brane of the first cell were high, this current could hyperpolarize the visible-type cell. Preliminary experiments designed to test this hypothesis, involving the placement of two separate microelectrodes inside neighboring cells, indicate that such cells are not coupled. This evidence argues against an electrical interaction model.

The second alternative is that the initial hyperpolarizing component of the receptor potential is an inhibitory postsynaptic potential (IPSP) (*5*), that is, the result of an inhibitory chemical synapse of a visible-type cell upon an ultraviolet-type neighbor, or vice versa. Several lines of evidence suggest that this is not the case: we cannot reverse the polarity or significantly change the magnitude of the hyperpolarizing component by hyperpolarizing or depolarizing the cell with extrinsic current; we find no difference in the hyperpolarizing component when K_2SO_4 electrodes are used in place of KCl electrodes; both depolarizing and biphasic responses have comparable latencies; and finally, in the two-electrode experiments referred to earlier, the depolarization of one cell with extrinsic current would be expected to produce an IPSP in a neighboring cell if the presumed synapses existed, but these IPSP's have not been found. Thus, we feel that the evidence to date is most consistent with the hypothesis that two photopigments in each cell are involved in the generation of receptor potentials; alternatively, one pigment in two different states (*6*) would also suffice.

It also seems unlikely that the repolarization of the membrane of one cell can be effected through an electrical interaction by depolarization of a neighboring cell. Here, the electrical interaction hypothesis would require that extrinsic current produce the same effect as light, but this is not the case (Fig. 3E).

The hypothesis that the repolarization is the result of an inhibitory chemical synapse of the visible-type cell onto the ultraviolet-type cell is also unlikely. If the IPSP due to visible light were produced by ion-conductance changes, as has been found for IPSP's elsewhere (*5*), then upon cessation of the visible light stimulus, the potential ought to return again to the trajectory of the slow recovery of potential seen after a bright ultraviolet stimulus; this is not found (Fig. 1, D and F; Fig. 3, C and F). Here again, we feel that the data, showing that visible light both diminishes the depolarization elicited by ultraviolet light and causes the cell to repolarize rapidly to its resting level following cessation of stimulation (despite the small steady-state depolarization which may be produced by visible light), suggest that two photopigments, or two states of the same pigment, are involved in the mechanism producing the receptor potential.

JOHN NOLTE
JOEL E. BROWN
Department of Biology and
Research Laboratory of Electronics, Massachusetts Institute of Technology, Cambridge 02139

T. G. SMITH, JR.
National Institute of Neurological Diseases and Blindness, Bethesda, Maryland

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30 August 1968

Genetic Implications of Common Region Sequence Comparisons of Lambda Immunoglobulin Chains Differing at Position 190

Abstract. *The common regions of two lambda chains (amino acid residues 109 to 213) have been partially sequenced. These two human immunoglobulin chains have lysine at position 190, but are otherwise identical in their common-region sequence to four reported lambda chains that have arginine at position 190. The single amino acid interchange at position 190 may be explained either by an ambiguous codon at this position or by a gene duplication so recent that only a single mutational event has occurred.*

Amino acid sequence information from homogeneous immunoglobulins, that is, those produced by plasmacytomas of man and mouse, indicates that immunoglobulin light chains contain a virtually constant COOH-terminal sequence (common region characteristic of chain type lambda or kappa) and an NH_2-terminal sequence specific to each plasma cell clone (*1*). The common region of lambda chains (CL region) from human immunoglobulins extends from residues 109 to 213 [according to the numbering of fully sequenced lambda protein Sh (2)] and has an amino acid interchange (arginine-lysine) at position 190 (Oz marker) (*3*); that is, about 75 percent of 107 lambda (Bence Jones) proteins have arginine at this position [Oz (−)] and 25 percent have lysine [Oz (+)] (*4*). Ein (*4*) has demonstrated that ten randomly chosen normal persons have both the lysine and arginine forms of the common region in their lambda chains and has indicated that this interchange is probably not due to allelic forms of a single gene. One might explain such an interchange by either of two models, (i) the presence of an ambiguous codon at position 190 or (ii) gene duplication followed by mutation. If the gene duplication is not a recent event, one might expect to find additional mutated sites. Thus, if the gene duplication at the common region is not recent, the arginine and lysine common regions should differ from one another at multiple positions just like the beta and delta chains of human hemoglobin which differ at ten positions (*5*).

Only four common regions of lambda chains have been completely sequenced; they are identical, and all have arginine at position 190 (and are termed CL-arg proteins) (*2*, *6*). We set out to see whether or not the common regions from two proteins with lysine at position 190 (that is, CL-lys proteins) were identical to their arginine counterparts (except for the interchange at position 190) in order to distinguish between a very recent gene duplication or translational ambiguity on the one hand and a gene duplication which occurred early in immunoglobulin evolution on the other hand.

Aminoethylated lambda chains (*7*)

were digested with 1 percent *l*-(1-tosylamido-2-phenol)ethyl chloromethyl ketone–trypsin, and peptide maps were prepared by two methods (*8, 9*) (Fig. 1). These maps were treated with a dilute ninhydrin spray and heated in 80°C oven for 6 minutes, the resulting ninhydrin-positive spots were cut out, and the peptides were eluted with water. Portions of the peptides were hydrolyzed for 16 hours at 110°C and examined by high voltage electrophoresis on paper (*10*). Additional aliquots of the appropriate peptides were then hydrolyzed for 24 hours at 110°C and analyzed on the Beckman (model 120 C) amino acid analyzer. The NH_2-terminal residue of each peptide was determined with the dansyl technique (*11*). Peptides containing tryptophan were detected by Ehrlich's reagent (*12*). Amide content was determined for certain peptides (for example, those with a single acidic group) by electrophoresis with amino acid standards at *p*H 6.5.

All of the common-region peptides can be isolated from the two procedures

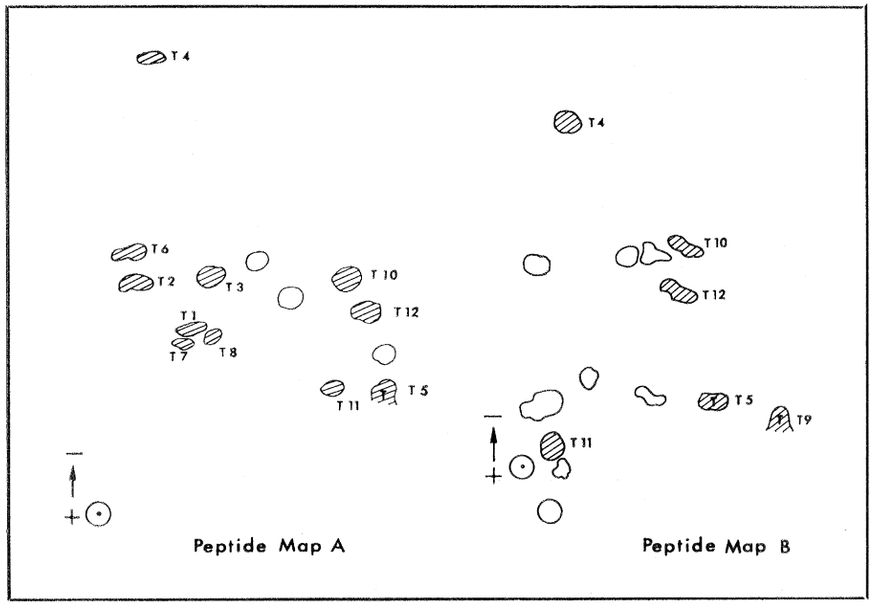

Fig. 1 (left). Tracings of tryptic peptide maps of lambda chain HS 5. Shaded peptides are from the common region and are labeled as indicated in Fig. 2. The unshaded peptides come from the variable region; *T* indicates tryptophan-containing peptides (Ehrlich's stain). Peptide map A was developed according to the procedure of Katz, Dreyer, and Anfinsen (*8*) and map B according to the procedure of Baglioni (*9*).

```
                T12↓
        108     110                          120
Sh      Gly-Gln-Pro-Lys-Ala-Ala-Pro-Ser-Val-Thr-Leu-Phe-Pro-Pro-Ser-Ser-Glu-Glu-Leu-Gln-
HS 5    Ser,Gln,Pro,Lys)Ala(Ala,Pro,Ser,Val,Thr,Leu,Phe,Pro,Pro,Ser,Ser,Glx,Glx,Leu,Glx,
HS 92   Gly,Gln,Pro,Lys)Ala(Ala,Pro,Ser,Val,Thr,Leu,Phe,Pro,Pro,Ser,Ser,Glx,Glx,Leu,Glx,

        T11↓            T10↓*
        130                          140
Ala-Asn-Lys-Ala-Thr-Leu-Val-Cys-Leu-Ile-Ser-Asp-Phe-Tyr-Pro-Gly-Ala-Val-Thr-Val-Ala-Trp-
Ala,Asx,Lys)Ala(Thr,Leu,Val,Cys)Leu(Ile,Ser,Asp,Phe,Tyr,Pro,Gly,Ala,Val,Thr,Val,Ala,Trp,
Ala,Asx,Lys)Ala(Thr,Leu,Val,Cys)Leu(Ile,Ser,Asp,Phe,Tyr,Pro,Gly,Ala,Val,Thr,Val,Ala,Trp,

 T9↓                T8↓                                  T7↓
150                          160                                  170
Lys-Ala-Asp-Ser-Ser-Pro-Val-Lys-Ala-Gly-Val-Glu-Thr-Thr-Thr-Pro-Ser-Lys-Gln-Ser-Asn-Asn-
Lys)Ala(Asp,Ser,Ser,Pro,Val,Lys)Ala(Gly,Val,Glu,Thr,Thr,Thr,Pro,Ser,Lys)Gln(Ser,Asn,Asn,
Lys)Ala(Asp,Ser,Ser,Pro,Val,Lys)Ala(Gly,Val,Glu,Thr,Thr,Thr,Pro,Ser,Lys)Gln(Ser,Asn,Asn,

 T6↓                                          T5↓      T4↓
                     180                          190
Lys-Tyr-Ala-Ala-Ser-Ser-Tyr-Leu-Ser-Leu-Thr-Pro-Glu-Gln-Trp-Lys-Ser-His-ARG-Ser-Tyr-Ser-
Lys)Tyr(Ala,Ala,Ser,Ser,Tyr,Leu,Ser,Leu,Thr,Pro,Glx,Glx,Trp,Lys)Ser(His,LYS)Ser(Tyr,Ser,
Lys)Tyr(Ala,Ala,Ser,Ser,Tyr,Leu,Ser,Leu,Thr,Pro,Glx,Glx,Trp,Lys)Ser(His,LYS)Ser(Tyr,Ser,

 T3↓*                        T2↓                      T1
                200                          210      213
Cys-Gln-Val-Thr-His-Glu-Gly-Ser-Thr-Val-Glu-Lys-Thr-Val-Ala-Pro-Thr-Glu-Cys-Ser
Cys)Glx(Val,Thr,His,Glx,Gly,Ser,Thr,Val,Glx,Lys)Thr(Val,Ala,Pro,Thr,Glu,Cys,Ser)
Cys)Glx(Val,Thr,His,Glx,Gly,Ser,Thr,Val,Glx,Lys)Thr(Val,Ala,Pro,Thr,Glu,Cys,Ser)
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Fig. 2. The common region of two CL-lys proteins compared with Bence Jones protein Sh, a CL-arg protein. Amino terminal residues of each peptide were determined by the dansyl method (*11*). Numbering is that of protein Sh (*2*). *Aminoethylation of cysteine residues generally permits trypsin cleavage at aminoethylcysteine residues. Such a cleavage did not occur between the penultimate aminoethylcysteine and the COOH-terminal serine.

for obtaining peptide maps (see Fig. 1). The amino acid compositions of these peptides for proteins HS 92 and HS 5 agree with the sequences in Fig. 2 within experimental error (maximum deviation ± 0.2 residue per mole). The peptide compositions and amino-terminal residues are identical in all three proteins except for the arginine-lysine interchange at position 190. The two CL-lys regions appeared to be identical to their CL-arg counterparts except for the interchange at position 190 (*13*).

A lambda common gene duplication early in immunoglobulin evolution seems unlikely in that the CL-lys regions have diverged from their CL-arg counterparts by only a single amino acid interchange. Rather, this virtual identity of sequences in CL-lys and CL-arg regions suggests a genetic model of the lambda common region with an ambiguous codon at position 190 or a model of recent gene duplication followed by a single mutational event.

LEROY HOOD
DANIEL EIN

National Cancer Institute,
Bethesda, Maryland 20014

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14. The amino acid analyses were carried out by M. Miller. We thank J. Williams for technical assistance and Dr. J. L. Fahey for reviewing the manuscript.

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Specificity of Ribonuclease Ch from Chalaropsis Species

Abstract. *A ribonuclease is produced by the fungus* Chalaropsis *sp. that appears to have an absolute specificity for 3′-guanylic acid residues in ribonucleic acid.*

In the course of isolating a staphylolytic enzyme from the fungus *Chalaropsis* sp. (*1*), it was discovered that a ribonuclease was also produced by this organism (*2*, *3*). This ribonuclease, which will be designated ribonuclease Ch, appears to have an absolute specificity for 3′-guanylic acid (3′-GMP) residues in ribonucleic acid.

The ribonuclease Ch used to establish the specificity was prepared by column chromatography on Amberlite CG-50 (*3*). Yeast RNA, purified according to Frisch-Niggemeyer and Reddi (*4*), was used as substrate. After incubation with ribonuclease Ch, cyclic phosphates were hydrolyzed with dilute HCl at 4°C and monophosphate groups were removed with ribonuclease-free alkaline phosphatase (Worthington). Samples were then hydrolyzed with 0.3*N* KOH at 37°C to give mixtures of 2′- and 3′- nucleotides and nucleosides. The specificity is indicated by the nucleosides that are produced.

Results of two-dimensional paper chromatography of a hydrolyzate of RNA by ribonuclease Ch are shown in Fig. 1. The products are the three nucleotides 2′- and 3′- adenylic acid, cytidylic acid, and uridylic acid (2′- and 3′- AMP, CMP, and UMP), and one nucleoside, guanosine. These results indicate that ribonuclease Ch cleaves RNA at 3′-GMP residues. Column chromatography, according to the procedure of Blattner and Erickson (*5*), fully corroborated these results (*6*). Guanosine was obtained from thin layer chromatograms for identification pur-

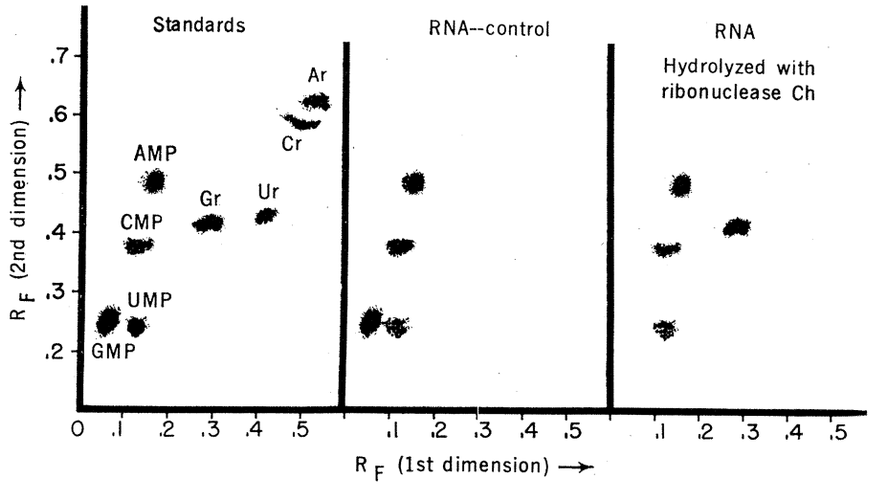

Fig. 1. Two-dimensional chromatography of RNA which has been hydrolyzed with ribonuclease Ch. RNA (50 mg) was dissolved in 2.5 ml of 0.02*M* phosphate buffer, *p*H 7.0, and 0.5 ml was removed as control. Ribonuclease Ch (10 μg protein in 5 μl buffer) was added and the sample was incubated at 37°C. Aliquots of 0.5 ml were removed at 10 minutes, 60 minutes, and 16 hours. Reactions were terminated by adding 50 μl of 1*N* HCl. Cyclic phosphates were hydrolyzed by holding the samples at the acid *p*H for 16 hours at 4°C. Tris-HCl buffer (0.5 ml of 0.4*M*, *p*H 7.9) containing 20 μg of ribonuclease-free alkaline phosphatase was added to the samples, which were then incubated at 37°C for 16 hours. KOH was added to 0.3*N* (60 μl of 5*N* KOH) and the samples were incubated 24 hours at 37°C. The *p*H of the samples was adjusted to 4.5 with 3*N* $HClO_4$, and $KClO_4$ was removed by centrifugation. Sample volumes were adjusted to 1 ml, and 20 μl aliquots were chromatographed in a descending fashion for 16 hours on Whatman No. 1 paper. The first-dimension solvent was isopropanol, concentrated NH_4OH, water (7 : 1 : 2, by volume) and the second dimension solvent was isobutyric acid, concentrated NH_4OH, water (66 : 1 : 33, by volume). The solvents were adapted from Wyatt (*11*). Contact prints of ultraviolet absorbing compounds were made with Ilford R4-1P photographic paper and a shortwave ultraviolet lamp. Images were reversed by conventional photography. Only that portion of each chromatogram which contained ultraviolet-absorbing compounds is shown. The nucleotides GMP, UMP, CMP, and AMP are the 2′- and 3′-derivatives, and Gr, Ur, Cr, and Ar represent the corresponding ribosides, guanosine, uridine, cytidine, and adenosine, respectively. The 10- and 60-minute samples were identical to the 16-hour sample shown.