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The paper shows that in the absence of membranes, the primary translation product of immunoglobulin light chain mRNA is a putative precursor. The extra fragment at the N-terminus is proposed to "provide a signal" to discriminate free and membrane-bound polysomes, and, hence, to initiate protein secretion. [The *SCI*® indicates that this paper has been cited in more than 565 publications.]

A Signal for Protein Secretion

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By the early 1970s, it became increasingly clear that to understand antibody diversity, it would be necessary to move beyond amino acid sequencing of myeloma proteins. We took the view that further understanding would come from structural studies of mRNA, at a time when sequencing of small transfer RNA was moving into larger entities, and when sequencing of DNA was not yet in the realm of possibilities. The earliest attempt at isolation of immunoglobulin light chain messenger RNA was by Peter Fellner, a student of Fred Sanger, collaborating with me. Although a failure, we decided to join forces and try again. Friendship, which included walking and camping for extended weekends in Wales and in the Lake District, was a critical ingredient of our collaboration. We were soon joined by Tim M. Harrison. The initial attempts were very depressing, largely because affinity methods for purification of mRNA were not at that time possible (the polyA tail of mRNA had not been discovered), and we had no specific assay for immunoglobulin mRNA. The picture changed, thanks to two developments.

First, J. Stavenezer and R.C.C. Huang[1] demonstrated in vitro synthesis of light chain in a rabbit reticulocyte cell-free system, thus providing an assay. Second was the arrival in our laboratory of Mike B. Mathews, who had developed an alternative in vitro protein synthesizing system using Krebs II ascites extracts. We set out to try both methods. Our first success was in fact with the latter.[2] However, it was the use of the rabbit reticulocyte lysate (set up with help from Tim Hunt and Elma Cartwright) which gave us our novel and surprising result.

We expected the ^{35}S-labeled light chain (fractionated on the then-recently described Laemmli gels) would co-migrate with authentic light chain. Instead, there was a product about 1,500 daltons larger. The Krebs II system, by contrast, gave a product of identical size to light chain. The critical difference between the two systems proved to be the presence of microsomes in the Krebs II ascites extract and their absence in the reticulocyte lysate. The idea that the difference reflected a precursor-product relationship, although speculative, was correctly predicted as being due to an "enzymatic activity required during, rather than after (protein) synthesis." This seemed to tie up neatly with the discrimination between membrane-bound and free polysomes. The signaling device whereby this was achieved was proposed to be 15-20 extra amino acids, which we showed were at the N-terminus of the putative precursor. This was serendipity. A tiny difference in the position of two bands in a gel that could have been easily overlooked, or ascribed to some artifact, was correctly interpreted as real and set in the context of a central problem in cell biology, of how proteins were secreted—a problem we had not set out to solve.

The discrimination between free and membrane-bound polysomes was, at the time, a subject of considerable debate. All sorts of ideas had been proposed to explain this, including one (proposed by G. Blobel and D.D. Sabatini,[3] but unknown to us) based on a signal peptide. However, what was important in our paper, perhaps more than the idea or even than the demonstration of the existence of the precursor, was the way in which knowledge advanced subsequently. It is interesting to look back at the literature at the time and see that, until our paper, the problem of secretion was centered on the studies of membrane-bound and free polysomes. Afterwards, the approach changed completely into a study of in vitro synthesis, either in the presence or absence of membranes. This opened the way to a full-scale biochemical analysis of the components involved, culminating in the complex series of events involved in the attachment of the translation machinery to the membranes, initiated by the synthesis of the signal peptide, and resulting in the vectorial release of secreted and membrane proteins across the endoplasmic reticulum membrane.[4]

1. **Stavenezer J & Huang R C C.** Synthesis of a mouse immunoglobulin light chain in a rabbit reticulocyte cell-free system. *Nature (London) New Biol.* 230:172-6, 1971. (Cited 185 times.)
2. **Brownlee G G, Harrison T M, Mathews M B & Milstein C.** Translation of messenger RNA for immunoglobulin light chains in a cell-free system from Krebs II ascites cells. *FEBS Lett.* 25:244-8, 1972.
3. **Blobel G & Sabatini D D.** Ribosome-membrane interaction in eukaryotic cells. (Manson L A, ed.) *Biomembranes.* New York: Plenum, 1971. Vol. 2. p. 193-5. (Cited 285 times.)
4. **Verner K K & Schatz G.** Protein translocation across membranes. *Science* 241:1307-13, 1988. (Cited 125 times.)

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