are separate from the surface membrane. Hence phototransduction in both the *Aplysia* neuron and the vertebrate rod requires the release of a transmitter to couple photon absorption by pigment to current flow across the plasma membrane. It is also noteworthy that the light-induced morphological alterations of these large granules, which ultrastructurally resemble lysosomes, may be preceded by a depletion of calcium and other elements.

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6. Calcium EGTA buffers were prepared by the method of R. W. Meech [*J. Physiol. (Lond.)* **237**, 259 (1974)]. The intracellular *p*H in R_2, which we measured with an H^+ glass microelectrode, ranges from 7.2 to 7.3. At *p*H 7.26 the apparent association constant for Ca EGTA is $10^{7.25}$ [J. Bjerrum, G. Schwarzenbach, L. G. Sillén, in *Stability Constants* (The Chemical Society, London, ed. 2, 1964), part 1, pp. 76 and 90; H. Portzehl, P. C. Caldwell, J. C. Rüegg, *Biochim. Biophys. Acta* **79**, 581 (1964)]. This calculation is only approximate for intracellular Ca since it ignores the effects of any intracellular Mg, which would increase the level of free Ca. A plot of the change in membrane conductance against the free Ca contained in a variety of Ca EGTA buffers injected into R_2 indicates a control level of about $1.1 \times 10^{-7} M$, which is slightly less than the values of $3 \times 10^{-7} M$ reported for squid [P. F. Baker, A. L. Hodgkin, E. B. Ridgway, *J. Physiol. (Lond.)* **218**, 709 (1971)] and $9 \times 10^{-7} M$ reported for *Helix* by Meech (see above). Illumination increases membrane conductance by 15 percent (*2*), and for such an increase the free intracellular Ca should have doubled.
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Partial Amino Acid Sequence of the Precursor of Immunoglobulin Light Chain Programmed by Messenger RNA in vitro

Abstract. *The five proteins programmed in a cell-free system by a mouse kappa light chain messenger RNA were labeled with [^3H]leucine and subjected to amino acid sequence analyses. In all five proteins, 20 amino acid residues precede the amino terminus of the mature protein, indicating that there is one major point for the initiation of messenger RNA translation. The abundance (30 percent) of leucine residues in the extra piece (leucine at positions 6, 7, 8, 11, 12, and 13) indicates that this moiety is hydrophobic. Furthermore, it seems that the precursor may have an additional extra piece at the carboxyl terminus.*

The possibility that the messenger RNA (mRNA) coding for the MOPC-321 immunoglobulin light (L) chain directs the synthesis of an L-chain precursor was initially suggested from the size of the cell-free products and from two-dimensional peptide mapping of their tryptic digests (*1*). In a cell-free system this mRNA programmed the synthesis of five proteins with molecular weights of about 28,700, 25,300, 19,700, 18,200, and 17,200. Despite the fact that none of these proteins are of the same size as the authentic L chain (24,020 molecular weight) (*2*), the peptide patterns they yield are composed almost entirely of L-chain peptides. Sequence analysis of one of the cell-free products showed that it contains an extra piece composed of 20 amino acid residues coupled to the NH_2-terminus of the mature protein (*1*). We report here radioactive amino acid sequence analyses of all of the five cell-free products that were labeled with [^{3}H]leucine and suggest how they are interrelated.

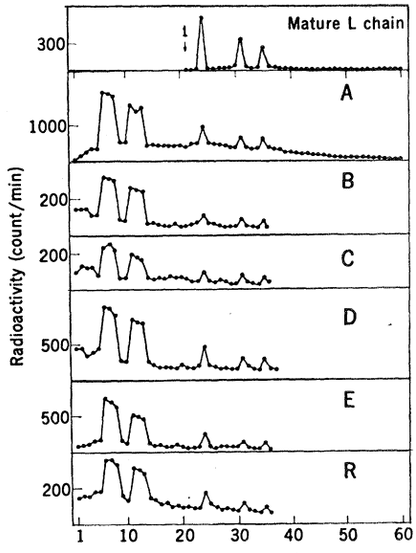

Fig. 1. Radioactivity determined from each cycle of Sequenator analyses of the mature M-321 L-chain marker (^{14}C, upper panel) and of cell-free products (^{3}H, samples A to R, lower panels) programmed by MOPC-321 L-chain messenger RNA. Molecular weights of the samples analyzed are given in Table 1. Numbers on the abscissa correspond to the cell-free products; the first Sequenator cycle for the mature L chain is indicated by the arrow.

The preparation of polysomes from MOPC-321 mouse myeloma, the isolation of L-chain mRNA from polysomes specifically precipitated with antibodies to L chain, and the translation of mRNA in the Krebs 2 ascites cell-free system have been described (*1*, *3*). More than 95 percent of the protein programmed by this mRNA was M-321 L chain (*1*). In our study the cell-free products labeled with [^{3}H]leucine (29.8 c/mmole) were resolved by electrophoresis on sodium dodecyl sulfate–10 percent polyacrylamide gels with 0.42*M* tris · HCl (*p*H 9.18) as the running buffer (*4*). After the electrophoresis (3 hours; 2 ma per tube), 110 slices per gel were prepared. The material was eluted by shaking each slice in 0.5 ml of 8 m*M* tris · HCl (*p*H 9.18) for 24 hours at room temperature. Portions (25 μl) were counted; the radioactive pattern obtained showed five peaks that corresponded to the five protein bands previously characterized in the cell-free reaction mixture (*1*). The peaks were collected separately. They are designated here A, B, C, D, and E (Table 1). To gain information on the totality of cell-free products, the small amount of material that was eluted between each of the peaks A to E was also collected and pooled to yield sample R. The radioactivity eluted in samples A to E and sample R was 72 percent of the amount applied to the gel. Nonradioactive M-321 L-chain protein (6 mg) was added to each sample. Sample A was precipitated with 10 percent trichloroacetic acid, washed once with 5 percent trichloroacetic acid and three times with a mixture of 95 percent acetone and 5 percent water (by

volume). Samples B to R were made 4*M* in guanidine hydrochloride and dialyzed exhaustively against 0.01*M* ammonium bicarbonate. The samples were then sequenced (*5*). Portions of the thiazolilinones for each cycle of the Sequenator degradations were counted in a liquid scintillation counter. In some analyses the sequence of the unlabeled M-321 carrier protein was also quantified by gas chromatography.

In order to evaluate the applicability of the Sequenator for analyzing labeled cell-free products, we first analyzed [^{14}C]leucine-labeled M-321 L chain that was synthesized by intact cells. The authenticity of this marker was ascertained from sizing on sodium dodecyl sulfate–polyacrylamide gels and from the two-dimensional peptide mapping of the tryptic peptides (*1*). We subjected the marker to 45 degradation steps in the amino acid Sequenator (Fig. 1). Labeled material was detected in steps 4, 11, and 15, as was expected from the chemical sequence of M-321 L chain [leucine residues located in positions 4, 11, 15, 50, and so on (*2*)]. Furthermore, we ascertained that the ^{14}C label was exclusively in leucine by hydrolyzing the thiazolilinone derivatives and identifying the labeled product on the amino acid analyzer.

The amino terminal sequence of each of the samples resolved from the cell-free reaction mixture was then determined. All samples yielded an identical pattern of radioactivity, which was different from that of the mature protein. Peaks of radioactivity occur at steps 6, 7, 8, 11, 12, 13, 24, 31, and 35 (Fig. 1).

The yield (*5*) from each of the Sequenator runs (Table 1) shows that only a fraction of the labeled material was analyzed (40 percent of sample A), and that this fraction varied in the different samples. This could be due to variable Sequenator efficiencies (see chemical yield) or due to differences in the availability of the α-amino groups of the proteins. We cannot rule out the possibility that each sample contains variable amounts of proteins with blocked α-amino groups.

All the cell-free products (but not the [^{14}C]leucine-labeled marker) showed considerable radioactivity background (Fig. 1). The pattern of this radioactivity is similar to that seen during the course of a normal sequence determination, except that in our products it is higher. The background radioactivity that occurs in all Sequenator analyses has been attributed to the generation of new α-amino groups by nonspecific cleavage of the peptide chain during the course of the degradative cycle (*5*, *6*). Thus, the increased background might be explained if we assume that in the extra piece (which is rich in [^{3}H]leucine) there are peptide bonds that have an increased tendency for nonspecific cleavage. That immunoglobulin associated with an unusual chain length is easily hydrolyzed has been reported. A cell line derived from MPC-11 synthesizes an L chain that has 12 extra residues at the NH_2-terminus. Two bonds in this extra piece are hydrolyzed under mild conditions that do not normally promote peptide bond cleavage (*7*).

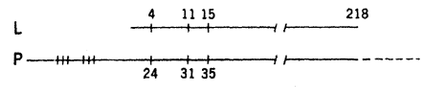

Fig. 2. Schematic representation of the precursor (*P*) of the mature MOPC-321 L chain (*L*). Vertical lines denote leucine residues determined at the amino terminus of both proteins. In addition to the extra 20 amino acid residues at the NH_2-terminus, the precursor may have another extra piece (broken line) at the COOH-terminus. Numbers represent positions in the polypeptide chain.

The extra piece in our precursor is different from that in the MPC-11 variant. The latter is secreted by intact cells, and half of the extra piece has the same sequence as the NH_2-terminus of the normal MPC-11 L chain (*7*). On the other hand, the major fraction of the M-321 precursor is short-lived in vivo because it was not detectable in the cell cytoplasma. As was mentioned above, the [^{14}C]leucine-labeled L chain isolated from the tumor was indistinguishable from the mature L chain. Furthermore, the sequence of the mature M-321 protein does not contain three consecutive leucine residues anywhere in its amino acid sequence.

The detection of labeled leucine residues at positions 4, 11, and 15 in the [^{14}C]leucine-labeled marker serve as a basic control for the identification of the NH_2-terminus of the mature protein in unknown samples. The probability that this pattern of leucine residues occurs by chance is low (*8*). The leucine residues in positions 24, 31, and 35 in the cell-free products match with the leucine residues at positions 4, 11, and 15 in the mature protein (Fig. 1). This establishes that the M-321 mRNA directs the synthesis of a precursor in which 20 amino acid residues are coupled to the NH_2-terminus of the mature protein (Fig. 2). Others have reported that preparations containing kappa L-chain mRNA obtained from myelomas M-41 (*9*), M-21 (*10*), and M-70E (*11*) direct the synthesis of proteins larger than the mature L chain; however, the location and precise sizing of the extra piece were not determined.

The 20 residues in the extra piece at the NH_2-terminus do not account for the size of the largest cell-free product. Peak A (28,700 molecular weight) corresponds in size to the mature protein plus about 40 amino acid residues. These findings raise the possibility that the precursor may contain at the COOH-terminus an additional extra piece which is also cleaved in the process of protein maturation (Fig. 2).

The five protein bands range in size from about 28,700 to 17,200 molecular weight, yet in all of them the same NH_2-terminal sequence was detected. Thus, we may conclude that portions of

Table 1. Sequenator results of MOPC-321 mouse kappa L chain. The [^{14}C]leucine-labeled L-chain marker was synthesized by intact cells and was subjected to 45 degradative cycles. Samples A to R are the cell-free products programmed by the M-321 L-chain mRNA. They were labeled with [^{3}H]leucine and subjected to at least 36 degradative cycles.

Sample	Molecular weight	Amount analyzed (count/min)	Sequenator yield (%) extrapolated to cycle 1	
			Radioactive*	Chemical†
^{14}C-labeled L chain	24,020	120,000	97	91
A	28,700	120,000	40	64
B	25,300	21,000	51	‡
C	19,700	86,000	23	‡
D	18,200	27,000	91	‡
E	17,200	80,000	28	‡
R	28,000 to 18,000	37,000	26	‡

* Yield based on 13 leucine residues per molecule for the ^{14}C-labeled L chain and 19 leucine residues per molecule (13 + 6) for the cell-free products. † Yield of M-321 nonradioactive carrier. ‡ Not done.

the COOH-terminal region are missing in the proteins that are smaller than the largest cell-free product (peak A). This can arise from initiation of mRNA translation at one point followed by premature termination of translation at several discrete points. Premature termination resulting in the synthesis of discrete protein bands was observed in the cell-free translation of encephalomyocarditis virus mRNA (*12*). The above reasoning may explain the peptide map of the tryptic digest of the cell-free products, which contained 27 of 28 L-chain peptides plus 4 additional peptides (*1*). The additional peptides might come from the extra piece as well as from new tryptic peptides generated at the COOH-terminus as a result of incomplete synthesis of the L chain. The apparent loss of one peptide is probably due to a modified NH_2-terminus in the precursor (*13*).

The synthesis of hemoglobin (*14*), and perhaps that of immunoglobulin L chain (*10*), is initiated by a specific methionyl-tRNA. The finding that all L-chain cell-free products share an identical NH_2-terminal sequence (Fig. 1) provides further evidence that in eukaryotes, as in prokaryotes (*15*), there is one major point for the initiation of mRNA translation.

Although the sequence of the extra piece is incomplete, the fact that 30 percent of it is composed of leucine residues indicates that it would be quite hydrophobic. Indeed experiments on the sequence of M-321 and M-41 precursors labeled with six ^3H-labeled amino acids show that in both cases at least 60 percent of the extra piece is composed of hydrophobic amino acids. The marked hydrophobicity suggests that the role of the extra piece is to favor interaction of the precursor with the endoplasmic membranes or the cell wall (or both). Furthermore, the NH_2-terminal extra piece in the M-321 and M-41 L-chain precursors differs both in size and in amino acid sequence (*16*).

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Solar Nitrogen: Evidence for a Secular Increase in the Ratio of Nitrogen-15 to Nitrogen-14

Abstract. *Solar wind nitrogen, implanted in lunar soil samples, exhibits isotopic variations that are related to the time, although not to the duration, of implantation, with earlier samples characterized by lower ratios of nitrogen-15 to nitrogen-14. An increase in the solar nitrogen-15 content during the lifetime of the lunar regolith is probably caused by spallation of oxygen-16 in the surface regions of the sun.*

Interaction between the solar wind and the moon leads to the implantation of solar wind species in the surfaces of lunar grains (*1, 2*). This effect is revealed by the presence within lunar soils of such elements as carbon, nitrogen, hydrogen, and the noble gases, which are effectively absent from lunar rocks. Although virtually all lunar soils are enriched in these elements, there is considerable intersample variation in the elemental abundance ratios and isotopic compositions. These variations have generally been associated with lunar fractionation mechanisms (*3*). No evidence has been found for any change in the composition of the solar wind over the lifetime of the lunar regolith, although short-term variations in the ratio of hydrogen to helium have been observed and related to solar flare activity (*4*).

Elemental and isotopic fractionations on the lunar surface reflect primarily the preferential loss of light species as a result of mass-dependent mechanisms such as thermally activated diffusion (*1, 3, 5*). Thus, the hydrogen content of lunar soils is depleted below the solar proportion relative to helium (*5*), and similar effects have been observed for other solar wind elements. Figure 1a shows the relationship between carbon and nitrogen abundances for soils from the Apollo 16 landing site (*6*). The strong correlation suggests a common origin for both elements, and the slope of the line for the Apollo 16 soils differs from that representative of the solar abundances, yielding a value for the ratio of carbon to nitrogen (atomic) of 1.5 ± 0.1, as compared with the solar value of 3.2 (*7*). Similar relationships exist between the nitrogen abundance and the abundance of hydrogen or of those noble gas isotopes of solar rather than spallogenic origin, and all show depletion relative to nitrogen in comparison with solar abundances, thus suggesting that nitrogen is the most efficiently retained solar wind element. This efficiency may approach 100 percent as a result of the reactivity of atomic nitrogen and the stability of bonds formed between nitrogen and the prevalent lunar cations (*8*). Calculations based upon the solar wind flux and nitrogen abundances in regolith of known thickness are consistent with 100 percent efficiency (*9*).

Nitrogen is clearly retained more efficiently than carbon and should therefore show a smaller range of isotopic fractionation than carbon if such fractionations are caused by loss from the lunar surface. Actually the ob-