

The signal hypothesis matures with age

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Secretory proteins are targeted for secretion by sequences in their mature domains, as well as by N-terminal signal peptides.

For over 40 years, researchers have known that the majority of membrane and secretory proteins are targeted for secretion by an N-terminal signal peptide that is subsequently cleaved off to generate the mature form of the protein. But Chatzi et al. now reveal that sequences in the mature regions of secretory proteins are also crucial for directing them to the translocation machinery and mediating their passage from the cytoplasm to the extracellular space (1).

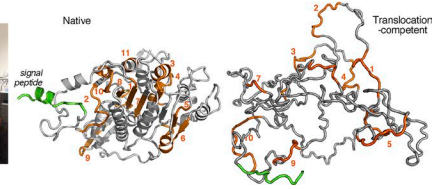
Günter Blobel won a Nobel Prize for his “signaling hypothesis” explaining how signal peptides target secretory proteins to a translocase channel embedded in the membrane of the endoplasmic reticulum (2). Once the proteins have passed through this channel into the lumen of the ER, the signal peptide is removed by proteases and the mature protein is secreted from the cell. A similar process takes place in bacteria, where cleavable signal peptides direct secretory proteins to the SecA–SecYEG translocase complex embedded in the bacterial plasma membrane (3).

The signal peptide is generally considered to be sufficient for secretory protein targeting. “Undoubtedly, the signal peptide is really important,” says Tassos Economou, from Katholieke Universiteit Leuven in Belgium. “But several lines of evidence suggest that additional targeting information may reside in the mature domains of secretory proteins.”

Economou’s group has provided some of this evidence themselves. In 2009, they demonstrated that bacterial secretory proteins lacking their signal peptides could still bind to the SecA–SecYEG complex with high affinity (4). Though the signal peptide further enhances this affinity, its essential role is to activate the translocase complex to transport the secretory protein across the membrane. In fact, signal peptides can even play this role in trans, stimulating the translocation of secretory protein mature domains to which they are not covalently linked (4).



Focal Point (left to right) Katerina Chatzi, Marios Sardis, Lily Karamanou, Tassos Economou, Alexandra Tsirigotaki, and colleagues reveal that bacterial secretory proteins, such as PhoA (pictured), are targeted for secretion by multiple hydrophobic patches (orange) in their mature domains, as well as by their N-terminal signal peptides (green). These mature targeting signals are buried in the fully folded form of the protein (left), but are exposed in the unstructured, secretion-competent form (right), allowing them to bind to hydrophobic patches on the translocase subunit SecA. Once bound to the translocase, the mature targeting signals are subsequently required for the protein’s translocation across the cell membrane. Photos courtesy of the authors.



Economou and colleagues, led by graduate students Katerina Chatzi and Marios Sardis and senior scientist Lily Karamanou, set out to identify targeting signals in the mature domains of bacterial secretory proteins (1). The researchers realized that the mature domains of proteins such as the alkaline phosphatase PhoA contain multiple stretches of hydrophobic amino acids that can bind to the SecA component of the bacterial translocase. One or two of these hydrophobic patches were sufficient to target the proteins to the translocase, but deleting or mutating all of them greatly reduced the proteins’ affinity for SecA, particularly if they also lacked an N-terminal signal peptide.

“The signal peptide isn’t enough for secretion.”

These hydrophobic residues would generally be buried on the inside of fully folded secretory proteins, but Chatzi et al. found that proteins targeting the bacterial translocase are largely unstructured. The buried hydrophobic stretches therefore become exposed on the surface of the secretory proteins, where they can interact with conserved hydrophobic patches on the surface of the SecA receptor, including one patch that lies next to SecA’s previously identified signal peptide-binding cleft (5).

Surprisingly, the mature domain targeting signals don’t just mediate binding to SecA; they are also required for the protein’s subsequent translocation across the bacterial membrane. Secretory proteins lacking their mature targeting signals could still bind to SecA through their signal peptides but they weren’t secreted *in vivo* or *in vitro*. Mutating the hydrophobic patches on SecA similarly impaired protein secretion.

“So, the signal peptide isn’t enough for secretion, and neither are the targeting sequences in the mature domain,” Economou says. “You need both for the translocase to be activated and secretion to occur.” This “dual key mechanism” allows the translocase to distinguish genuine secretory proteins from cytoplasmic proteins with exposed hydrophobic residues. The researchers now want to investigate how the mature targeting signals contribute to protein translocation.

It remains to be seen whether eukaryotic secretory proteins also carry targeting information in their mature domains. Economou suspects that this may be the case, at least for proteins that are translocated into the ER posttranslationally.

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4. Gouridis, G., et al. 2009. *Nature.* 462:363–367.
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