Eukaryotic release factor 1 (eRF1) abolishes readthrough and competes with suppressor tRNAs at all three termination codons in messenger RNA

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ABSTRACT

It is known from experiments with bacteria and eukaryotic viruses that readthrough of termination codons located within the open reading frame (ORF) of mRNAs depends on the availability of suppressor tRNA(s) and the efficiency of termination in cells. Consequently, the yield of readthrough products can be used as a measure of the activity of polypeptide chain release factor(s) (RF), key components of the translation termination machinery. Readthrough of the UAG codon located at the end of the ORF encoding the coat protein of beet necrotic yellow vein furovirus is required for virus replication. Constructs harbouring this suppressible UAG codon and derivatives containing a UGA or UAA codon in place of the UAG codon have been used in translation experiments in vitro in the absence or presence of human suppressor tRNAs. Readthrough can be virtually abolished by addition of bacterially-expressed eukaryotic RF1 (eRF1). Thus, eRF1 is functional towards all three termination codons located in a natural mRNA and efficiently competes in vitro with endogenous and exogenous suppressor tRNA(s) at the ribosomal A site. These results are consistent with a crucial role of eRF1 in translation termination and forms the essence of an in vitro assay for RF activity based on the abolishment of readthrough by eRF1.

INTRODUCTION

Termination of protein synthesis occurs when the ribosome and requires specific release factors (RFs) and GTP (for recent reviews see 1–3). In higher eukaryotes, the situation concerning peptide chain termination remained largely unexplored following the identification and partial purification from rabbit reticulocytes of one RF recognizing any of the three termination codons (4,5). Recently, the rabbit RF was purified to homogeneity and four of its proteolytic peptides sequenced (6). These peptides were identical or very similar to peptides deduced from the amino acid sequences derived from the human TB3-1, Xenopus laevis Cl1, Saccharomyces cerevisiae sup45 and Arabidopsis thaliana sup45-like cDNAs. Expression of the human and X.laevis genes in yeast and Escherichia coli respectively, yielded proteins endowed with RF activity. Since all these proteins possess similar amino acid sequences, it was proposed that they belong to a highly conserved protein family designated eRF1 (6). No GTP binding domain was detected in the eRF1 family (6), and indeed, eRF1 is functional towards all three termination codons located in a natural mRNA and efficiently competes in vitro with endogenous and exogenous suppressor tRNA(s) at the ribosomal A site. These results are consistent with a crucial role of eRF1 in translation termination and forms the essence of an in vitro assay for RF activity based on the abolishment of readthrough by eRF1.

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It is known that the context 5′ and more significantly 3′ of the termination codon influences the efficiency of termination and suppression (reviewed in 1,13). In addition, the nature of the last two amino acids of the growing peptide chain affects the efficiency of suppression and possibly also of termination (2,14). In view of the drawbacks of the original RF assay, an assay system was developed that circumvents the limitations outlined above. Pure eRF1 was used, and a natural mRNA was the source of termination signal: it contains a naturally-occurring suppressible termination codon that served to evaluate readthrough, as opposed to release of fMet from fMet-tRNA.

A suppressible UAG codon is present in the genome of beet necrotic yellow vein furovirus (BNYVV). RNA2 of the tetrapartite RNA genome of this plant virus, is 4612 nucleotides (nt) long opposed to release of fMet from fMet-tRNA.

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Readthrough is detected in vitro (16) and in vivo (17). The readthrough protein is required for transmission of the virus by its fungus vector (18). It is also involved in virus assembly (19) and is present in trace amounts in purified virus particles (20).

Starting from a construct containing the cDNA corresponding to the BNYVV CP ORF and the readthrough domain, transcripts were synthesized in vitro and used as templates for in vitro translation studies in the absence or presence of exogenous eRF1. The results demonstrate that eRF1 prevents readthrough, that this occurs also when the UAG codon is replaced by UGA or UAA, and that eRF1 competes with suppressor tRNAs (tRNAsu*) for all three termination codons.

MATERIALS AND METHODS

Constructs

The starting wild-type plasmid pB218 (designated here pB218-TAG) was used for the transcription experiments; it includes the fragment corresponding to BNYVV RNA2, from nt 1 to 2715 cloned between the T7 and T3 promoters of a Blue Scribe vector (21). This insert contains an Ncol and an MnlI restriction site. The resulting BNYVV RNA fragment contains the 5′ untranslated region, the CP and readthrough domains, as well as 495 nt following the termination codon at the end of the readthrough domain.

Mutated cDNAs of pB218-TAG in which the TAG codon of the CP was replaced by TGA or TAA, were obtained by the polymerase chain reaction (PCR). Primer 1 was: 5′-CCAATTACCATGGACA-CCTGTTCAGGTAAGATTACACCATTCCGGACAAATGC\-AATTGCGTGC-3′. It is homologous to nt 660–722 of viral RNA2 except for the TGA (bold) which replaced the TAG in pB218-TAG, and the introduction of a T residue in place of a C residue to form the BspEI site (underlined) for screening purposes, and it contains an Ncol site (underlined). Primer 2 was identical to primer 1 except that the TGA in primer 1 was replaced by TAA. Primer 3 was 5′-CTGTAGCACGGCTGGTGCAGC-3′. It is complementary to nt 1130–1150 of viral RNA2, and contains an MnlI restriction site (underlined). An additional mutated cDNA derived from pB218-TAG was produced. In this cDNA, the wild-type TAG codon was maintained, but the C residue following the TAG, was replaced by a G residue, yielding pB218-TAGG. This was achieved with primer 4 which was identical to primer 1 except that the TGAC was replaced by TTAG. Primers 1, 2 or 4 were used in conjunction with primer 3.

Cloned Pfu DNA polymerase (Stratagene) was used for PCR reactions according to the supplier’s recommendations. The PCR products were inserted into the NcoI and MnlI restriction sites of pB218-TAG. The sequence of the resulting constructs pB218-TGA, pB218-TAA and pB218-TAGG was verified by the dideoxynucleotide chain termination method (22) using the USB sequencing kit.

mRNAs synthesized by in vitro transcription

Conditions were as described (23). After linearization of the wild-type and mutated plasmids by BamHI, transcription was performed with T7 RNA polymerase (BRL) and m7GpppG (Pharmacia), and the integrity, size and concentration of the transcripts estimated. The wild-type transcript is designated tB218-UAG and the mutated transcripts tB218-UAG, tB218-UAA and tB218-UAGG.

Release factors and suppressor tRNAs

Escherichia coli-expressed X. laevis and human His-tagged eRF1 were purified as described (6). The factors were tested for RF activity using the fMet release assay (6,12). Escherichia coli-expressed X. laevis His-tagged eRF3 was purified and assayed as described (7).

Suppressor tRNAs were partially purified from human 293 cells transiently expressing plasmids ptRNAam, ptRNAop or ptRNAoc which contain respectively the amber, opal or ochre derivatives of a human tRNA^ser\* gene (24). Plasmid ptRNAwt contains the wild-type version of this gene. These plasmids (24) were modified as described (25). The 293 cells at 50% confluency were transfected with 15 µl of plasmid DNA per 100 mm plate and 48 h after transfection total cellular RNAs were extracted as described (26). The RNA samples were electrophoresed in 7 M urea–10% acrylamide gels. After etidium bromide staining, the RNA band containing the tRNA^ser\* isoacceptors, localized by autoradiography.

RESULTS

Capped tB218-UAG containing the BNYVV CP ORF and the readthrough domain was added to a reticulocyte lysate without or
We have developed an assay system to evaluate eukaryotic termination of protein synthesis versus readthrough in vitro using pure eRF1, suppressor tRNA and a naturally-occurring suppressible UAG codon embedded in the genome of a plant RNA virus.
that of tetraplet termination signals. Moreover, a natural protein is terminated as opposed to hydrolysis of fMet-tRNA which probably never occurs in a natural system.

The ability of eRF1 on its own to compete efficiently with tRNA\textsuperscript{Asu}\textsuperscript{*} of various nonsense codons in vitro (this work) and in vivo (25) implies that association of eRF1 with eRF3 is probably not required for productive eRF1 binding to the ribosomal A site. Recent data on yeast eRF1 (Sup45p) mutants (29) agree with this interpretation. In these studies, reduction of the level of Sup45p in yeast cells caused the appearance of a suppressor phenotype, while the level of Sup35p (equivalent to eRF3) remained unaltered. The results presented here are also in line with those obtained with a \textit{Salmonella typhimurium} strain (30) carrying a UGA suppressor allele in the gene encoding RF2 (a prokaryotic analog of eRF1). In these experiments, reduction in the level of RF2 was responsible for the suppressor phenotype. In view of all these findings (7,25,29,30; this work) the earlier data (31 and references therein) regarding the antisuppressor activity of eRF3 on its own requires further experiments.

Suppression of termination during translation is a strategy frequently encountered among animal and plant RNA viruses for the synthesis of their proteins (reviewed in 32–34). To date only suppressible UAG and UGA codons have been encountered. The codon context surrounding the suppressible amber codon in \textit{BNYVV} RNA2 is identical to the one surrounding the suppressible amber codon in tobacco mosaic tobamovirus (TMV) RNA (35) over 12 nt (C\textsuperscript{AAUAGCA}AAUU). In the case of TMV in which readthrough is required to produce the RNA-dependent RNA polymerase (36), it has been demonstrated that the two codons downstream of the UAG signal are important determinants of suppression in vivo (37), and in vitro (38). Moreover, it has been demonstrated that translational termination efficiency is strongly influenced by the base following the termination codon (39). A and G being preferred over C and U. Indeed, mutating the nucleotide following the suppressible UAG codon in \textit{BNYVV} RNA2 from a C to a G resulted in barely detectable readthrough protein as compared with the wild-type transcript. Thus it is not surprising that in the \textit{BNYVV} and TMV RNAs in which readthrough is required, a C residue follows the termination codon, thereby presumably decreasing the possibility of termination at this position.

Addition of excess \textit{X.laevis} eRF3 over eRF1 enhanced the release activity of eRF1. Alone, eRF3 had no effect on the efficiency of readthrough, and conversely, eRF1 alone was sufficient to inhibit readthrough. Earlier it was shown that even in the absence of eRF3 and GTP, eRF1 on its own was active in promoting termination codon-dependent fMet-tRNA hydrolysis (7). The finding described here, that eRF1 in the absence of exogenous eRF3 efficiently competes with endogenous and exogenous suppressor tRNA(s) implies that recognition of termination codons and peptidyl-tRNA hydrolysis are predominantly linked with eRF1. These experiments show the contribution of eRF3 in stimulating the antisuppressor activity of eRF1 though this effect is obviously of secondary importance. Our experiments indicate that in this \textit{in vitro} translation system, eRF1 rather than eRF3 limits the efficiency of termination.

Readthrough of termination codons requires the positioning of a suppressor tRNA in the ribosomal A site. Our results indicate that exogenous eRF1 efficiently competes at the A site with endogenous and exogenous suppressor tRNA(s) for the termination codon in a natural mRNA-ribosome complex. This observation
emphasizes the functional resemblance between tRNAs and eRF1, and implies that eRF1 could also be structurally similar to aminoacyl-tRNA. This suggestion is in line with the recently proposed tRNA-protein mimicry concept (40) and its extension to RFs (2,41).

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REFERENCES