Ribosome-Binding and Activity of Release Factor 2 in the Presence of L-Tryptophan

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Introduction

During protein synthesis, Release factor 2 (RF2) recognizes stop codons terminating the synthesis of proteins by inducing hydrolyzing the last peptidyl-tRNA within the ribosome, releasing the newly produced proteins from the ribosomes. The TnaC-peptidyl-tRNA^{Pro} generated during translation of the *tnaC* gene resists hydrolysis induced by RF2 in the presence of L-tryptophan (L-Trp)[1] . To determine if L-Trp inhibit RF2-recognition of the stop codon we produced and purified a wild type and a non-functional mutant Q252G RF2 proteins from *Salmonella typhimurium* to test their binding and activity in ribosomes containing TnaC-tRNA^{Pro}.

Purification of the RF2 proteins

In the first step, IPTG-regulable T7-plasmids from containing His-tagged *Salmonella typhimurium* RF2 genes were transformed into BL21 cells, which expresses the T7 RNA polymerase that need it to transcribe the RF2 genes[1]. Cells were grown overnight in the presence of IPTG and broken-down using sonication. Both wild type and mutant proteins were purified using cobalt columns.

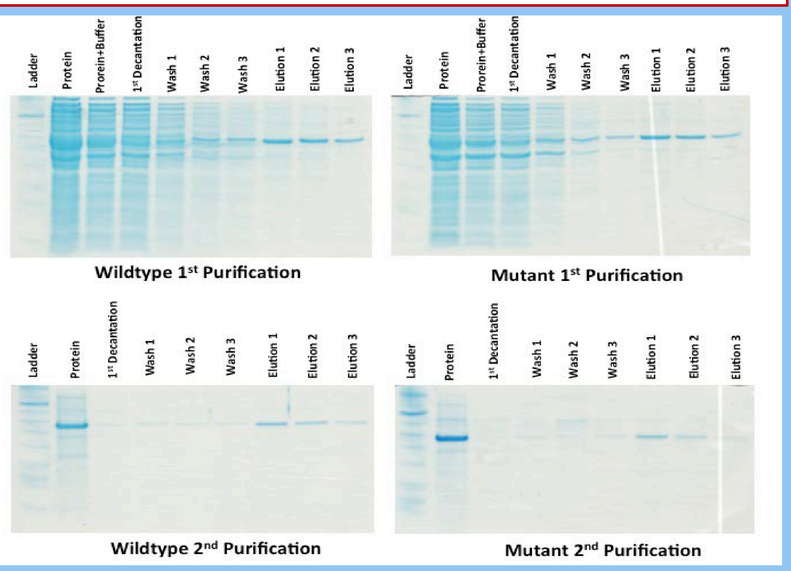


Figure 1: Purification of RF2. RF2 protein corresponds to 42 kD.

Testing the binding and activity of RF2

In order to test the binding and activity of the purified proteins, coupled transcription-translation *in vitro* reactions were performed to produce arrested ribosomes containing TnaC-tRNA^{Pro} in the absence of RF2 proteins (Figure 2). To analyze the activity of our purified proteins we added them to the *in vitro* reactions to induce hydrolysis of the TnaC-tRNA^{Pro} (Figure 3). To analyze the binding of our purified proteins we performed toe-printing assays after adding the pure proteins to the *in vitro* reactions (Figure 4).

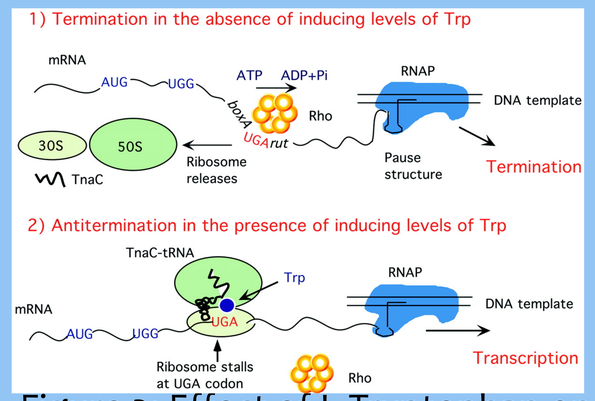


Figure 2: Effect of L-Tryptophan on TnaC-tRNA^{Pro} in ribosomal translation.

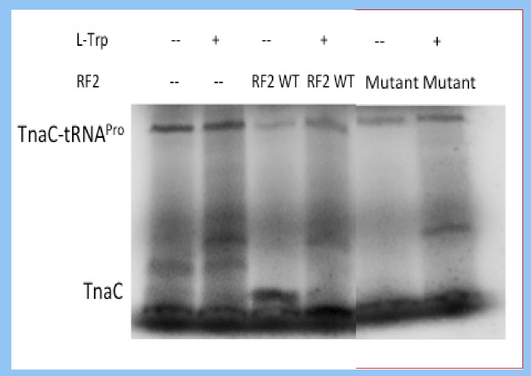


Figure 3: RF2-hydrolysis assays of TnaC-tRNA^{Pro}. In the absence of activity of RF2 a strong TnaC-tRNA^{Pro} band should be observed. In the presence of activity of RF2 a strong TnaC band should be observed.

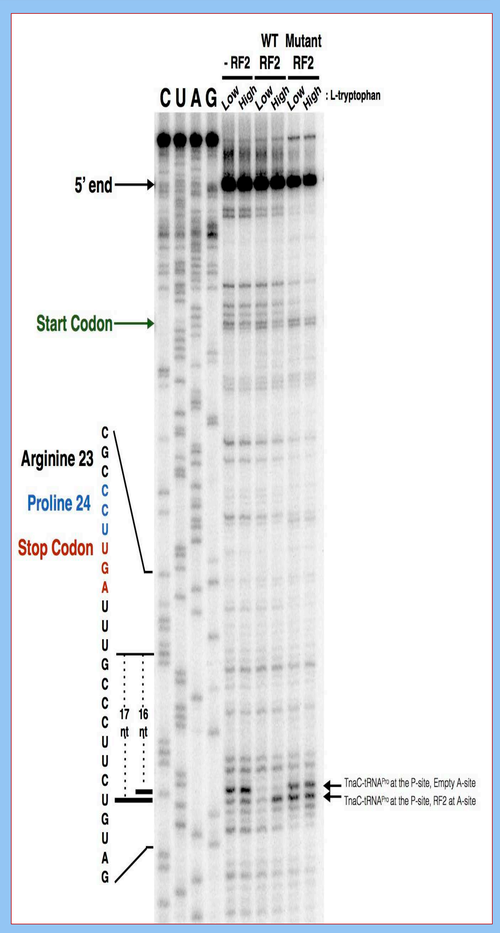


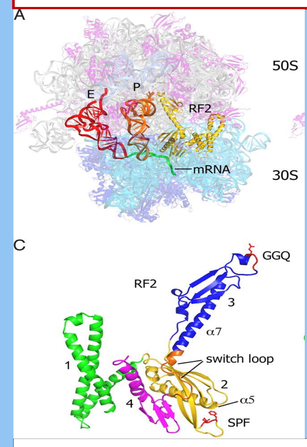
Figure 4: RF2-binding assays. In absence of bound-RF2 proteins a strong signal is observed at 16 nt. In the presence of bound-RF2 proteins a strong signal is observed at 17 nt.

Discussion

Our observations indicated that in the presence of L-Trp, the wild type and mutant RF2 proteins cannot hydrolyze the TnaC-tRNA^{Pro} (Figure 3, compare lane 3 with lanes 4 and 6). Also, the mutant RF2 cannot induce hydrolysis of this peptidyl-tRNA (Figure 3, compare lane 3 with lane 5). Therefore, L-Trp blocks the activity of RF2. However, our data indicated that both wild type and mutant RF2 can bind within the ribosome in the presence of L-Trp (Figure 4, compare lane 6 with lanes 8 and 10). Then, L-Trp cannot block the binding of these two RF2 proteins.

Conclusion

Because L-Trp blocks hydrolysis but not RF2 binding, we suggest that L-Trp affects allosterically the ribosome and/or the hydrolysis reaction induced by RF2 .



References
1. Gong and Yanofsky, 2001. PNAS , 98:8997
2. Ardrei Korostelev, 2008. PNAS, 50:105

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