Connectase
An enzyme that fuses proteins in a specific manner

Dr Adrian Fuchs, post-doctoral researcher at the Max Planck Institute for Developmental Biology, and his team of researchers have discovered an enzyme that can fuse two proteins at specific recognition sites. This reaction enables the engineering of proteins with new characteristics, for example by labeling them with a detectable marker or by manipulating their interaction behaviour. Such applications are useful both in academic research and biotechnology.

Proteins are central to almost all biological processes taking place within living organisms. However, proteins cannot last forever; they need to be regularly broken down and replaced. Controlled protein turnover is an essential activity for all living cells. A key facilitator within this pathway are proteasomes, large enzymes that degrade unfolded or damaged proteins through proteolysis, a chemical reaction that breaks peptide bonds.

Proteasomes are near universal. They are found inside all eukaryotes (higher organisms, such as protists, fungi, plants and animals) and archaea (single-celled organisms often found in extreme environments), and in some bacteria too.

Due to their huge physiological relevance, Dr Adrian Fuchs and his team at the Max Planck Institute for Developmental Biology became interested in understanding more about their function and evolution. It was through this research that they discovered Connectase, a new protein with fascinating biotechnological potential.

**HOW WAS CONNECTASE DISCOVERED?**

Dr Fuchs and his team were carrying out a bioinformatic analysis (a type of analysis used for understanding large and complex sets of biological data) when they discovered a protein that is very divergent from the proteasome, but still distantly related to it: Connectase. Curiously, this protein could only be found within methanogenic archaea. These microorganisms obtain their energy from producing large quantities of methane, a greenhouse gas, and are therefore key factors affecting our planet’s climate.

The researchers had expected Connectase to be a multi-subunit protease like the proteasome. However, their structural analysis showed that Connectase subunits do not assemble into proteasome-type complexes but rather exist as single protein chains, so-called monomers. Even more surprising, these monomers did not exhibit any proteolytic activity. Unlike other members of the proteasome family, Connectase showed only ligase activity. A ligase is an enzyme that can catalyse the joining of two large molecules by forming a new chemical bond. That essentially means it connects proteins together, rather than breaking them apart.

**HOW DOES CONNECTASE FUNCTION?**

During further experiments with methanoarchaenal cell extract, the researchers identified a physiological substrate of Connectase in methyltransferase A (MtrA). MtrA is a key enzyme in methanogenesis, the pathway by which archaea produce methane. This pathway is thought to be one of the oldest processes in living organisms for energy generation and carbon fixation and an important contributor to the greenhouse effect.

The researchers were able to show that Connectase specifically interacts with MtrA and is able to bind and modify it. Further studies showed that only a short recognition motif in MtrA, the Connectase recognition motif, is required for this interaction. In fact, when this recognition motif was incorporated in a variety of other unrelated proteins, Connectase interacted with them just like with MtrA. The team was surprised to find that in both cases, Connectase does not only bind but also cleave this sequence into two fragments.

However, the reaction remained incomplete, no matter how the researchers altered the reaction conditions. This led them to the conclusion that the reaction must be reversible. It appeared that the recognition sequence was being constantly cleaved and resynthesized.

In order to prove this theory, Dr Fuchs and his team fused the recognition sequence to the N-terminal end of one protein and to the C-terminal end of another protein. Indeed, upon addition of Connectase, both proteins were fused together, with the recognition sequence bridging their N- and C-termini. This experiment showed that Connectase can be used to replace a protease like the proteasome.

**ARCHAEA WERE FOUND IN VOLCANIC HOT SPRINGS.**
Pictured here is Grand Prismatic Spring of Yellowstone National Park.

Archea are single-celled organisms often found in extreme environments.
Connectase is a unique protein ligase that can specifically ligate proteins bearing a recognition sequence when presented with two proteins that have this sequence. This is highly attractive for a variety of biotechnological applications. Connectase is easy to handle and control the location of proteins for different applications, such as the cell surface or in solution, making it a versatile tool.

**WHAT ARE THE BIOLOGICAL IMPLICATIONS OF THIS DISCOVERY?**

The unprecedented activity of Connectase was exciting for Dr Fuchs and his team, as it showed how versatile protein-like proteins could be. It also helped them to understand the link between their diverse reactions: proteasome substrates are made as precursors that must be activated (“autolysis”) before they can degrade proteins (“proteolysis”) or shuffle peptides (“splicing”). All these reactions are mechanistically related and the Connectase reaction is a fourth, unprecedented variation of this reaction scheme that sheds light on the different proteasome functions. It is yet enigmatic how exactly the ligase activity of Connectase is affecting methane production. For this process, it is essential that the Connectase target MtrA is located at the membrane, a lipid layer that encapsulates the cell. The Connectase recognition sequence is located between the functional core of MtrA and its membrane anchor. Consequently, the Connectase reaction may control or alter methane production, by separating and re-ligating the two MtrA fragments. While these exciting prospects are subject to ongoing studies, the capability of Connectase to ligate proteins makes it highly attractive for a variety of biotechnological applications.

**WHAT ADVANTAGES DOES CONNECTASE HAVE OVER OTHER ENZYMES?**

There are several existing methods that we can use to ligate proteins, but they all come with different advantages and limitations and are therefore useful in different settings. Connectase-mediated ligation is not quite as traceless as some of these other ligation methods. They necessitate a ~20 amino acid recognition sequence, so Connectase will only be used in cases where the presence of this sequence can be tolerated. In these cases, however, Connectase offers a combination of simplicity and efficiency that is hard to rival.

**CONCLUSION**

In summary, Connectase represents a fascinating scientific find. From a purely theoretical perspective, it’s interesting to study its unique characteristics and differences from other proteasome structures. Still, most exciting is the potential for Connectase’s unique characteristics to be used in a range of future biotechnological applications.

**WHAT BIOTECHNOLOGICAL APPLICATIONS COULD CONNECTASE HAVE?**

The most obvious use of Connectase would be to fuse different types of protein together to create entirely new ones. This could lead to hybrid proteins that would have been difficult to produce by conventional means, such as new antibody conjugates with a diverse set of binding sites. It is yet enigmatic how exactly the ligase activity of Connectase is affecting methane production. For this process, it is essential that the Connectase target MtrA is located at the membrane, a lipid layer that encapsulates the cell. The Connectase recognition sequence is located between the functional core of MtrA and its membrane anchor. Consequently, the Connectase reaction may control or alter methane production, by separating and re-ligating the two MtrA fragments. While these exciting prospects are subject to ongoing studies, the capability of Connectase to ligate proteins makes it highly attractive for a variety of biotechnological applications.

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