Role of Ribosome Recycling Factor as a Ribosome Releasing Factor

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Abstract

The objective of this presentation is to study the *in vivo* actions of ribosome recycling factor (RRF) and compare them with those found *in vitro*. RRF is known to catalyze three separate reactions: release of tRNA and mRNA from the post-termination complex (PoTC), and splitting of the ribosome of the PoTC. To study the mechanism of RRF reaction *in vivo*, we used *E. coli* harboring temperature sensitive (ts) RRF and assayed by following downstream reading of translationally coupled ORF. At the non-permissive temperature, ribosomes remain on the termination codon of the junction sequence of coupled ORFs and translate downstream ORF lacking a Shine-Dalgarno (SD) sequence. The readings were in all three frames due to thermal frameshift at the termination codon. When upstream ORF was short, translation of the downstream reading was abolished, suggesting that the **ribosomes released by RRF** are moving toward the SD sequence of the upstream ORF. The thermal frame shift at the stop codon was also stopped by the nearby upstream SD sequence. Our data suggest that the ribosome-bound mRNA may take a secondary structure around the junction sequence. This structure can affect the reading of downstream ORF. For *in vitro* studies, we used mRNA that incorporated different radioactively labeled amino acids based on the frameshift at the junction sequence, UAAUG, of two translationally coupled ORFs. In the absence of RRF, the ribosome stayed on the mRNA and translated in frame with the termination codon UAA. In the presence of RRF, amino acid incorporation occurred in frame with the start codon AUG. This suggests that **RRF releases the ribosome** from UAA and the released ribosome binds to AUG and begins translation. With the use of tethered, unsplittable ribosomes (Ribo-T) in the *in vitro* system, we showed that complete ribosomal splitting is not required for the action of RRF. Therefore, the main role of RRF in the ribosome recycling reaction appears to be the **release of ribosomes from mRNA**.

Results

In vivo studies of RRF action

Figure 1. System used to study the behavior of ribosomes at the end of ORFs under the influence of RRF/EF-G. Translation of *LacZ* is a marker for downstream-ORF reading. All d-ORF reading is performed by ribosomes from the u-ORF.

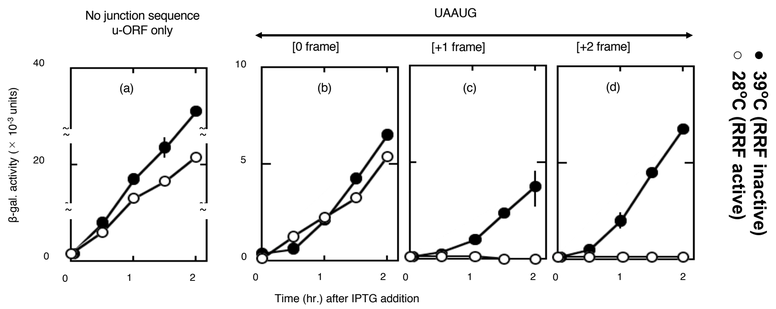

Figure 2. Ribosomes are released and rebind to AUG in the presence of RRF. When tsRRF is temperature inactivated, ribosomes remain on mRNA and undergo thermal frameshift.

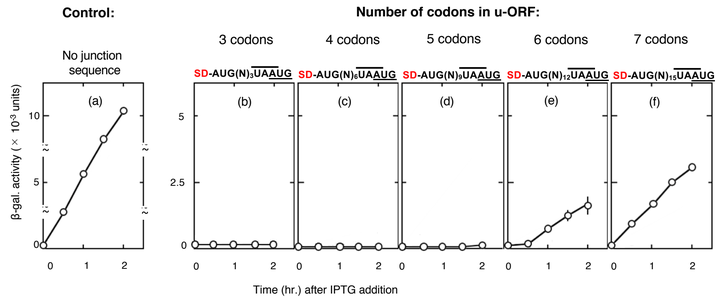

Figure 3. Ribosomes released from junction sequence by RRF are attracted by upstream SD sequence. Shorter u-ORF results in no reading of d-ORF and released ribosome rereads u-ORF only.

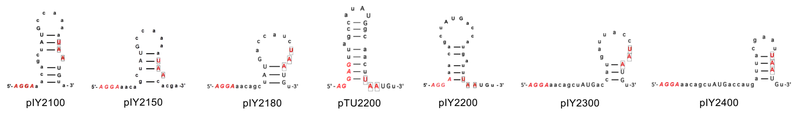

Figure 4. Possible secondary structures of various mRNA used in this study.

Table 1. LacZ expression at 39°C is dependent on spatial distance between the upstream SD sequence and UAA of the u-ORF --- mRNA can take on secondary structures on the ribosome in the absence of RRF

Junction Sequence	Spatial Distance (nucleotides) between SD and UAA	LacZ expression (%) at 39°C
pIY2100, AUAA	3 (short)	58 (high)
pIY2150, AUAA	6 (short)	71 (high)
pIY2180, UAAUG	16 (long)	8 (low)
pTU2200, UAAUG	0 (short)	38 (high)
pIY2200, UAAUG	0 (short)	38 (high)
pIY2300, UAAUG	13 (long)	11 (low)
pIY2400, UAAUG	13 (long)	18 (low)

Short spatial distance between UAA and SD gives high % expression when RRF is temperature-inactivated. Long spatial distance gives low % expression when RRF is inactive.

In vitro studies of RRF action

AUUUAAUACGACUCACUAUAGGGAAUUCAAAAAUUUAAAAGUUAACAGGUAUACAUACU
17 bases T7 promotor — SD
AUG UUU ACG AUU ACU ACG AUC UUC UUU ACG AUC UUC UUU ACG AUU ACU ACG AUC
M F T I T T I F F T I F F T I T T I
UUC UUU ACG AUU UUC UUU ACG AUU ACU ACG AUC ACU ACG AUC UUC UUU ACG
F F T I F F T I T T I T T I F F T

In the presence of RRF (0 frame) - - - - - *UAA* **UG** CGU CUG CAG GCA UGC AAG CUA A_{24} AGC
M R L Q A C K L K_8 S

In the absence of RRF (+1 frame) - - - - *UAA* **UG**C GUC UGC AGG CAU GCA AGC UAA AAA_{21}AGC
C V C R H A S

Figure 5. mRNA sequence used for the *in vitro* study of the role of RRF

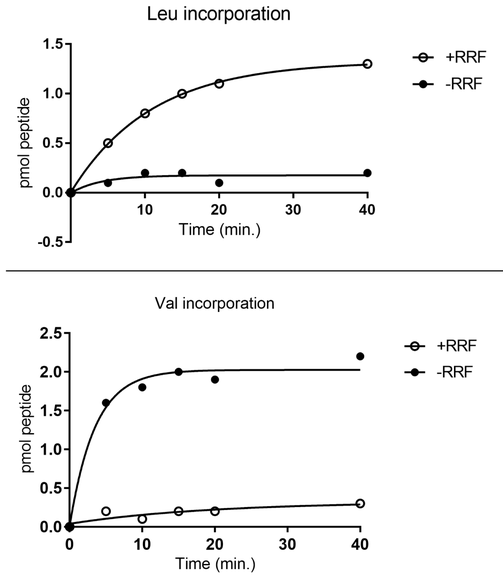

Figure 6. The ribosome is released and rebinds to AUG, in frame with Leucine. Leucine is incorporated into polypeptide in the presence of RRF.

Figure 7. Absence of RRF causes ribosome to remain on UAA, in frame with Valine. Addition of RRF inhibits Valine incorporation.

Figure 8. Ribo-T (Mankin's ribosome) are not split by RRF/EF-G. WT 70S ribosomes are split into 50S and 30S subunits in the presence of RRF/EF-G.

Figure 9. Complete ribosome splitting is not necessary for ribosome release by RRF. Leucine incorporation still occurs in the presence of RRF with Ribo-T.

Conclusion

All data (*in vivo* and *in vitro*) support the concept that RRF releases ribosomes from mRNA of the PoTC without necessarily splitting the ribosome into subunits.

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