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In vivo and *in vitro* studies of RRF (ribosome recycling factor) revealed that its major function is to release mRNA from the post-termination complex and not splitting of the ribosomal subunits

In prokaryotes two adjacent ORF are often linked with an overlapping combination of termination and initiation codons with a total of 4 or 5 nucleotides, for example UAAUG or AUGA. In these junctions, ribosomes at the stop codon are released by RRF and some of them re-bind to the nearby AUG and start translating the downstream ORF. In the absence of RRF, the ribosome at the stop codon remains on the mRNA and would read it in frame with the termination triplet. We studied the role of RRF in these junctions *in vitro* and *in vivo*. For *in vivo* studies, we used an *E. coli* strain with a temperature sensitive mutation of RRF, so that we could inactivate the function of RRF at the non-permissive temperature (39°C). We show that for correct reading of the downstream ORF, AUG is essential. The shorter the upstream ORF the lower will be the downstream reading. Introduction of a complementary sequence to the 3'-terminal regions of 16S rRNA into the mRNA increased downstream reading from AUGA. Shortening of the upstream ORF to 4 codons completely abolished the downstream reading of UAAUG. This suggests that if Shine-Dalgarno (SD) sequence is near the termination codon, the RRF-released ribosome is attracted by the SD sequence to the extent that it loses the downstream movement after it is released. For *in vitro* studies, we used the PURE system, so that we could omit RRF in the reaction mixture. We have confirmed, *in vitro*, the essence of the *in vivo* observation described above using a mRNA having the following sequence: "GGGAAUUCAAAAAUUUAAACAGGUAUACAUACU AUG UUU ACG AUU ACU ACG AUC UUC UUU ACG AUC UUC UUU ACG AUU ACU ACG AUC UUC UUU ACG AUU ACU ACG AUC UUC UUU ACG AUU ACU ACG AUC UUC UUU ACG UAAUG CGU CUG CAG GCA UGC AAG CUA A24A" (Bold character is the junction sequence broken underline is the Shine-Dalgarno sequence). In the presence of RRF, the downstream reading starts from AUG causing the incorporation of [14C]-Leucine (CUG and CUA). On the other hand in the absence of RRF, the first triplet read was UGC of UAAUG CGUC and [14C]-Valine was incorporated due to the codon GUC. Upstream reading was detected by [3H]-phenylalanine incorporation (due to UUUs and UUCs). With this assay, we also showed that Fusidic Acid could inhibit RRF at lower concentration than that necessary to inhibit translocation (monitored by the incorporation of [3H]-phenylalanine), causing the inhibition of the incorporation of [14C]-Leucine. Moreover, using ribosome with tethered subunits (1), we were able to show that the splitting of the ribosomal subunits was not necessary in the recycling reaction, demonstrating that recycling of the ribosome take place also in the absence of the splitting of the ribosomal subunits.

Speaker Biography

Akira Kaji is a Professor of Microbiology, School of Medicine, University of Pennsylvania. He has contributed to the deciphering of genetic code by his discovery of the fact that the complex of poly-U with ribosome binds specifically to tRNA specific for phenylalanine. He also discovered RRF.

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