid-eluted peptides from HEL/I-E^d	80

A20 cells were grown in RPMI medium supplemented with 5% fetal calf serum, 100 μg ml^{-1} streptomycin, 100 U ml^{-1} penicillin G, and 5×10^{-5} β-mercaptoethanol. A20 cells were pulsed for 24 h with 1 mg ml^{-1} HEL (Grade I, Sigma). I-E^d molecules were purified from both pulsed and unpulsed cells by affinity chromatography on 14.4.4 anti-I-E^d monoclonal antibody column[8]. To prepare the acid-eluted peptides, I-E^d (350 μg) was purified from 1.5×10^{10} HEL-pulsed A20 cells (HEL/I-E^d), acetonitrile-precipitated, treated for 30 min at 37 °C with 2.5 M acetic acid, and then loaded on a Sephadex G-10 column equilibrated with acetate buffer[11]. The peptide material eluted in the void volume was collected and lyophilized twice. Complexes between I-E^d and peptides prepared *in vitro* were obtained by dissolving the lyophilized peptides in 10 μl phosphate-buffered saline/100 mM Tris-HCl, pH 7.2, supplemented with 1 mg ml^{-1} sodium azide and a cocktail of protease inhibitors (16 mM EDTA, 2.6 mM 1,10-phenanthroline, 0.15 mM pepstatin A and 2 mM PMSF) (ref. 10), and 10 μl of a 1–2 mg ml^{-1} I-E^d preparation. After incubating for 1 d at room temperature, the samples were diluted to 250 μl with PBS containing 1 mg ml^{-1} sodium azide, 1% *n*-octyl-β-D-glucopyranoside (Sigma), 175 μg ml^{-1} L-α-phosphatidylcholine (Sigma), and 25 μg ml^{-1} cholesterol (Sigma). I-E^d-peptide complexes were inserted into planar membranes and tested in a T-cell stimulation assay as described[8,9].

The next step in this series of experiments would be to dissociate the antigenic determinants from the I-E^d molecules and biochemically characterize them. For this purpose, it was necessary to devise as sensitive an assay as possible. To decrease the amount of antigenic material required to trigger IL-2 release by the 1 H-11.3 T cell hybridoma, we formed I-E^d/peptide complexes *in vitro*, under conditions already established as optimal[10], and used these complexes, inserted into planar mem-