



Bacterial protein translocation in Crete

A Motor Driven by Chopsticks

Protein segregation in *E. coli* is well studied but still not fully understood. Therefore, it is still an exciting field for people like Thassos Economou.

White beaches, the azure sea of the Aegean and the beautiful peaks of the Ida Mountains. In addition, the historical sites of ancient Minoan culture and Greek mythology. “This is an absolutely worthwhile place to be,” Anastassios Economou enthuses but he refers to his working place not to the tourist attractions of his surroundings. He believes that research conditions here at the Institute of Molecular Biology and Biotechnology of the Foundation for Research and Technology Hellas (FORTH) in Heraklion, Crete are the best in the whole of Greece.

That’s why the scientist came here in 1995, returning to his home country after completing his PhD in Norwich, UK, followed by postdocs in Los Angeles and at the Dartmouth College in Hanover on the US east coast. At that time Anastassios – or Tassos, as he calls himself – Economou had already focussed on the topic that he is still working on: the molecular mechanisms of protein segregation, mainly in *E. coli*.

Molecular escorts

In each cell, about 30% of the proteins are made that end up either in the cell membrane or are secreted to the outside world, explains Economou. “The process of getting proteins out of the cytoplasm is essential for life, as well as for the pathogenicity of bacteria,” says the biologist.

How can a cell decide which proteins are going to stay in the cytoplasm, and which ones are going to go out? How do the latter exit the cell? These are the questions Economou and his team work to elucidate.

“Proteins which are going to go out have something like a target address in their sequence. Usually at the amino-terminus,” Economou explains. “Thus, they are actually pre-proteins, as they do not have their final sequence yet. This address, in turn, can be recognized by secretion piloting escorts. As soon as that target signal comes out of the mouth of the ribosome during translation, these factors recognize and bind to it.

A soluble cytosolic protein lacks this signal, thus it is not seen by the factors and escapes into the cytoplasm.”

Once the escort factors have grabbed the secretory chain of an outward addressed protein, the complex goes to the membrane and the protein is exported through a channel that allows the hydrophilic protein to snake across the lipid bilayer. To do so effectively they have help in the form of energy input, provided by an ATPase that pushes – or in some cases pulls – the protein across. Having arrived at the outer side, the proteins don’t need the target address anymore, thus, a special protease cuts it away. Finally, the protein looks like a typical soluble protein as you would also find in the cytoplasm.

Not only across, also sideways

What players are involved in this process? In *E. coli*, secretory and membrane proteins are translocated across the membrane by the Sec translocase system, consisting of several components. The pore that allows crossing through the membrane is the trimeric protein SecYEG. “These channels are very unusual and unique,” Tassos points out. “They do not only allow vectorial transport, just across the membrane, but can open laterally as well, sideward to the lipid bilayer. A very important thing for membrane proteins!” If this channel senses an engaged protein which is hydrophobic and thus designated to be a membrane protein, it will open laterally and let it go, if not, it remains closed like a cylinder, explains the 43-year-old. “The molecular mechanisms of this are not yet 100% understood,” says Economou, “but we are coming closer”.

A sensing enzyme

However, a lot of the focus of Economou and his team lies elsewhere. The team works to understand the motor protein of the system, the ATPase called SecA that pushes the secretory proteins to the Sec-machine. “This is a very complicated but

fascinating enzyme,” Economou thinks. It physically contacts every body involved in the reaction, *i.e.* the pore, the lipid bilayer, the secretory protein, some of the targeting factors that bring the secretory protein from the cytoplasm to the membrane, it recognizes itself and forms dimers... “SecA obviously plays a very central role in the reaction,” the biologist points out, “Therefore, it is essential for bacterial life!”

How this enzyme senses secretory peptides, and how it converts chemical energy in the form of ATP into work, is what Economou and Co. are interested in. “This is extremely crucial to understand the whole reaction,” he says.



“Thassos” Economou (back, r.) and his team

For years, the scientists worked to resolve the protein’s structure. “Only when you know what the enzyme looks like, can you ask some rational questions about how it might work,” Economou asserts. By means of crystallography and – in collaboration with other groups – by nuclear magnetic resonance (NMR), the researchers worked to get an atomic resolution of the enzyme structure, with success. Today the structure of SecA is very well characterized, “At the core of the protein there is the ATPase motor, the so called DEAD motor. Having the shape of a clam shell consisting of two domains this binds ATP. Depending on whether ATP or ADP or nothing is occupying the motor, it has a different shape,” says

Economou, "If it is occupied with ATP, the clam shell is shut as it is protecting a viable pearl in there."

The researchers actually understand that this motor can sense nucleotides and depending on the nucleotide's state it starts moving. This is nothing special; many other ATPases have such a core as well. However, the question remains: How did the motor learn to work on pre-proteins and to push them through the membrane?

Complicated relaxation

Recently, Economou and his team came a bit closer to the answer (*EMBO J.* 26, p. 2904). The enzyme must have surfaces, where the pre-proteins go and dock, so that SecA knows that a new protein has just arrived. Furthermore, the protein binding must have a way of triggering the motor to start working. There are two additional domains, the Pre-protein Binding domain (PBD) and the so called C-domain, Economou says. These work like chopsticks held by hand. When the pre-proteins are binding to the PBD, its conformation is changing and it starts moving. This movement causes localized changes to the outside rim of the closed clam shell motor and starts prying the motor open. Once the motor becomes loosened the ADP sitting there can leave and the motor is empty.

"This is absolutely essential for the translocation!" says Economou. As soon as ATP comes along the reaction will start. The central motor region of this mechanism is a salt-bridge called Gate1, which controls the opening and closing of the nucleotide cleft. "Now, we

understand how pre-proteins that bind to this enzyme can exploit the chopsticks to communicate with the motor and thus send a signal to the motor to force it to become open," Tassos Economou summarises their recent results.

Fishing ligands

As a next step, the Greek scientists plan to get granular on interactions of SecA with its ligands, like pre-proteins, pieces of the pore, other escort proteins, ATP, etc. "We will combine our traditional biochemistry and biophysical tools with new NMR methods developed by our colleague Babis Kalodimos at the Rutgers University in New Jersey, which allow us to look at this

in real time," says Economou with gleaming eyes.

With this, the scientists want to get atomic resolution information of how the enzyme moves and where exactly all the ligands bind. "Essentially, you can get a picture down to the level of looking inside the protein," says Economou.

In addition to their basic research on bacterial protein trafficking the researchers in Heraklion explore possible applications for their results. "One of our goals is to develop novel antibiotics that inhibit SecA," says Economou. This life-essential protein for bacteria would be a suitable target, the scientists thought. Especially as humans have no SecA, and thus, such antibiotics would selectively affect bacteria.

"In collaboration with Pfizer we have identified a number of candidate compounds and have already started a high throughput screen," Economou tells us.

Watch your glucose

The researchers still have another idea: to generate biosensors. For this purpose they use sensitive proteins to detect a given situation in the body. Economou gives an example, "We work to develop a biosensor for diabetic patients that will be implanted into the body and will sense the level of glucose in the blood. This system would be connected to the appropriate wireless electronic components, like a chip in the computer," plans Tassos Economou. The implantable component in the body will send out readings of glucose levels to a watch or a telephone.

Implantation just ahead

With this project, Economou's group is part of a large European consortium called Cezzane. A lot of specialists are involved, including polymer-scientists, electronic and optics experts, and so on. The Greeks' part of this project is the very front end of the system. "We've taken a protein from *E. coli* called glucose binding protein and added two fluorescent dyes," Economou explains. Depending on the level of glucose present, the dyes admit different light that can be measured by a detector.

"We made the protein and demonstrated in the lab that it works very nicely," says Economou. Next step would be to immobilize this protein in a small device. Tassos Economou knows that they are just at the beginning of a very long and difficult path. However, they plan to implant a biosensor in a rat within the next four years.

SUSANNE DORN

