Editorial

Translation Termination in Eukaryotes: from Simplicity to Complexity

(protein biosynthesis / termination of translation / eukaryotes / release factors)

L. L. KISSELEV

Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia

Abstract. The last forty years of molecular biology and biochemistry were enormously rich in discoveries, which were not foreseen even by the most eminent scientists of that time. The findings of 1993–2000 profoundly enlarged our views on translation termination, clearly showing that our previous understanding was enormously oversimplified. Now the structural basis is created for much better insight into functions of termination factors. The story of translation termination in eukaryotes could be taken as an illustration of a general trend of molecular biology: “From simplicity to complexity”. However, genuine knowledge requires that after this stage a third phase has to be reached, which is “From complexity to clarity”. This has not yet been achieved in translation termination and therefore makes this topic quite attractive for researchers.

The border between the two centuries, even more, between two millennia is highly provocative to those of us who have been involved in science for many decades. My scientific carrier started in 1959 when my first paper was published in the Russian journal “Doklady Akademii Nauk SSSR” (Proceedings of the Academy of Sciences of the USSR) (Severin et al., 1959). Since that time I have been heavily engaged in molecular biology and biochemistry running my own research, teaching my young colleagues and watching what is going around in the field of molecular bases of life. The last forty years of molecular biology and biochemistry were enormously rich in discoveries, which were not foreseen even by the most eminent scientists of that time. Let me provide you with a few examples of such kind. In 1970, F. Crick published his vision of “Molecular Biology – 2000” (Crick, 1970), in which he discussed the coming 30 years of molecular biology, trying to predict the most remarkable events in this field. Surprisingly, the discovery of reverse transcrip-

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Corresponding author: Lev L. Kisselev, Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, ul. Vavilova 32, Moscow, 117984, Russia. Tel.: (095) 1356009; Fax: (095) 135–1405; e-mail: kissel@imb.ac.ru.

Abbreviation: RF - translation termination factor.

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neered components of the translation machinery, greatly improved our knowledge of the structure of the ribosome and its nucleic acid and protein components. Second, big differences were found between eukaryotes and prokaryotes in the translation apparatus, which were virtually ignored at the initial phase of research. Third, we reached the point when it became evident that our knowledge and understanding of protein biosynthesis is very naive and incomplete. What looked clear 30 years ago seems to be mysterious now. I shall take an example of translation termination in eukaryotes to illustrate this issue in more detail.

At the end of the 1960s the concept was formulated postulating the following major points (Caskey, 1977). Translation termination is governed by the presence of the termination (stop, nonsense) codons at the ribosomal A site, where a termination factor should also be present to trigger the hydrolysis of peptidyl-tRNA located at the ribosomal P site. The process requires two proteins (polypeptide chain release factors) in prokaryotes and only one factor in eukaryotes. The translation termination is a GTP-dependent process. In its essence the scheme holds true until nowadays, but the mechanism of termination was not elucidated as well as how the termination codon transduces the signal to the peptidyl transferase center of the ribosome to induce hydrolytic reaction and release a free nascent polypeptide chain.

Nearly a quarter of the century nothing happened in this field although the primary structures of termination factor(s) (RFs) from bacteria had been deciphered. Breaking news emerged in 1993–1995 when, firstly, the claimed structure of eukaryotic translation termination factor or eRF (Lee et al., 1990) appeared to be incorrect due to misassignment of the cloned DNA (Frolova et al., 1993), and the genuine structure of eRF was established (Frolova et al., 1994). Secondly, a new translation termination factor, termed (e)RF3, was first characterized for prokaryotes (Grenzmann et al., 1994; Mikuni et al., 1994) and, soon after, for eukaryotes (Zhouravleva et al., 1995). Unexpectedly, the primary structure of prokaryotic RF1/RF2 and eukaryotic eRF1 appeared to be profoundly different, showing no indication for a common ancestor (Frolova et al., 1994; Kisselev et al., 2000). RF3 and eRF3 appeared to be GTP-binding proteins possessing a ribosome-dependent GTPase activity (Frolova et al., 1996; Freistrother et al., 1997). A complex was revealed between eRF1 and eRF3 (Stansfield et al., 1995; Zhouravleva et al., 1995), which is absolutely necessary for eRF3 GTPase activity (Frolova et al., 1996, 1998).

All these findings profoundly enlarged our views on translation termination, clearly showing that our previous understanding was enormously oversimplified. Now the structural basis was created for much better insight into functions of termination factors.

Careful examination of more than 40 class-1 RF sequences available at that time from all three kingdoms of the living matter ended up with a discovery of a ubiquitous, absolutely conserved GGQ tripeptide (Frolova et al., 1999). Its universal occurrence in all bacterial, archaeal and eukaryotic class-1 RFs points to its biological significance. In fact, site-directed mutagenesis of G183 and G184 (numbering for human eRF1) leads to complete abolishment of RF activity in vitro (Frolova et al., 1999). It was supposed that the lack of the amino acid side chains in GG facilitates the access of the water molecule to the hydrolysable ester bond in peptidyl-tRNA. These data were confirmed by in vivo studies undertaken with yeast eRF1 (Song et al., 2000). The mini-domain containing GGQ occupies one of the extremities of the elongated eRF1 protein (Song et al., 2000), which is fully consistent with its proposed role in triggering peptidyl-tRNA hydrolysis (Frolova et al., 1999). The eRF1 mutants being completely inactive as release factors retain their affinity to the ribosome and possess high competition capacity toward wild-type eRF1 (Frolova et al., 1999). These mutants are valuable tools for studying translation (Kisselev, 1999) and may be used to generate "stalled" ribosomes carrying polypeptidyl-tRNA of equal size not released from the ribosome charged with a given mRNA.

The domain organization of eRF1 was examined in solution (Frolova et al., 2000) and in a crystal state (Song et al., 2000). The N domain composed of 275 amino acid residues remains active in vitro as a release factor in the absence of the C domain, while the C domain is used to form a complex with the C domain of eRF3 (Merkulova et al., 1999; Frolova et al., 2000; Kisselev et al., 2000). The N domain ("core" factor) (Frolova et al., 2000; Kisselev et al., 2000) is composed of domains N (1) and M (2) (Song et al., 2000), the first being involved in interactions with mRNA and the ribosome (Bertram et al., 2000; Kisselev et al., 2000), whereas the second domain is involved in triggering peptidyl-tRNA hydrolysis at the peptidyl transferase centre (Frolova et al., 1999; Song et al., 2000). Consequently, the three major functions of eRF1 – mRNA recognition, peptidyl-tRNA hydrolysis and eRF3 binding – are physically associated with the distinct domains of eRF1. The location of the recognition site toward stop codons at the eRF1 molecule remains obscure and is one of the major issues that has to be solved in the future.

It was proposed that the termination mechanism involves the orientation of a water molecule toward peptidyl-tRNA at the peptidyl transferase centre of the ribosome due to its non-covalent binding to the amide group of Gln 185 located at the GGQ tripeptide (Song et al., 2000). Although this hypothesis looks reasonable, the experimental data on site-directed mutagenesis of Q185 do not prove it: Q185G and Q185R mutants of eRF1 remain partially active as release factors, although