How to make a stop at a Stop Sign

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Proteins are the tools in a cell and one can find proteins everywhere. While we backe a cake we use the proteins of yeast to work for us. The question then arrives, who is making these proteins? All life forms as we know them today have a giant machine in their cells. This machine is called Ribosome. A copy of the famous DNA is the blueprint for the ribosome to produce proteins. The machinery of protein synthesis is very important. Since the bacterial ribosome differs in its details from the ribosome those we human have. In medicine we use the ribosome as a target for antibiotics to stop the bacteria to make its tools to survive.

Stop! That's the keyword of this work presented here. When does a ribosome know to stop? Very often the blueprint for the ribosome is longer than the protein has to be and if the protein is longer than it should be, it will be non-functional and goes straight into the trash bin of a cell.

Thus nature has developed stop signs to signal the ribosome to stop. There are three stop signs that we call after their genetic letter UGA, UAG and UAA. The ribosome however is not able to stop the synthesis it needs some factors that reads this stop signals, we call them release factor (they release the protein from the machine to be free and to do its work). In the present work it is shown how good these factors are reading these stop signals. It turns out that they are very precise in recognizing the stop signals. In bacteria, we have two factors to recognize the stop signals. Here a mutant (one small part of this protein has been changed) is shown that can recognize all three stop codons but still allows the machinery to synthesise the protein. The risk in designing a release factor is that it won't allow the machine to produce a full length protein, because it would release the protein during its synthesis. In the master thesis it is shown that the designed release factor does not release randomly.

Another question I wanted to answer with this Master Thesis was how many release factors of each kind to we have I a bacterial cell? It turned out that the so called release factor 2 is much more abundant in bacteria than its counterpart release factor 1.

Last but not least I showed that a small molecule called GDP has to be absent so that the release factor can be removed from the ribosome, this was earlier in the literature known but still challenged by many people.

All in all we understand now how these factors recognize the stop signals, how many of these factors are existing in the cell, we have further evidence that the small molecule GDP inhibits the recycling of these release factors and most excitingly we created a release factor that stops at all three stop signs and does not interfere with the production of a protein by the large ribosome.

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