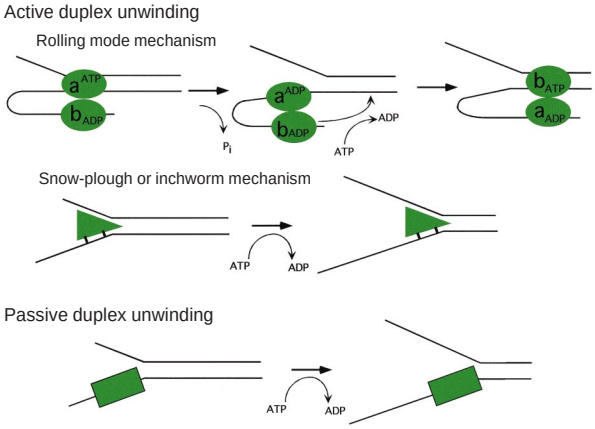

Fig. 3 Proposed models for the action of helicases. In the rolling model, an oligomeric helicase binds alternating to single and double stranded nucleic acid, where the ATP-bound proteins have a high affinity for double stranded nucleic acid. In the snow-plough model, the RNA helicase is moving along the fork and uses energy from NTP hydrolysis to melt hydrogen bonding between the two nucleic acid strands. By contrast, in the passive unwinding model, the local denaturation by thermal fluctuation could be fixed by a single stranded RNA (ssRNA) binding protein. The movement of the ssRNA binding protein along the single stranded nucleic acid would be an ATP-dependent reaction.

In conclusion, genetic experiments have been able to assign individual RNA helicases to specific cellular processes. Biochemical experiments have shown that in the presence of ATP and Mg^{++} some of these proteins are able to dissociate short RNA duplexes. With the report by Jankowsky *et al.*[1] our view of RNA helicases has expanded. RNA helicase have now been shown to migrate in a directional fashion, in distinct steps along the substrate RNA without dissociating from it.

Despite this important step forward, we remain at the beginning of our understanding of RNA helicase function. Indeed, we do not know how helicases unwind duplex molecules. Do they act as an active snow-plough, as a rolling oligomer tearing the substrate apart or by following in an ATPase-dependent manner the spontaneous denaturation of the duplex (Fig. 3)? In the case of active DNA helicases it has been shown that they exist in oligomeric complexes. So far there is little information on this concerning RNA helicases, and even in the case of 'monomeric' RNA helicases it cannot be excluded that oligomerization is induced by contact with the RNA.

Thus, it may turn out — depending on the molecular environment or their tertiary structure — that some RNA helicases unwind duplex substrates in a processive fashion, whereas others do not. Moreover, the RNA helicases are highly specific and cannot be freely interchanged. Thus, they most certainly possess specificity determinants and/or interact with other components that let them work in a controlled manner on the right substrate and at the right time.

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The servant with the scissors

In 1978, Werner Arber (Biozentrum der Universität, Basel, Switzerland), Dan Nathans and Hamilton Smith (both at Johns Hopkins University School of Medicine, Baltimore, Maryland, USA) were awarded the Nobel Prize in Physiology or Medicine for the discovery of "restriction enzymes and their application to problems of molecular genetics". Almost immediately, the application of these enzymes to genetics led to "new and far reaching results". In fact, it is hard to imagine what the biological sciences would look like today without restriction maps, cloning and the ability to alter genes at will, to name just a few everyday tools of the trade. But how did this crucial discovery come about?

While studying a phenomenon known as 'host controlled restriction of bacteriophages', Arber and Dussoix[1–3] found that it provided bacteria with a defense mechanism against invading foreign DNA, such as viral DNA. This process, which was shown to be a property of the recipient bacteria, could be divided into two parts: restriction and modification. Restriction involved the endonucleolytic cleavage of DNA at specific DNA sequences. Because this would restrict viral growth, these enzymes came to be known as restriction enzymes. Modification involved nucleotide methylation at these same specific DNA sequences in the genome. In this way, the bacteria's own DNA was protected from cleavage because it was methylated while the inappropriately or unmodified

foreign DNA was destroyed. Arber and Dussoix proposed that both processes were catalyzed by specific restriction and modification enzymes and that the DNA contained the specific sites to bind both types of enzymes. Over twenty years later the remarkable specificity of restriction enzymes is just now being understood (see pages 134 and 89 in this issue).

Smith[4–7] verified Arber's hypothesis by purifying both bacterial restriction and modification enzymes and showing that they specifically cut DNA and methylated the DNA, respectively. Nathans[8–10] pioneered the application of restriction enzymes to genetics by surveying the ability of known restriction enzymes to cleave the DNA of Simian Virus 40, one of the simplest animal viruses that can transform cultured cells. Clearly, all three were instrumental in the development of modern molecular biology.

While this brief account does highlight some of the major contributions of the three recipients, Arber's daughter, Silvia (then 10 years old), did a better job of explaining why her father was chosen as a Nobel Laureate[11] with "The tale of the king and his servants":

"When I come to the laboratory of my father, I usually see some plates lying on the tables. These plates contain colonies of bacteria. These colonies remind me of a city with many inhabitants. In each bacterium there is a king. He is very long, but skinny. The king has many servants. These are thick and short, almost like balls. My father calls the king DNA, and the servants enzymes. The king is like a book, in which everything is noted on the work to be done by the servants. For us human beings these instructions of the king are a mystery.

My father has discovered a servant who serves as a pair of scissors. If a foreign king invades a bacterium, this servant can cut him in small fragments, but he does not do any harm to his own king.

Clever people use the servant with the scissors to find out the secrets of the kings. To do so, they collect many servants with scissors and put them onto a king, so that the king is cut into pieces. With the resulting little pieces it is much easier to investigate the secrets. For this reason my father received the Nobel Prize for the discovery of the servant with the scissors."

Boyana Konforti

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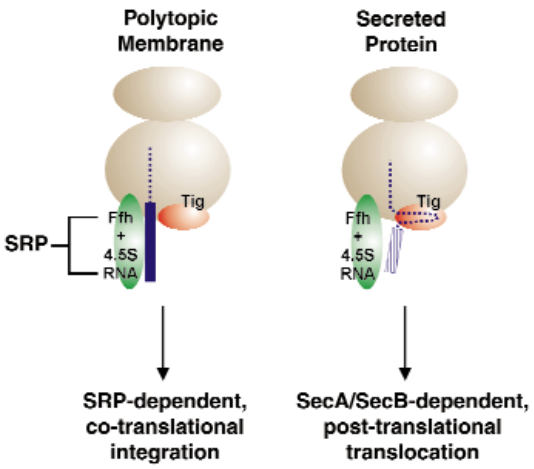
Picking pathways

Many proteins synthesized in the cytoplasm of a cell are secreted outside the cell or are transported into specific cellular compartments. For proteins that are secreted or targeted to the membrane, the sorting information is usually encoded in the N-terminal segment of the protein, called the signal sequence. Signal sequences vary in length and actual amino acid sequence, but share a common feature — a central hydrophobic patch flanked on either side by polar regions. Interestingly, in prokaryotes, secreted proteins and membrane proteins containing multiple transmembrane helices (polytopic membrane proteins) appear to be translocated via different pathways — targeting of secreted proteins requires the proteins SecA and SecB, while integration of polytopic membrane proteins involves an RNA–protein complex, called the signal sequence recognition particle (SRP). Thus, one intriguing question is: how does the translocation machinery interpret the signal sequences and thereby target the proteins to the correct locations?



To address this question, Beck *et al.* (*EMBO J.*, **19**: 134–143) used an *in vitro* translation system and crosslinking techniques to identify ribosome-associated factors that can distinguish between a polytopic membrane protein and a secreted protein. Their results indicate that the translocation pathway is selected early during protein synthesis, with two factors playing a determining role. For a polytopic membrane protein (left panel), the signal sequence (blue box) emerging from the ribosome (brown ellipsoids) binds to SRP (green ellipsoid) and the subsequent membrane integration occurs simultaneously with protein synthesis. In contrast, trigger factor (Tig, red ellipsoid), a chaperone protein tightly associated with the ribosome, binds to the nascent chain of a secreted protein (right panel) and prevents SRP binding to the signal sequence (blue hashed box). As a result, the protein is directed to the SecA/SecB-dependent translocation pathway after synthesis is complete.

The results of Beck *et al.* suggest that trigger factor and SRP bind to different regions of the nascent polypeptide chain. However, despite having different interaction sites, binding of these two factors appears mutually exclusive. Their study therefore leads to new questions. What are the features recognized by trigger factor, and is this recognition sequence-specific? Does trigger factor binding persist throughout protein synthesis? How does trigger factor binding exclude SRP–signal sequence interactions? A better understanding of how proteins are targeted to different compartments will emerge as these questions are addressed.

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