Essentiality of class I Release Factors in prokaryotic translation termination

By Matteo Libero Baroni

Translation is the biological process that leads to the formation of polypeptide chains (proteins), assembled according to the genetic information encoded by the messenger RNA (mRNA) sequence to be translated. This process occurs at any moment of our lives and during the life cycle of any organism living on this planet. The formation of proteins is a multi-step process which sees the interplay of several factors and it progresses through three major steps: Initiation, Elongation and Termination. Each of these steps comprises several "players" which make the process possible: these are called "protein factors" or simply factors. Therefore, we can find initiation factors, elongation factors and termination factors. The present work focuses on the last part of the process (the termination) and it studies how "important" these factors are for assuring a proper termination of translation. The termination of translation makes possible that the protein newly formed during the "elongation" is cut off from the translation machinery (responsible for assembling proteins according to the genetic information encoded in the DNA). The "cutting-off" is made possible by two protein factors, Release Factor 1 and Release Factor 2, which together form the class I Release Factors. These two proteins are very similar in structure and function: they evolved from a common ancestor and they perform the same action, with the unique difference that they "cut" in response to different sequences: RF1 cuts when it encounters the mRNA codon with the sequences "UAA" or "UAG" and RF2 cuts when it finds the sequences "UAA" or "UGA". It seems that even if these factors are performing the same action, they show some differences in "how" they do it. RF2 cuts more efficiently than RF1 and RF2 is also much more abundant in the cell.

It was demonstrated that RF1 can cut also on the codon that should be specific for RF2 (the mRNA sequence "UGA"). The premises for RF1 to cut on UAA, UAG as well as UGA is that there is a much higher amount than there would normally be inside a bacterium. This work aims, through extensive genetic engineering, to increase the level of RF1 inside *E.coli* to see if it is possible to survive without RF2. In this way the cell will have only one factor (RF1) to perform the release of proteins. The outcome of this genetic modification would give insight into the mechanisms regulating the process of translation termination and it would elucidate how excess of RF1 can overcome the lack of RF2. The present work demonstrated that it is possible to increase the intracellular level of RF1, and it is possible to disrupt the gene encoding for RF2. *E.coli* cells, when trying to delete RF2 encoding gene (called *prfB*), showed the capacity to duplicate it and to insert it in another location of the genome, thus resulting in a cell having a disrupted version of *prfB* (after genetic engineering) and a fully functional copy, as result of gene duplication.