Surprising Discovery with a Small RNA

A series of lucky finds revealed a small RNA to be involved in protein secretion

When Peter Walter of the Rockefeller University gave an unscheduled presentation at a Cold Spring Harbor meeting on RNA processing earlier this year he caused considerable delight and surprise. His results, gathered in the weeks just prior to the meeting, showed that a small RNA molecule, designated 7*S*, was an essential component in the export from cells of secretory proteins.

The study of the numerous families of small RNA molecules has become highly fashionable, particularly because of their putative involvement in processing messenger RNA. Despite all this effort, none of the small RNA's has been ascribed a function, apart of course from the long-established ribosomal 5*S* and 5.8*S* RNA's and transfer RNA's. The discovery of a function for 7*S* RNA was therefore an important "first," especially as its role in protein secretion was entirely unexpected.

The series of events that led Walter to the Cold Spring Harbor meeting followed a classic, and pleasing, pattern of scientific discovery, involving a combination of chance observations and coincidental meetings.

Walter joined Günter Blobel in 1977 at the Rockefeller University where he hoped to characterize some of the components involved in protein secretion. In 1980 Walter and Blobel submitted a paper to the *Proceedings of the National Academy of Sciences* that described the composition of a protein complex that mediated communication between the protein to be exported and a receptor in the endoplasmic reticulum.

The complex interacts with a signal sequence that is tagged onto the front of all proteins that are to be secreted from their cell of origin. The sequence is between 15 and 30 amino acids in length and, although it varies in composition between different proteins and organisms, its overall physical characteristics always conform to a common pattern. Without the signal sequence a protein cannot exist through the endoplasmic reticulum to the cell's exterior.

Walter and Blobel called the complex the signal recognition protein. It is made up of six polypeptides, with molecular weights of 72,000, 68,000, 54,000, 19,000, 14,000, and 9,000. "We found that the complex was very compact and, by antigenicity tests, was evolutionarily conserved over many different species," says Walter. "We purified it to homogeneity. And we thought we'd characterized it too."

During the rest of 1980 and through 1981 Walter and Blobel, with some additional help from Ibrahim Ibrahimi, worked out the properties of the signal recognition protein. These are described in a series of papers in the *Journal of Cell Biology* published in November 1981.

"It has been possible to dissect this protein translocation system into its component parts," says Walter, "so we now have a good picture of what each of them does. You can think of this signal recognition particle as an adapter between the cytoplasmic translation machinery and the translocation machinery in the membrane."

> The discovery of a function for 7*S* RNA was therefore an important "first," especially as its role in protein secretion was entirely unexpected.

When a messenger RNA encoding a secretory protein engages with the two subunits of the ribosome and the other components of translation, the first part of the polypeptide to emerge is the signal sequence. The signal recognition particle immediately binds with the ribosome, presumably interacting with the signal sequence in some way. Now, if in an experimental environment the endoplasmic reticulum membrane is absent, translation is arrested at this point. Addition of the membrane, which has a 72,000-dalton protein, the SRP-receptor, embedded in it, releases the arrested translation when the signal recognition particle makes contact with this intracellular receptor molecule. Protein manufacture and export proceed as a continuous process.

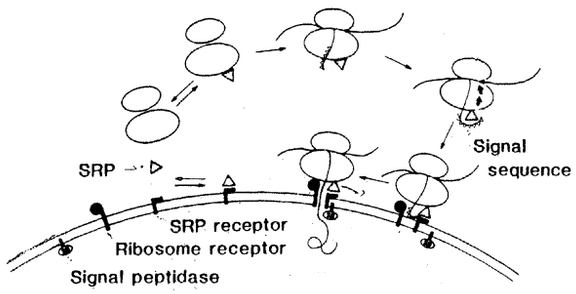
"Having got this far in our studies," says Walter, "the thing we were principally interested in was the various recognition processes between the ribosome, the receptor, and particle." Work therefore continued, and during one preparation of the signal recognition protein in March of this year Walter noticed what appeared to be nucleic acid associated with the protein. "We happened to notice a particularly strong absorption around 260 nm in the eluate we wanted in the preparation. Normally we wouldn't have monitored at this wavelength. It was just a lucky observation."

Some intense work quickly showed the nucleic acid to be a small RNA molecule that was an integral component of the particle, not just one loosely associated with it. "This was of course very exciting, and a little embarrassing too. Having claimed to have purified and characterized the particle we were now finding it contained a whole new component." The complex is composed of the six polypeptides plus one molecule of the RNA.

Isolation of the RNA from the signal recognition particle was relatively easy. It yielded a main sequence 260 nucleotides long with a shorter sequence, presumably a product of degradation, some 245 nucleotides long. Walter and Blobel were aware of, but not familiar with, the many families of small RNA's. They wondered whether theirs was one already known, and so began some partial sequencing to see if any matched. By the beginning of May they had fully sequenced 40 residues from the 3′ end as well as producing a partial sequence (A and G residues only) for about 150 residues from the 3′ end.

At this point Walter visited Yale to give a seminar in the Department of Cell

0036-8075/82/1119-0777$01.00/0



Protein translocation

The signal recognition particle acts as a transient third unit of a ribosome in linking protein translation with export from the cell. Details of the interaction between the RNA component of the particle and the other components of the system have yet to be established.

Biology. As it happened, Elisabetta Ullu, who had joined Alan Weiner's laboratory at Yale just a few weeks earlier from the European Molecular Biology Laboratory in Heidelberg, was at the seminar. Ullu had been working on the structure of 7*S* RNA from humans and she had a paper in press, with Shona Murphy and Marialuisa Melli, on its sequence (derived from its complementary DNA).

During the seminar, Walter described the discovery of the mysterious small RNA and showed some data on its size. Ullu immediately saw a striking resemblance with the size of the 7*S* RNA which she was about to publish. After the seminar, they made a detailed comparison of all available information and found that the two molecules were identical as far as could be established.

The Cold Spring Harbor meeting on RNA processing was due to be held 2 weeks later, and a speaking slot was quickly arranged for Walter to present his work there. This coincided with the publication of Ullu's paper in *Cell*. Harris Busch and his colleagues from Baylor College of Medicine, Houston, also published a sequence for 7*S* RNA, this one from rat, about the same time. Human and rat 7*S* RNA are essentially identical in structure.

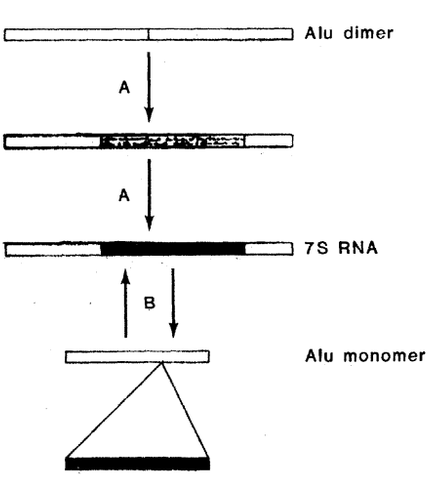
The structure of 7*S* RNA is intriguing, particularly because of its relationship to the most common repeated sequence of all, Alu. There are perhaps half a million copies of this 300-base-pair sequence in the human genome, accounting for around 5 percent of the DNA. In humans, Alu is apparently made up of two 140-base-pair sequences joined head to tail; in addition, there is a 31-base-pair insert in the right-hand monomer. The 7*S* RNA is similar in length to human Alu, and its sequence is homologous with Alu for 100 nucleotides from the 5′ end and 40 nucleotides from the 3′ end.

"There are obviously several possible ways in which the 7*S* structure might have arisen," says Ullu. "It might have arisen from an Alu dimer in which the central portion diverged in structure. Or the middle section might have inserted in an Alu monomer." There is some support for this latter suggestion in that the core section of 7*S* is flanked by short direct repeats, a molecular signature often associated with insertion.

Ullu has compared the 7*S* sequence over a wide phylogenetic distance, stretching from humans to sea urchins. She finds that there is a strong conservation of the core section of the molecule, but that the flanking Alu-like regions have diverged considerably.

The striking conservation of at least part of the 7*S* molecule is not surprising because the function in which it is involved is extremely primitive. The machinery for protein secretion is therefore likely to be similar across wide phylogenetic distances. For instance, Walter and Blobel, together with their co-worker Matthias Müller, recently constructed the following molecular chimera: messenger RNA for prokaryotic beta-lactamase (a secretory protein), plant ribosomes (from wheat germ), and mammalian microscomal membranes. The system worked with high fidelity.

The probable antiquity of the 7*S* RNA, and the apparent ease with which the whole molecule is packaged within the signal recognition particle, leads Ullu and Walter to speculate that Alu might have derived from 7*S* rather than 7*S* having picked up part of Alu. "It's a possibility one can at least think about," she says. "There are still many questions to ask about 7*S* RNA."



Possible origin of 7S RNA

7S RNA might have arisen by sequence divergence of the central section of an Alu dimer (route A) or by the insertion of the core sequence into an Alu monomer (route B). Route B is reversible and might instead explain the origin of Alu. Short repeats flanking the core section offer support for a insertion.

The current estimate for the number of 7*S* genes in the human genome is somewhere between 500 and 1000. It appears, however, as if only about ten or fewer of these are transcribed. Most sequences, in common with an emerging pattern with small RNA, are pseudogenes.

With the discovery of a small RNA as an integral part of the signal recognition particle, the question of interaction between the various components of the protein secretion machinery became even more interesting. It is possible that the 7*S* RNA serves only as a structural core on which the six polypeptides assemble. Walter considers this unlikely, however, and guesses that the RNA is directly involved in interparticle recognition. "We are trying to determine whether there is RNA-RNA interaction between the particle and the ribosome or some specific sequences on the messenger. Or whether it is an interaction between a section of the 7*S* RNA and a protein component of the ribosome or some part of the signal sequence. There are many possibilities."

When they first purified the particle Walter and Blobel designated it formally as the signal recognition protein (SRP). With the discovery of the 7*S* RNA as part of the complex the initials were retained but the formal name was changed to signal recognition particle. The combination of protein and RNA in the SRP, and its role in mediating protein translocation, virtually gives it the status of a third ribosomal subunit, even if the association is only transitory. And for those working on small RNA's, the recognition of the SRP for what it really is has emphasized the probable importance of RNA-protein combinations for many of the still-uncharacterized small RNA's.

—ROGER LEWIN

Additional Readings

1. R. Lewin, "Big problems faced in RNA processing," *Science* **216**, 1208 (1982).
2. W. Y. Li, R. Reddy, D. Henning, P. Epstein, H. Busch, "Nucleotide sequence of 7*S* RNA. Homology to Alu RNA and 4.5*S* RNA," *J. Biol. Chem.* **257**, 5136 (1982)
3. E. Ullu, S. Murphy, M. Melli, "Human 7*SL* RNA consists of a 140 nucleotide middle-repetitive sequence inserted in an Alu sequence," *Cell* **29**, 195 (1982).
4. P. Walter and G. Blobel, "Purification of a membrane-associated protein complex required of protein translocation across the endoplasmic reticulum," *Proc. Natl. Acad. Sci. U.S.A.* **77**, 7112 (1980).
5. P. Walter, I. Ibrahimi, G. Blobel, "Translocation of proteins across the endoplasmic reticulum I–III," *J. Cell Biol.* **91**, 545 (1981).
6. P. Walter and G. Blobel, "7*SL* Small cytoplasmic RNA is an integral component of the signal recognition particle," *Nature (London)* **299**, 691 (1982).