COMMENTARY

Proposing a mechanism of action for ataluren

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Protein synthesis follows the advice given in *Alice in Wonderland*: "'Begin at the beginning,' the King said gravely, 'and go on till you come to the end: then stop.'" (1). For most protein synthesis, "the beginning" is the first methionine codon (AUG) encountered by the ribosome downstream of the cap, which is situated at the 5′ end of the mRNA (Fig. 1). "The end" is a signal that consists of one of three termination or stop codons: UAG, UAA, or UGA. The path between the initiating AUG and the proper stop codon delineates the open reading frame of the encoded protein.

Nonsense mutations occur when a point mutation in a sense codon, which codes for an amino acid, is altered to one of three stop codons. This results in a truncated protein product due to premature termination of mRNA translation, hence the term premature termination codon (PTC) (Fig. 1). When PTCs are encountered by the elongating ribosome, mRNAs are subjected to an accelerated degradation process through a mechanism referred to as nonsense-mediated decay (NMD) (2). NMD is a conserved surveillance mechanism that involves the recruitment of several proteins to degrade and reduce the expression of nonsense-containing mRNAs (3). This process engenders minimal expression of the defective gene, that is, reduced amounts of truncated, and usually inactive, protein are synthesized. Indeed, it is thought that NMD has evolved to reduce the generation of truncated proteins that could function in a dominant-negative manner and compromise the activity of the complexes in which they serve as subunits.

Nonsense suppression is a process by which PTCs can be functionally overridden by alterations in protein synthesis that affect the efficiency of termination. For instance, whenever a ribosome positioned with a nonsense codon in its A site incorporates a near-cognate tRNA, rather than engaging the termination machinery, it bypasses translation termination and results in stop codon readthrough. PTCs encountered during protein synthesis are the underlying cause of a large number of genetic disorders caused by nonsense mutations. Approximately 10–15% of all human genetic diseases are thought to result from PTC mutations (4, 5); diseases associated with PTC mutations include cystic fibrosis, β-thalassemia, Duchenne muscular dystrophy (DMD), hemophilia, and some forms of cancer (6, 7).

Ataluren, previously known as PTC124, is a bioactive molecule that is thought to modulate the translation machinery (8, 9). The compound allows for the readthrough of PTCs during mRNA translation and thereby facilitates the production of full-length functional proteins (8). This drug (under the name Translarna) has been approved in the European Union for the treatment of nonsense mutation DMD and is currently being evaluated in the clinic for treatment of other diseases, including cystic fibrosis, aniridia, and mucopolysaccharidosis I (10).

In PNAS, Roy et al. (11) address the efficacy and mechanism of action of ataluren. This study shows that ataluren-mediated readthrough of different PTCs (UAG, UAA, and UGA) can be observed with multiple reporter systems in human cells as well as yeast, and identifies the specific amino acids inserted during nonsense suppression when premature termination is bypassed. Enhancement of near-cognate tRNA insertion by ataluren favors a subset of tRNAs, which generally leads to the incorporation of specific amino acids at the PTC (Gln, Lys, and Tyr at UAA and UAG codons, and Trp, Arg, and Cys at the UGA codon). These results show that tRNA selection is attributable to base mispairing at codon positions 1 and 3, that is, not just at the classic wobble position, and to the preferred use of certain nonstandard base pairs, such as U–G. Combined with a previous study (12), and with the observation that similar readthrough specificities appear to be operating endogenously (albeit at much lower efficiency), the data demonstrate that the rules of near-cognate tRNA insertion at PTCs are conserved in eukaryotes. Strikingly, Roy et al. (11) show that the proteins resulting from such nonsense suppression retain substantial function, notwithstanding the different amino acids inserted at the PTCs during ataluren-mediated readthrough. These proteins included CFTR (cystic fibrosis transmembrane

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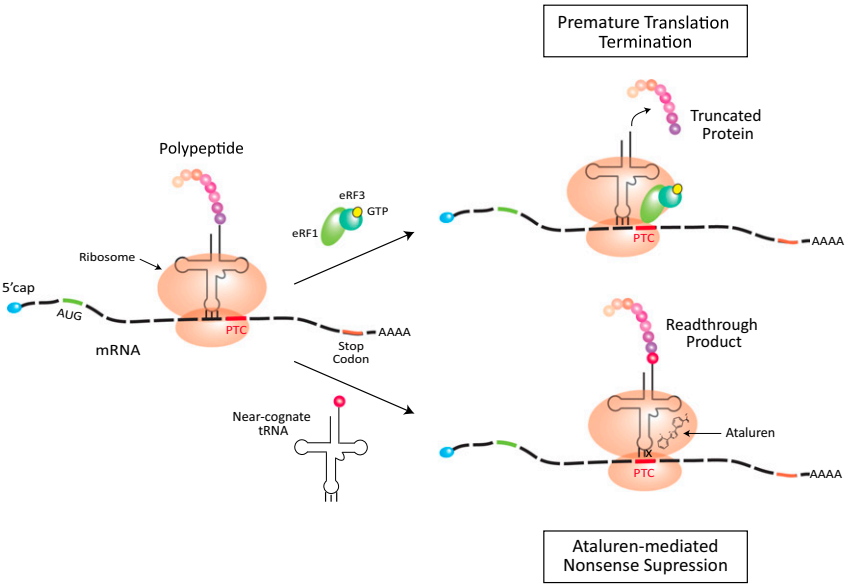

Fig. 1. Schematic diagram illustrating ataluren's proposed mechanism of action. In normal circumstances, ribosomes move along the mRNA linking amino acids into protein until arriving at the stop codon. When a ribosome encounters a premature stop codon (PTC) due to a nonsense mutation, eukaryotic release factors [eRF1 (green) and eRF3 (turquoise) in complex with GTP (yellow)] are recruited and translation is terminated prematurely, generating a truncated protein. Ataluren is proposed to interact with the ribosome and facilitate the recruitment of near-cognate tRNAs, which supresses the nonsense mutation and allows for the readthrough of a PTC and synthesis of a full-length protein. The amino acid colored in red on the near-cognate tRNA is incorporated into the readthrough protein product. The X on the tRNA in the ataluren-mediated nonsense suppression model indicates mispairing at codon position three. The 5'cap is shown in blue, ribosomes are shown in brown, and amino acids in multi-colors on the polypeptide.

conductance regulator), the protein defective in nonsense mutation CF.

The observation that ataluren can stimulate the insertion of near-cognate tRNAs that resemble those inserted endogenously, and the earlier observation that ataluren does not promote readthrough of normal stop codons (8), indicate that the protein products generated by its use are unlikely to be antigenic, a property important for the drug's efficacy as a therapeutic. This appears to be a unique asset of ataluren, because Roy et al. (11) demonstrate that other readthrough-promoting drugs (G418 and gentamicin) generate products that deviate significantly from endogenous readthrough proteins and are therefore more likely to have dominant-negative functions or elicit an immune response.

The data from Roy et al. (11) also address an earlier controversy. Previous studies reported that ataluren's nonsense suppression activity might be attributable to stabilization of the luciferase enzyme used as a reporter (13, 14). The confirmation of ataluren's readthrough activity in numerous nonluciferase systems (including those in Roy et al.) (11) and the demonstration that the hypothetical inhibitory molecule, PTC124-AMP, is rapidly converted to ataluren under conditions of in vitro translation, effectively alleviate this concern.

A significant question concerning ataluren is its molecular mechanism of action. Ataluren's influence over specific near-cognate tRNA selection implies that this drug may target the ribosome (Fig. 1). This conclusion is supported by results in Roy et al. (11) where they showed that the aminoglycoside, Tobramycin, which binds to the ribosomal A site (15), engendered a dose-dependent reduction of ataluren's readthrough activity. Further research investigating the physical interaction between ataluren and the ribosome, including 3D structural studies, will be important for the development of more potent ataluren derivatives in the future.

Another important unanswered question is the mechanistic and molecular explanation for the exquisite preference of ataluren for the insertion of amino acids at PTCs, as opposed to the canonical stop codons that occur at the ends of all mRNA coding regions. One possibility raised by earlier studies (16) is that normal termination and premature termination differ in a key aspect; thus, ataluren may function at one, but not the other. Clearly, understanding the nature of ataluren's target preference may also elucidate an important enigma: the mechanistic distinction between standard termination codons and PTCs, which might further illuminate how the terminal step of protein synthesis takes place.

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