



**HAL**  
open science

# Deciphering the molecular mechanism of stop codon readthrough

Martine Palma, Fabrice Lejeune

► **To cite this version:**

Martine Palma, Fabrice Lejeune. Deciphering the molecular mechanism of stop codon readthrough. Biological Reviews, In press, 10.1111/brv.12657 . inserm-02972847

**HAL Id: inserm-02972847**

**<https://inserm.hal.science/inserm-02972847>**

Submitted on 20 Oct 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# 1 **Deciphering the molecular mechanism of stop codon readthrough**

2

3 Martine Palma and Fabrice Lejeune<sup>\*</sup>

4 *Univ. Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, UMR9020 – UMR 1277 -*

5 *CANTHER – Cancer Heterogeneity, Plasticity and Resistance to Therapies, F-59000 Lille,*

6 *France*

7

8

9 <sup>\*</sup>Author for correspondence (E-mail: [fabrice.lejeune@inserm.fr](mailto:fabrice.lejeune@inserm.fr); Tel.: 33-320871059 ).

10

## 11 **ABSTRACT**

12 Recognition of the stop codon by the translation machinery is essential to terminating  
13 translation at the right position and to synthesizing a protein of the correct size. Under certain  
14 conditions, the stop codon can be recognized as a coding codon promoting translation, which  
15 then terminates at a later stop codon. This event, called stop codon readthrough, occurs either  
16 by error, due to a dedicated regulatory environment leading to generation of different protein  
17 isoforms, or through the action of a readthrough compound. This review focuses on the  
18 mechanisms of stop codon readthrough, the nucleotide and protein environments that  
19 facilitate or inhibit it, and the therapeutic interest of stop codon readthrough in the treatment  
20 of genetic diseases caused by nonsense mutations.

21

22 **Key words:** stop codon readthrough, mechanism, translation termination, premature  
23 termination codon, readthrough activators, molecules, screening.

24

## 25 **CONTENTS**

26	I. Introduction
27	II. Translation termination
28	III. Different types of stop codon readthrough
29	(1) Non-programmed translational readthrough
30	(2) Programmed translational readthrough
31	(3) Induced translational readthrough
32	IV. Parameters influencing stop codon readthrough
33	(1) Cis elements activating stop codon readthrough
34	(2) Trans elements activating readthrough
35	(a) Proteins and RNAs
36	(i) Factors involved in the general process of stop codon readthrough
37	(ii) Factors involved in specific stop codon readthrough events
38	(b) Small molecules
39	(c) Cell environment
40	V. Therapeutic interest of stop codon readthrough
41	VI. New developments and therapeutic targets
42	VII. Conclusions
43	VIII. Acknowledgements
44	IX. References
45	
46	<b>I. INTRODUCTION</b>
47	For cells to function properly, genetic information must be faithfully expressed in RNAs or
48	proteins. A key step in the gene expression pathway is translation from messenger RNA
49	(mRNA) to protein. Translation obeys very specific rules, such as starting at an initiation
50	codon (very often an AUG codon) and stopping at one of the three stop codons UAA, UAG,

51 or UGA, in order to ensure accurate protein length. Sometimes, however, rules can be  
52 bypassed, and translation is no exception. Under specific conditions, ribosomes may ignore  
53 stop codons, continuing with translation and extending the C-terminal part of the nascent  
54 protein. The C-terminally extended protein may acquire a new functional domain in this way.  
55 The absence of termination codon recognition is called stop codon readthrough. Although this  
56 can constitute a decoding error, stop codon readthrough can also be a means of expressing  
57 different protein isoforms and may represent a therapeutic solution for some pathologies.  
58 This review discusses the different molecular factors that regulate translation termination and  
59 stop codon readthrough, mainly in higher eukaryotes. It also outlines the potential of this  
60 mechanism in terms of basic science and clinical applications to advance understanding of  
61 pathways of gene expression and in the development of therapeutic approaches for nonsense-  
62 mutation-related genetic diseases. Potential clinical applications of a stop codon readthrough  
63 strategy have recently been discussed in several reviews (Morais, Adachi & Yu, 2020;  
64 Dabrowski, Bukowy-Bieryllo & Zietkiewicz, 2018; Sharma, Keeling & Rowe, 2020; Bezzeri  
65 *et al.*, 2020), hence we focus here on the molecular mechanisms leading to stop codon  
66 readthrough.

67

## 68 **II. TRANSLATION TERMINATION**

69 Translation is a process that promotes amino acid polymerization leading to a peptide  
70 sequence based on an mRNA sequence. The ribosome carries out translation with the help of  
71 cofactors, ensuring accurate decoding of the open reading frame (ORF) (Dever, Kinzy &  
72 Pavitt, 2016; Merrick, 1992; Kapur, Monaghan & Ackerman, 2017). Misincorporation of an  
73 amino acid occurs at a very low rate. For example, misincorporation of a lysine instead of an  
74 arginine during translation of protamine mRNA has been estimated at only 0.06% to 0.2%  
75 (Mori *et al.*, 1985). Site A of the ribosome is where the transfer RNA (tRNA) carrying an

76 amino acid hybridizes with a codon *via* its anticodon sequence, thus bringing to the growing  
77 polypeptide chain the next amino acid to be incorporated (Fig. 1A). tRNAs are subject to  
78 numerous post-transcriptional modifications that stabilize their tertiary and quaternary  
79 structures and also favour codon–anticodon interaction in the ribosome by changing the  
80 polarity of the modified base and allowing additional interactions with the ribosome  
81 (Grosjean & Westhof, 2016). It is established that post-transcriptional modifications of tRNAs  
82 play a role in the fidelity of codon recognition, and their absence can lead to increased codon  
83 recognition by near-cognate tRNAs (Blanchet *et al.*, 2018).

84 Translation termination occurs when site A of the ribosome reaches one of three stop codons  
85 (UAA, UAG or UGA) in frame with the translation initiation codon (Fig. 1A). In human cells,  
86 the only tRNA to recognize these stop codons is tRNA<sup>[Ser]<sup>Sec</sup></sup>, which carries the amino acid  
87 selenocysteine and pairs with UGA stop codons in a dedicated environment such as a  
88 selenocysteine insertion sequence (SECIS) combined with the presence of SECIS binding-  
89 protein 2 (SBP2) (Labunsky, Hatfield & Gladyshev, 2014). When the ribosome reaches a  
90 stop codon, competition occurs between the translation termination complex and near-cognate  
91 tRNAs recognizing two of the three bases constituting the stop codon. Likely because of a  
92 lower energetic stability of stop codon recognition by a near-cognate tRNA, the translation  
93 termination complex is recruited in more than 99.9% of cases (Floquet *et al.*, 2012; Rajon &  
94 Masel, 2011). UAG and UAA stop codons share the same near-cognate tRNAs, which differ  
95 from those recognizing UGA stop codons. The amino acids glutamine, tyrosine, and lysine  
96 are incorporated during UAG or UAA readthrough, whereas arginine, cysteine, and  
97 tryptophan can be incorporated during UGA readthrough, consistent with their positions in the  
98 genetic code table (Feng *et al.*, 1990; Roy *et al.*, 2015). Yet our understanding of stop codon  
99 misdecoding by near-cognate tRNAs is far from complete, since other amino acids have also  
100 been found to be incorporated during readthrough, according to the nonsense mutation and its

101 nucleotide context. In particular, leucine is the main amino acid incorporated during  
102 readthrough of the UGA nonsense mutation at position 1282 (mutation W1282X) of the cystic  
103 fibrosis transmembrane conductance regulator (CFTR) gene (Xue *et al.*, 2017). It is important  
104 to note that the protein synthesized may or may not be functional according to the amino acid  
105 incorporated at the site of the nonsense mutation, if this position is crucial for the function or  
106 stability of the protein.

107 The translation termination complex is composed of at least two subunits, called eukaryotic  
108 release factors (eRFs) 1 and 3 (Fig. 1A). The eRF1 subunit mimics a tRNA and enters the A  
109 site of the ribosome to recognize the stop codon (Muramatsu *et al.*, 2001; Song *et al.*, 2000).  
110 In eukaryotic cells, interestingly, eRF1 recognizes all three stop codons, whereas in  
111 prokaryotic cells, two release factors (RFs) are necessary: RF-1 recognizes UAA and UAG  
112 stop codons, whereas RF-2 recognizes UAA and UGA stop codons. To understand how eRF1  
113 recognizes all three stop codons, Brown *et al.* (2015) used cryo-electron microscopy and a  
114 catalytically inactive eRF1 to show that glutamic acid at position 55 and tyrosine at position  
115 125 of eRF1 discriminate between purines and pyrimidines at the second and third base  
116 positions of a codon. They thus play a crucial role in the ability of eRF1 to recognize stop  
117 codons (Brown *et al.*, 2015). The eRF3 subunit is a GTPase whose activity is stimulated  
118 mainly by interaction with either the polyA-binding protein (PABP), when translation  
119 termination occurs at the physiological stop codon, or the nonsense-mediated mRNA decay  
120 (NMD) factor UPF3X (also called UPF3B), when translation termination occurs at a  
121 premature termination codon (PTC) (Neu-Yilik *et al.*, 2017). When eRF3 catalyses  
122 conversion of GTP to GDP, it induces a conformational change in the structure of eRF1,  
123 promoting translation termination through release of the nascent peptide chain and  
124 replacement of eRF3 by the ATPase ABCE1 (ATP Binding Cassette Subfamily E Member 1).  
125 ABCE1 will then hydrolyse ATP to ADP to promote recycling of the two ribosomal subunits

126 (Fig. 1A). In addition to the stop codon and to certain proteins located downstream of the stop  
127 codon (PABP or UPF3X for instance), the nucleotide environment around the stop codon, and  
128 particularly certain post-transcriptional modifications, may influence the translation  
129 termination process. A transcriptome-wide mRNA methylation analysis has revealed a high  
130 level of methylation at position 6 of adenosine (m6A) at the end of the ORF and the beginning  
131 of the 3' untranslated region (UTR), in the vicinity of the stop codon. This deserves further  
132 investigation and clarification, as it suggests a possible role of this mark in translation  
133 termination (Meyer *et al.*, 2012; Li *et al.*, 2014).

134

### 135 **III. DIFFERENT TYPES OF STOP CODON READTHROUGH**

136 Although translation termination must be a very efficient process to ensure the correct protein  
137 size, under certain conditions or at a very low rate, a near-cognate tRNA can be recruited to  
138 the A site of the ribosome when the latter reaches a stop codon. The consequence of this is  
139 that translation continues until a later stop codon that promotes translation termination. This  
140 event is called stop codon readthrough (Fig. 1B). Several types of readthrough can occur at  
141 stop codons, depending on the presence of regulatory elements or of readthrough-promoting  
142 molecules (Fig. 2).

143

#### 144 **(1) Non-programmed translational readthrough**

145 In the absence of any readthrough molecules, readthrough of any physiological stop codon or  
146 PTC can occur at a basal level. This readthrough can be considered a translation error and is  
147 referred to here as non-programmed translational readthrough (Fig. 2A). This type of  
148 readthrough is a rare event, calculated as less than 0.1% in more than 80% of cases (Rajon &  
149 Masel, 2011; Floquet *et al.*, 2012; Fearon *et al.*, 1994). Interestingly, the likelihood of non-  
150 programmed translational readthrough depends on the identity of the stop codon. It is highest

151 at the UGA stop codon, whereas translation termination is most efficient at the UAA stop  
152 codon. In the case of PTCs, this very low readthrough rate is partly due to competition  
153 between near-cognate tRNAs and release factors, in favour of the former, but it is also due in  
154 part to activation of the surveillance mechanism provided by NMD, which results in silencing  
155 of the gene (Gupta & Li, 2018; Lejeune, 2017; Kurosaki & Maquat, 2016; He & Jacobson,  
156 2015). Non-programmed translational readthrough thus occurs on the fraction of PTC-  
157 carrying mRNAs that escape NMD (Kuzmiak & Maquat, 2006).

158 Although the amount of protein generated by non-programmed translational readthrough is  
159 minute compared to the amount of protein produced without readthrough of a physiological  
160 stop codon or from the wild-type mRNA in the case of a PTC, the consequences of non-  
161 programmed translational readthrough can be considerable. For example, the phenotypes of  
162 patients suffering from the same pathology and carrying the same nonsense mutation may  
163 vary even though they should be identical due to silencing of the mutant gene. Some cystic  
164 fibrosis patients with a nonsense mutation that one would assume to cause a severe phenotype  
165 due to the absence of the CFTR protein actually show mild pulmonary damage (Cutting *et al.*,  
166 1990; Kerem *et al.*, 1990). The level of non-programmed translational readthrough has been  
167 proposed to explain this mild phenotype, allowing some functional full-length CFTR protein  
168 to be synthesized.

169

## 170 **(2) Programmed translational readthrough**

171 The second type of readthrough is called programmed translational readthrough (Fig. 2B). It  
172 targets specific mRNAs (Loughran *et al.*, 2018; Freitag, Ast & Bolker, 2012; Dunn *et al.*,  
173 2013) and is a proteome-expanding mechanism allowing synthesis of specific protein  
174 isoforms with particular functions. It is thus a way to synthesize two isoforms of a protein  
175 from one mRNA. A specific regulatory mechanism intervenes at the physiological stop codon



176 either to terminate translation at the first stop codon in phase with the initiation codon starting  
177 the ORF or to promote its readthrough so as to terminate translation at one of the downstream  
178 stop codons in phase with the initiation codon. This type of readthrough has been identified in  
179 viruses, fungi, *Drosophila* spp., and mammals. In human cells, programmed translational  
180 readthrough is thought to be a very rare event, reported to affect the expression of only a  
181 dozen genes: the opioid receptor Kappa 1 (OPRK1), opioid related nociceptin receptor 1  
182 (OPRL1), aquaporin 4 (AQP4), mitogen-activated protein kinase 10 (MAPK10), peroxisomal  
183 lactate dehydrogenase B (LDHB), malate dehydrogenase (MDH1), vitamin D receptor (VDR),  
184 vascular endothelial growth factor A (VEGFA), myelin protein zero (MPZ), and beta-globin  
185 genes (Loughran *et al.*, 2014, 2018; Chittum *et al.*, 1998; Yamaguchi *et al.*, 2012; Eswarappa  
186 *et al.*, 2014; Schueren *et al.*, 2014; Geller & Rich, 1980). It appears more frequent in other  
187 species. In *Drosophila melanogaster*, for example, the expression of several hundred genes is  
188 regulated *via* this readthrough process (Lin *et al.*, 2007). Similarly, some 5% of yeast genes  
189 appear to be subject to programmed translational readthrough (Kleppe & Bornberg-Bauer,  
190 2018). An *in silico* analysis aiming to identify physiological stop codons putatively subject to  
191 programmed translational readthrough suggests that this process may actually occur more  
192 frequently than previously thought in many species, including humans (Jungreis *et al.*, 2016;  
193 Dunn *et al.*, 2013). Consistent with these results, ribosome profiling performed on human  
194 foreskin fibroblasts revealed 42 genes as potentially subject to programmed translational  
195 readthrough. Interestingly, this process has been shown to generate C-terminally extended  
196 proteins very efficiently, with the amount of protein formed exceeding the amount of  
197 unextended product protein by up to 30% (Loughran *et al.*, 2014; Singh *et al.*, 2019). This  
198 suggests that specific translation termination regulation occurs at these physiological stop  
199 codons and that cis elements and trans-acting factors must be involved in promoting an

200 exceptionally high rate of readthrough. Some such elements have been identified and are  
201 described in Section IV.

202

### 203 **(3) Induced translational readthrough**

204 The third type of stop codon readthrough is PTC readthrough promoted by certain molecules  
205 (Fig. 2C), here termed induced stop codon readthrough. When the ribosome reaches a stop  
206 codon, the presence of such molecules favour recruitment of near-cognate tRNAs instead of  
207 the translation termination complex. In early studies, aminoglycosides were shown to  
208 facilitate this type of readthrough process in bacteria and yeast (Singh, Ursic & Davies, 1979).  
209 The first evidence of induced nonsense mutation readthrough in mammalian cells was  
210 reported a few years later in a study of G418 and paromomycin aminoglycosides (Burke &  
211 Mogg, 1985). Since then, both aminoglycoside and non-aminoglycoside molecules have been  
212 identified as readthrough molecules (see Section IV) that might potentially be used to treat  
213 nonsense-mutation-related pathologies.

214 Several lines of evidence indicate that induced translational readthrough occurs at PTCs and  
215 not at physiological stop codons. The nucleotide and protein environments around  
216 physiological stop codons have been evolutionarily selected to facilitate translation  
217 termination. This is not true of PTCs, since they appear through mutation in an environment  
218 selected to promote translation and not translation termination. While PTCs do promote  
219 translation termination, they are more sensitive to readthrough than physiological stop codons.  
220 Although several studies using readthrough molecules have shown the absence of readthrough  
221 at physiological stop codons (Benhabiles *et al.*, 2017; Trzaska *et al.*, 2020; Welch *et al.*, 2007),  
222 it remains necessary to demonstrate for each new molecule that it does not impact translation  
223 termination at physiological stop codons.

224 Interestingly, the efficiency of translational readthrough induced by a molecule may be related  
225 to the level of non-programmed translational readthrough (basal level of readthrough)  
226 occurring at the stop codon in the absence of that molecule: the higher the level of basal  
227 readthrough, the more efficient the readthrough promoted by molecules such as  
228 aminoglycosides (Floquet *et al.*, 2012), which should be taken into account when  
229 implementing a therapeutic approach for a given nonsense mutation.

230

#### 231 **IV. PARAMETERS INFLUENCING STOP CODON READTHROUGH**

232 The basal readthrough level varies from one stop codon to another, as shown in various  
233 studies (Fearon *et al.*, 1994; Floquet *et al.*, 2012; Rajon & Masel, 2011). The identity of the  
234 stop codon influences this level, but other elements acting in cis or trans can also modulate the  
235 efficiency of PTC or physiological stop codon readthrough. Such elements can influence all  
236 three types of stop codon readthrough.

237

##### 238 **(1) Cis elements activating stop codon readthrough**

239 Stop codon readthrough efficiency can be influenced by various factors, including the identity  
240 of the stop codon and the nucleotide sequence surrounding it (Bidou *et al.*, 2004; Howard *et*  
241 *al.*, 2000). Results from studies where a PTC was introduced into a reporter gene show that  
242 the UGA stop codon is the most permissive to readthrough and the UAA stop codon is the  
243 least permissive (Bidou *et al.*, 2004; Floquet *et al.*, 2012; Howard *et al.*, 2000; Wangen &  
244 Green, 2020; Manuvakhova, Keeling & Bedwell, 2000). However, these properties can be  
245 altered by the nucleotide context of the stop codon (Bonetti *et al.*, 1995; McCaughan *et al.*,  
246 1995; Cassan & Rousset, 2001). In particular, counting the first nucleotide of the stop codon  
247 as position +1, the nucleotide at position +4 strongly influences translation termination  
248 efficiency. It appears that a purine at this position, as found in about 90% of the most highly

249 expressed human genes, favours translation termination (Tate & Mannering, 1996), whereas a  
250 pyrimidine facilitates readthrough (Brown *et al.*, 1990; McCaughan *et al.*, 1995; Tate &  
251 Mannering, 1996). In particular, the presence of a cytosine at position +4 has been shown to  
252 allow, in most cases, the most efficient stop codon suppression (Floquet *et al.*, 2012; Howard  
253 *et al.*, 2000; Phillips-Jones, Watson & Martin, 1993; Wangen & Green, 2020), although this is  
254 not an absolute rule. The nucleotide environment most favourable to efficient readthrough  
255 also depends on the identity of the stop codon. Manuvakhova *et al.* (2000) found cytosine to  
256 be the most favourable nucleotide at position +4 for promoting readthrough of UGA and  
257 UAA stop codons, but readthrough of UAG stop codons was most efficient when the other  
258 pyrimidine, uracil, is present at that position.

259 The nucleotide immediately following the stop codon is not the only nucleotide that can  
260 influence readthrough efficiency. Several studies have demonstrated that some downstream  
261 nucleotides can favour readthrough. Recently, a study measuring stop codon readthrough by  
262 ribosome profiling showed that the two nucleotides immediately downstream of the stop  
263 codon strongly influence readthrough efficiency (Wangen & Green, 2020). The authors  
264 concluded that enrichment in adenosines or uridines in the vicinity of the stop codon favours  
265 stop codon readthrough, whereas enrichment in guanosines or cytosines favours translation  
266 termination. Other studies have demonstrated that both upstream and downstream sequences  
267 influence the readthrough rate at a stop codon. In particular, CAA sequences upstream and  
268 downstream of a UAG stop codon in the *ste6* gene in yeast or in a reporter gene have been  
269 shown to act synergistically to promote readthrough (Fearon *et al.*, 1994; Manuvakhova *et al.*,  
270 2000; Bonetti *et al.*, 1995; Xue *et al.*, 2014).

271 According to the reporter system used to investigate readthrough efficiency, the upstream and  
272 downstream consensus sequences favouring stop codon readthrough may differ slightly. For  
273 example, the downstream sequences CAR YYA (where R is a purine and Y is a pyrimidine)

274 and CAR NBA (where N is any of the four nucleotides and B can be U, C or G) seem to  
275 favour stop codon readthrough (Namy, Hatin & Rousset, 2001; Harrell, Melcher & Atkins,  
276 2002; Beier & Grimm, 2001). These hexanucleotide sequences are found in several virus and  
277 cell genes associated with the regulation of programmed translational readthrough (Skuzeski  
278 *et al.*, 1991). The upstream sequence can also affect readthrough efficiency. For example, one  
279 study found that the nucleotide sequence spanning positions –6 to +9 influences readthrough  
280 rate. In particular, positions –1 and +4 were crucial for readthrough activation, and the  
281 sequence U STOP C has been reported as the consensus sequence for efficient readthrough  
282 (Floquet *et al.*, 2012). Supporting the idea of involvement of the upstream sequence in  
283 promoting readthrough, a recent study on the glycosyltransferase gene *B4GALNT1* has  
284 demonstrated that the base triplet AGC, immediately upstream and downstream of the PTC, is  
285 required for efficient basal readthrough generated by the M4 nonsense mutation at amino acid  
286 228 (Yesmin *et al.*, 2020). Overall, all these studies indicate that stop codon readthrough  
287 efficiency is modulated by cis elements that have not yet been clearly identified and that are  
288 likely to differ among genes. It remains very difficult to predict with certainty the rate of  
289 readthrough of a stop codon without additional experimental data. For instance, when  
290 attempting to explain why a given readthrough molecule has variable effects on the same  
291 nonsense mutation located at different positions in a gene, one must consider the influence of  
292 the stop-codon-surrounding sequence on readthrough efficiency. This is especially true when  
293 the goal is to develop a therapeutic approach (Martorell *et al.*, 2020).

294 Besides the immediate primary sequence surrounding the stop codon, some secondary  
295 structures have been shown to facilitate stop codon readthrough. In the Moloney murine  
296 leukemia virus, a pseudoknot located eight nucleotides downstream of the stop codon  
297 separating the gag and pol ORFs promotes readthrough by about 5% of the ribosomes  
298 reaching this stop codon (Wills, Gesteland & Atkins, 1991). Since this discovery, efficient

299 readthroughs have been found to require other secondary structures (e.g. conserved hairpins)  
300 occurring at stop codons from various virus genomes (Firth *et al.*, 2011). The exact  
301 mechanism remains obscure, but these results suggest that certain proteins could be recruited  
302 by these secondary structures to promote readthrough.

303

## 304 **(2) Trans elements activating readthrough**

305 When the ribosome reaches a stop codon, it must terminate translation or ignore this  
306 translation termination signal by incorporating an amino acid and continuing translation of the  
307 ORF to a downstream stop codon. To understand the mechanisms underlying the ‘decision’ to  
308 stop translation or to continue, specific factors dedicated to stop codon readthrough have been  
309 sought. Some endogenous trans elements have been identified as proteins or RNAs required  
310 either for readthrough of specific stop codons or for the general readthrough mechanism.  
311 Molecules with the capacity to promote readthrough are of particular interest because of their  
312 potential in treating nonsense-mutation-related pathologies. Finally, the cell environment has  
313 been shown to influence readthrough efficiency, suggesting that regulation of the readthrough  
314 process may occur according to culture conditions.

315

### 316 *(a) Proteins and RNAs*

317 To date, only a few factors have been implicated in the readthrough process, some affecting  
318 all stop codon readthroughs and some that are specific to readthrough of one particular stop  
319 codon.

320

#### 321 *(i) Factors involved in the general process of stop codon readthrough*

322 As readthrough opposes translation termination and *vice versa*, it is not surprising that loss of  
323 function of proteins involved in translation termination leads to increased levels of stop codon

324 readthrough. For example, the proteins termination and polyadenylation 1 (TPA1) and Ccr4  
325 associated factor 1 (CAF1 also called POP2) involved in regulating the length of the poly(A)  
326 tail also participate in the translation termination process. In experiments using the firefly  
327 luciferase reporter gene carrying a PTC, loss of TPA1 or POP2 function in yeast resulted in  
328 increased basal readthrough levels (Keeling *et al.*, 2006). These proteins can thus be viewed  
329 as inhibitors of stop codon readthrough. However, in both yeast and human cells, the  
330 nucleotide context appears to determine whether TPA1 exerts a positive or a negative  
331 influence on stop codon readthrough (Loenarz *et al.*, 2014). In yeast, the balance between  
332 translation termination efficiency and stop codon readthrough efficiency can be altered by the  
333 expression levels of the inhibitor of translation termination 1 (ITT1) gene. This was  
334 demonstrated in experiments using a PGK1-STOP-LacZ reporter construct, in which  
335 overexpression of ITT1 caused increased basal levels of readthrough (Urakov *et al.*, 2001).

336 Another example of the antagonism between translation termination efficiency and stop codon  
337 readthrough activation is illustrated by the dead-box RNA helicase Dbp5/DDX19, which is  
338 involved in translation termination. Dbp5/DDX19 interacts with eRF1, bringing it into contact  
339 with eRF3 so as to promote translation termination (Fig. 1). If the function of Dbp5/DDX19 is  
340 impaired, eRF1 interacts prematurely with eRF3 leading to failure of the translation  
341 termination process, thus allowing near-cognate tRNAs to enter the A site of the ribosome,  
342 recognize the stop codon, and promote readthrough (Gross *et al.*, 2007; Mikhailova *et al.*,  
343 2017; Beissel *et al.*, 2019).

344 Although it makes sense that proteins involved in the translation termination process can  
345 interfere with stop codon readthrough, it is surprising to note the presence of translation  
346 initiation factors among the proteins that modulate stop codon readthrough. In both yeast and  
347 human cells, the eukaryotic initiation translation factor 3 (eIF3) seems to play a general role  
348 in programmed translational readthrough. The absence of functional eIF3 reduces the basal

349 level of readthrough of all three stop codons, provided they are in a readthrough-favourable  
350 nucleotide context (Beznoskova *et al.*, 2015). To promote readthrough, eIF3 appears to act as  
351 an inhibitor of eRF1 by interacting with the pre-termination complex and interfering with  
352 pairing between eRF1 and the third base of the codon. By preventing recognition of the stop  
353 codon by eRF1, eIF3 favours recognition of the stop codon by a near-cognate tRNA, thus  
354 promoting stop codon readthrough.

355 It is also possible to modulate the efficiency of stop codon readthrough by acting on the  
356 fidelity of the translation process. tRNAs are subject to various post-transcriptional  
357 modifications that prevent erroneous codon recognition by the tRNA. For example, the  
358 wobble effect, proposed to explain recognition of the first two bases of a codon by a near-  
359 cognate tRNA independently of the third nucleotide (Crick, 1966), is strongly increased when  
360 these post-transcriptional modifications are not present (Duechler *et al.*, 2016; Hagervall *et al.*,  
361 1990; Bednarova *et al.*, 2017; Agris *et al.*, 2017). Interestingly, the rules governing  
362 recognition of a stop codon by a near-cognate tRNA appear less restrictive than proposed by  
363 the wobble effect, as more tRNAs than expected can recognize a stop codon (Roy *et al.*, 2015).  
364 Not only can codon recognition by a near-cognate tRNA occur *via* the first two bases of the  
365 codon independently of the third nucleotide, but it can also occur *via* the last two bases of the  
366 codon independently of the first. Similarly, the discovery that leucine is the amino acid most  
367 abundantly incorporated at the nonsense mutation W1282X in the CFTR gene suggests that a  
368 tRNA may recognize a stop codon with a mismatch at the central base of the triplet forming  
369 the codon (Xue *et al.*, 2017).

370 Note also that, before being substrates of stop codon readthrough, PTC-carrying mRNAs are  
371 substrates of NMD. It is therefore tempting to connect these two mechanisms and to  
372 hypothesize that they share factors in common. In humans at least, this is indeed the case for  
373 some NMD factors: downregulation of the NMD factors Up frameshift (UPF) 1, 2, or 3X/3B



374 impairs readthrough, indicating that these factors are necessary for this process (Jia *et al.*,  
375 2017; Ivanov *et al.*, 2008). The exact roles of these NMD proteins in readthrough remain to be  
376 clarified, especially because contradictory reports have been published on this role of UPF  
377 proteins in other organisms such as yeast. It has notably been claimed that UPF proteins  
378 inhibit readthrough, since knockout of one UPF gene in yeast results in an increased  
379 readthrough rate (Salas-Marco & Bedwell, 2005; Wang *et al.*, 2001). However, another study  
380 failed to observe any effect of UPF gene knockout on the readthrough rate, suggesting that  
381 these proteins are not involved in readthrough (Harger & Dinman, 2004). The role of the UPF  
382 proteins in stop codon readthrough thus may differ between yeast and humans. This warrants  
383 in-depth investigations into the connection between NMD and stop codon readthrough.  
384 Translation takes place *a priori* throughout the cytoplasm, and since stop codon readthrough  
385 shares the same translation machinery, it can be expected also to occur in the cytoplasm,  
386 without any dedicated sites. Yet a recent study demonstrated that the cytoskeleton influences  
387 PTC readthrough (Jia *et al.*, 2017). In particular, basal readthrough is activated when  
388 formation of actin filaments is impaired. It thus seems that actin filaments are neither required  
389 for readthrough nor favourable to it, meaning that they may participate in some form of  
390 readthrough inhibition. The same study showed that non-programmed translational  
391 readthrough occurs at specific cytoplasmic foci different from P-bodies and named  
392 readthrough bodies (Jia *et al.*, 2017). Their results suggest that PTC-containing mRNAs are  
393 actively targeted either to degradation by NMD or to undergo PTC readthrough. Although  
394 UPF proteins have been found in readthrough bodies (unlike the P-body marker decapping  
395 protein 1a (DCP1a), which is involved in NMD), readthrough body characterization remains  
396 poor and no specific proteins have been identified.

397

398 *(ii) Factors involved in specific stop codon readthrough events*

399 To explain programmed translational readthrough on an mRNA, cis elements have been  
400 identified (see Section IV.1). Often these cis elements work together with or recruit factors to  
401 promote readthrough of a specific stop codon. For example, heterogeneous nuclear  
402 ribonucleoprotein (hnRNP) A2/B1, an RNA-binding protein involved in primary microRNA  
403 (pri-miRNA) processing and in the trafficking and assembly of the human immunodeficiency  
404 virus (HIV) genome (Alarcon *et al.*, 2015; Beriault *et al.*, 2004; Levesque *et al.*, 2006), is  
405 involved in programmed translational readthrough on VEGFA mRNA (Eswarappa *et al.*,  
406 2014; Houck-Loomis *et al.*, 2011). HnRNP A2/B1 interacts with an A2 response element  
407 (A2RE) located downstream of the physiological stop codon to promote stop codon  
408 readthrough. Mutating the A2RE sequence or downregulating hnRNP A2/B1 impairs  
409 readthrough of the physiological stop codon of VEGFA mRNA. Whether hnRNP A2/B1  
410 interacts with VEGFA pre-messenger RNA (pre-mRNA) or mRNA only has not yet been  
411 investigated, but given the nuclear localization of this protein, it could be an early mark for  
412 specific programmed translational readthrough.

413 More recently, Lethal 7a (Let7a) microRNA (miRNA) has been shown to promote  
414 programmed translational readthrough on Argonaute 1 (Ago1) mRNA, generating a longer  
415 isoform called Ago1x. The miRNA binds a sequence downstream of the physiological stop  
416 codon and upstream of the subsequent in-phase downstream stop codon. This sequence is  
417 sufficient to promote readthrough when introduced into a heterologous 3' UTR (Singh *et al.*,  
418 2019). This indicates that the mechanism is independent of the identity of the stop codon or of  
419 the mRNA, as long as a Let7a miRNA binding site is present downstream of the stop codon to  
420 be read through.

421 Both hnRNP A2/B1 and Let7a miRNA bind an RNA sequence located about 10 nucleotides  
422 downstream of the stop codon whose readthrough they promote. This suggests a possible  
423 interaction with the translation machinery pausing on the stop codon. In both cases,

424 interestingly, the rate of translational readthrough of the canonical stop codon reaches at least  
425 20%, which can be considered very efficient stop codon readthrough (Eswarappa *et al.*, 2014;  
426 Singh *et al.*, 2019). Unfortunately, the precise mechanism remains to be determined in order  
427 to understand how these trans elements impair translation termination and efficiently promote  
428 synthesis of a C-terminally extended protein.

429 All factors involved in translation termination or stop codon readthrough constitute targets for  
430 the development of therapeutic approaches to treating genetic diseases caused by nonsense  
431 mutations. By inhibiting the synthesis of these factors or in some cases by overproducing  
432 them, stop codon readthrough is activated. Readthrough activation could thus represent a  
433 potential way to correct a nonsense mutation responsible for a pathology. Molecules capable  
434 of targeting the trans factors described in this section could be sought in the framework of  
435 developing a therapeutic approach. Yet to date, as discussed in Section V, this is not yet a  
436 common approach to identify readthrough molecules. The strategy used focuses on searching  
437 for molecules that target the readthrough mechanism as a whole, rather than targeting a  
438 specific factor.

439

#### 440 *(b) Small molecules*

441 Because of their potential therapeutic interest, molecules activating PTC readthrough have  
442 been the focus of many studies. Historically, some members of the aminoglycoside family  
443 have shown the capacity to promote PTC readthrough (Burke & Mogg, 1985). This family of  
444 molecules is composed of a sugar substituted with one amino group. However, not all  
445 members of this family promote significant stop codon readthrough: gentamicin, geneticin  
446 (G418), paromomycin, neomycin, and lividomycin do have this effect (Table 1), but  
447 hygromycin, streptomycin, kanamycin, tobramycin, and amikacin do not (Manuvakhova *et al.*,  
448 2000). Aminoglycosides promote stop codon readthrough by interacting with the 16S

449 ribosomal RNA located in the decoding centre of the ribosome (Carter *et al.*, 2000; Ogle,  
450 Carter & Ramakrishnan, 2003; Prokhorova *et al.*, 2017; Zingman *et al.*, 2007). Besides  
451 aminoglycosides, several non-aminoglycoside molecules have also been shown to promote  
452 PTC readthrough (Table 2). PTC124/ataluren/translarna, an oxadiazole derivative, is the only  
453 molecule to have reached clinical phase II/III trials for the treatment of genetic diseases  
454 caused by nonsense mutations (Welch *et al.*, 2007; Kerem *et al.*, 2014). This molecule, which  
455 can rescue expression of genes carrying UGA, UAG, or UAA nonsense mutations, has a  
456 mode of action that remains to be clarified, but might either target the A site of the ribosome  
457 as do aminoglycosides or interact directly with the stop codon to promote readthrough (Roy *et*  
458 *al.*, 2016; Tutone *et al.*, 2019). Even though the efficacy of this molecule seems too low for  
459 clinical development aimed at treating, for example, cystic fibrosis or Duchenne muscular  
460 dystrophy (Haas *et al.*, 2015; Kerem *et al.*, 2014), it does illustrate the need to identify  
461 readthrough molecules that might enhance the treatment of nonsense-mutation-related genetic  
462 diseases (Kong *et al.*, 2019). Among other readthrough molecules, a dipeptide-like hydrazide  
463 antibiotic negamycin, originally purified from *Streptomyces purpeofuscus*, appears more  
464 potent than aminoglycosides and less toxic, as do negamycin derivatives (Arakawa *et al.*,  
465 2003; Taguchi *et al.*, 2014; Hamada *et al.*, 2019) (Table 2).

466 For more than 10 years, various screening systems have been used to identify compounds  
467 more potent than aminoglycosides and ataluren (Fig. 3). The readthrough compounds (RTCs)  
468 RTC13, RTC14, RTC204, RTC219, GJ071, GJ072, NV2907, NV2909, NV2899 and NV2913  
469 (Du *et al.*, 2009, 2013; Tutone *et al.*, 2020) (Table 2) have been identified and tested on  
470 different constructs and cell models of nonsense-mutation-related genetic diseases. In most  
471 cases, these molecules have shown a readthrough activity similar to that of ataluren or  
472 aminoglycosides such as gentamicin (G418) (Du *et al.*, 2009; Tutone *et al.*, 2020; Gomez-  
473 Grau *et al.*, 2015).

474 Among the compounds identified as promoting induced translational readthrough, some have  
475 remarkable characteristics. The anti-allergy and anti-asthma drug Amlexanox, for example,  
476 has shown the capacity to both inhibit NMD and activate readthrough of UGA, UAG, and  
477 UAA nonsense mutations (Atanasova *et al.*, 2017; Banning, Schiff & Tikkanen, 2017;  
478 Gonzalez-Hilarion *et al.*, 2012). The clinically approved molecule Escin has also been shown  
479 to exert this dual action (Mutyam *et al.*, 2016). In theory, such molecules should be more  
480 effective than molecules that only activate readthrough, since inhibiting NMD leads to an  
481 increased amount of RNA substrates for readthrough (Linde *et al.*, 2007; Gonzalez-Hilarion *et al.*,  
482 2012). It seems, however, that this is not an absolute rule, as some readthrough activators  
483 with no NMD-inhibiting action promote greater synthesis of full-length proteins than do dual-  
484 action molecules. For instance, the readthrough activators *Lepista flaccida* extract H7 and 2,6-  
485 diaminopurine, which do not inhibit NMD, correct UGA and UAA (extract H7) or UGA only  
486 (2,6-diaminopurine) more effectively than dual-action G418 (Benhabiles *et al.*, 2017; Trzaska  
487 *et al.*, 2020; Correa-Cerro *et al.*, 2005).

488 Clitocine is another molecule with a high readthrough-promoting capacity. However, this  
489 molecule, found in various mushroom species (including *Leucopaxillus giganteus* and *Lepista*  
490 *flaccida*) (Trzaska *et al.*, 2020; Kubo *et al.*, 1986; Wilde *et al.*, 2007; Ren *et al.*, 2008;  
491 Benhabiles *et al.*, 2017; Fortin *et al.*, 2006) unfortunately shows high toxicity, which limits its  
492 potential therapeutic development (Fortin *et al.*, 2006; Sun *et al.*, 2012). The mode of action  
493 of clitocine has been studied. Interestingly, this molecule, whose structure resembles that of a  
494 nucleoside, is incorporated into RNA molecules during transcription, substituting for  
495 adenosines. Like adenosine, clitocine can preferentially pair with uracil, but it has been  
496 proposed that the translation termination factor eRF1 weakly recognizes stop codons  
497 containing clitocine, increasing the chance of near-cognate tRNA recruitment (Friesen *et al.*,  
498 2017).

499

500 (c) *Cell environment*

501 Cell culture conditions can also influence readthrough efficiency. In ribosome profiling  
502 experiments performed on the neural cell line PC12, oxygen and glucose deprivation were  
503 shown to inhibit programmed translational readthrough of 18 mRNAs with a UGA  
504 physiological stop codon followed by a cytosine: Fkbp1a, Hadhb, Hs1bp3, Klc1,  
505 LOC102554884, Mdh1, Mrto4, Nedd8, Nudcd2, Plat, Polr2l, Ppp4c, Rfc2, Rnf111, Sec13,  
506 Slc7a1, Ssna1 and Thy1 (Andreev *et al.*, 2015). Interestingly, this regulation occurs very  
507 rapidly: less than 20 min after starting hypoxia and low glucose, programmed translational  
508 readthrough was strongly reduced. Although the mode of action is not clear, it could be  
509 related to the loss of protein hydroxylation at the decoding centre of the ribosome.  
510 Other cell culture conditions that seem to potentiate PTC readthrough notably include serum  
511 starvation. In a medium containing 1% serum, the PTC-readthrough efficacy of  
512 aminoglycosides can increase two- to threefold without modifying the translation activity on  
513 the mRNA substrates, as demonstrated using a dual-reporter green fluorescent protein (GFP)-  
514 blue fluorescent protein (BFP) construct (Wittenstein *et al.*, 2019). How serum starvation  
515 potentiates the readthrough activity of aminoglycosides is not yet understood, but given the  
516 influence of serum on gene expression, it seems likely that specific gene products acting as  
517 cofactors are overexpressed or repressed.

518

519 **V. THERAPEUTIC INTEREST OF STOP CODON READTHROUGH**

520 Favouring stop codon readthrough to correct a nonsense mutation represents an attractive  
521 approach to the treatment of certain genetic diseases. About 11% of patients with a genetic  
522 disease carry a nonsense mutation responsible for its pathology (Mort *et al.*, 2008). The use of  
523 readthrough molecules was first tested in patients with cystic fibrosis caused by a nonsense

524 mutation in the CFTR gene. The aminoglycoside gentamicin was administered intravenously,  
525 at 2.5 mg/kg every 8 h for seven days, to five patients carrying nonsense mutations and five  
526 patients without nonsense mutation in CFTR (Clancy *et al.*, 2001). The results showed a mild  
527 but encouraging rescue of CFTR expression and function. Another study on cystic fibrosis  
528 patients demonstrated that rescue of CFTR function depends strongly on the nonsense  
529 mutation: patients with the Y122X nonsense mutation seemed to respond better than patients  
530 carrying G542X, R1162X, or W1282X (Sermet-Gaudelus *et al.*, 2007). Aminoglycosides  
531 (particularly gentamicin) have also been tested in patients with Duchenne muscular dystrophy  
532 (DMD) carrying a nonsense mutation in the *DMD* gene encoding dystrophin. Gentamicin has  
533 been tested in several studies with different protocols. In one study, four DMD patients with a  
534 nonsense mutation in the *DMD* gene were treated daily for 14 days with gentamicin at 7.5  
535 mg/kg (Wagner *et al.*, 2001). This study concluded that the administered gentamicin treatment  
536 was unable to promote the synthesis of full-length dystrophin protein. In another study,  
537 positive results were obtained by intravenous administration of 7.5 mg/kg gentamicin, once or  
538 twice a week for 6 months, to patients carrying a nonsense mutation in the *DMD* gene, as  
539 compared to control patients carrying a frameshift mutation in the *DMD* gene (Malik *et al.*,  
540 2010). The treatment was well tolerated by patients, without any sign of toxicity. The level of  
541 dystrophin protein increased in gentamicin-treated patients carrying a nonsense mutation,  
542 indicating that readthrough of the nonsense mutation occurred. However, the physical strength  
543 of these patients was unaffected by the treatment, implying that dystrophin rescue was  
544 insufficient.

545 The non-aminoglycoside molecule ataluren has also been tested on cystic fibrosis and DMD  
546 patients carrying nonsense mutations. This molecule was well tolerated by patients. DMD  
547 patients received ataluren orally three times daily at 4–4–8 mg/kg, 10–10–20 mg/kg, or 20–  
548 20–40 mg/kg for 28 days. At the end of the study, dystrophin was detected in patients at the

549 lowest and middle doses but not in patients at the highest dose (Namgoong & Bertoni, 2016).  
550 After a 48-week exposure to ataluren or placebo, drug-treated patients showed no significant  
551 improvement in a 6-min walking-distance test as compared to the placebo group (Finkel *et al.*,  
552 2013; Bushby *et al.*, 2014). For patients with cystic fibrosis caused by nonsense mutations,  
553 several phase II and III clinical trials have been completed. Physical improvement was  
554 observed in patients having received ataluren *versus* placebo, although this improvement  
555 remained modest (Kerem *et al.*, 2008; Sermet-Gaudelus *et al.*, 2010; Lee & Dougherty, 2012).  
556 The results of the clinical trials performed with gentamicin or ataluren clearly demonstrate  
557 that it is possible to correct nonsense mutations by inducing PTC readthrough in patients.  
558 Although no cure for patients with nonsense-mutation-related genetic diseases is yet available,  
559 these encouraging results warrant both optimization of the tested protocols and a search for  
560 new therapeutic approaches.

561

## 562 **VI. NEW DEVELOPMENTS AND THERAPEUTIC TARGETS**

563 As described below, aminoglycosides have high potential to correct nonsense mutations. Yet  
564 because of their oto- and nephrotoxicity, their clinical development has been limited (Hock &  
565 Anderson, 1995; Mingeot-Leclercq & Tulkens, 1999; Swan, 1997; Greenwood, 1959; Heck,  
566 Hinshaw & Parsons, 1963; Matz, 1993; Wu *et al.*, 2001). To address this issue,  
567 aminoglycoside derivatives have been synthesized and tested for the capacity to correct  
568 nonsense mutations while showing lower toxicity. Several molecules meeting these criteria  
569 appear to be good candidates for treating genetic diseases caused by nonsense mutations, their  
570 efficacy having been demonstrated on cell lines and *in vivo* mouse models (Goldmann *et al.*,  
571 2012; Nudelman *et al.*, 2009, 2006; Shulman *et al.*, 2014; Wang *et al.*, 2012; Xue *et al.*, 2014).  
572 One of these molecules, named ELX-02 (from Eloxx Pharmaceuticals), has already  
573 successfully passed clinical phase I (Leubitz *et al.*, 2019), and clinical phase II is ongoing.



574 ELX-02 is much less toxic than gentamicin or G418, and no nephrotoxicity has been detected.  
575 Its readthrough efficiency is similar to that of G418, making this molecule an excellent drug  
576 candidate (Brasell *et al.*, 2019).

577 Regarding ataluren (from PTC Therapeutics), the quest to identify derivative molecules with  
578 higher efficacy has already begun. Molecules such as NV2445 and PTC414 seem to offer a  
579 slightly higher readthrough efficiency. Provided their toxicity is no higher than that of  
580 ataluren, they could represent optimized versions of this drug (Pibiri *et al.*, 2018; Moosajee *et*  
581 *al.*, 2016).

582 One of the biggest challenges is to identify low-toxicity molecules with a significantly higher  
583 readthrough efficiency than reference molecules such as G418. To identify readthrough  
584 molecules, many screening systems have been devised (Fig. 3). They are based on the use of  
585 either (i) one cDNA encoding a fluorescent protein or enzyme, in which a stop codon has  
586 been introduced to interrupt the ORF and prevent synthesis of the functional protein (Fig. 3A)  
587 (Du *et al.*, 2009; Manuvakhova *et al.*, 2000; Welch *et al.*, 2007; Burke & Mogg, 1985;  
588 Sogaard, Jakobsen & Justesen, 1999) or (ii) two cDNAs encoding fluorescent proteins or  
589 enzymes, separated by a stop codon (Fig. 3B) (Bidou *et al.*, 2004; Xue *et al.*, 2014; Cardno *et*  
590 *al.*, 2009). In the latter case, the level of protein produced from the first cDNA is used to  
591 normalize measurements between samples, and the level of protein produced from the second  
592 ORF reflects the readthrough efficiency. The advantage of these constructs is that they are not  
593 spliced downstream of the premature termination codon, so that the corresponding mRNA is  
594 not subject to NMD (Lejeune & Maquat, 2005). This makes it possible to identify molecules  
595 on the sole basis of their readthrough-promoting activity. However, with such constructs there  
596 is a risk of selecting molecules with low readthrough efficiency (and hence low therapeutic  
597 efficacy), as testing is done on mRNAs present in huge copy numbers. In addition, as the pre-  
598 mRNAs of more than 90% of human genes are subject to splicing, the corresponding PTC-

599 carrying mRNAs will be substrates for NMD, which will strongly reduce the copy number of  
600 substrates for readthrough. It is important to bear in mind that this is the prevailing situation in  
601 patient cells. To identify molecules capable of correcting nonsense mutations in these cells,  
602 screening systems should be designed with constructs subject to NMD. Such a screening  
603 system has been described: it consists of a cDNA encoding the firefly luciferase interrupted  
604 by an intron and an upstream PTC (Fig. 3C) (Benhabiles *et al.*, 2017). It has been shown that  
605 the firefly luciferase pre-mRNA is spliced and the mRNA degraded by NMD. The luciferase  
606 activity measured in this screening system thus depends on the efficiency of readthrough  
607 occurring on the number of firefly luciferase mRNAs that have escaped NMD. This screening  
608 system has been used successfully to identify *Lepista flaccida* fungus extract H7 and 2,6-  
609 diaminopurine as two highly potent correctors of nonsense mutations in human cells  
610 (Benhabiles *et al.*, 2017; Trzaska *et al.*, 2020).

611 Studies aiming to increase the effectiveness of readthrough compounds have identified some  
612 molecules that potentiate the readthrough activity of aminoglycosides (Table 3). Interestingly,  
613 these molecules do not promote readthrough by themselves. For example, the phthalimide  
614 derivative CDX5-1 increases about 180-fold the readthrough efficiency of G418 in both yeast  
615 and human cells (Baradaran-Heravi *et al.*, 2016). More recently discovered potentiators of  
616 aminoglycosides include the antimalarial mefloquine (Ferguson *et al.*, 2019) and 2-  
617 aminothiazole-4-carboxamides (Rabea *et al.*, 2019). How these molecules improve the  
618 readthrough efficiency of aminoglycosides is unclear, but by making it possible to administer  
619 lower doses of aminoglycosides, they should reduce aminoglycoside-linked toxic effects.

620 As the use of screening systems to identify molecules with readthrough activity takes time  
621 and money, new targeted approaches are being or could be developed to promote efficient  
622 readthrough. One such approach is based on the mechanism of action of 2,6-diaminopurine,  
623 recently shown to promote readthrough of UGA premature stop codons by interfering with the

624 function of filamentous temperature sensitive J1 (FTSJ1). This enzyme is a methyltransferase  
625 targeting position C34 of the tRNA carrying tryptophan and recognizing the UGG codon  
626 (Trzaska *et al.*, 2020). Decreased C34 methylation allows this tRNA to recognize also the  
627 UGA stop codon (Trzaska *et al.*, 2020). Hence, tRNA-modifying enzymes appear as  
628 interesting targets. This readthrough-activating strategy seems more effective than targeting  
629 the ribosome, but it remains to be validated through identification of new molecules targeting  
630 other tRNA-modification enzymes. Another potential target in the search for readthrough-  
631 promoting molecules is the ATP-binding domain 3/cytosolic thiouridylase subunit 1  
632 (ATPBD3/CTU1) protein. This tRNA-modifying enzyme notably acts at the anticodon loop  
633 of near-cognate tRNAs, enabling them to recognize a UAG or UAA stop codon (Blanchet *et*  
634 *al.*, 2018).

635 Pseudouridylation is another post-transcriptional modification occurring on RNA molecules  
636 that might be used to activate stop codon readthrough. Interestingly, pseudouridylation  
637 converts a uridine to a pseudouridine, which can pair with all four conventional bases  
638 (Kierzek *et al.*, 2014). Protocols have been designed to induce site-directed pseudouridylation  
639 at the uridine constituting the first base of all three stop codons (Wu, Huang & Yu, 2015). The  
640 idea is that the presence of a pseudouridine at this position will stabilize near-cognate tRNAs  
641 and increase basal readthrough of this stop codon.

642 A final approach worth mentioning is direct modification of tRNA anticodon sequences,  
643 enabling them to pair with stop codons. Such tRNAs, called suppressor tRNAs, have been  
644 investigated as potential therapeutics for almost 40 years (Temple *et al.*, 1982), and research  
645 is still ongoing. Very recently, investigators have described a high-throughput cloning system  
646 for identifying tRNAs that suppress nonsense mutations with high efficiency (Lueck *et al.*,  
647 2019). Interestingly, a comparative study of suppressor tRNAs and readthrough molecules  
648 such as gentamicin and G418 demonstrated a superior nonsense-mutation-correcting

649 efficiency of the tested tRNAs. A legitimate question is whether such molecules affect  
650 translation termination at physiological stop codons. Lueck *et al.* (2019), using ribosome  
651 profiling, showed that suppressor tRNAs do read through physiological stop codons in rare  
652 cases, but that this readthrough is much less efficient than PTC readthrough. A limitation of  
653 this very promising approach remains delivery of the suppressor tRNA to the cells of patients  
654 liable to benefit from correction of a nonsense mutation responsible for a genetic disease.

655

## 656 **VII. CONCLUSIONS**

657 (1) Stop codon readthrough is a natural process resulting in continuation of translation beyond  
658 the first stop codon encountered in phase with the translation initiation codon. The mechanism  
659 determining whether the translation machinery recruits a tRNA to promote translational  
660 readthrough, or the translation termination complex to the stop codon remains poorly  
661 understood.

662 (2) Several types of stop codon readthrough can be identified. Non-programmed translational  
663 readthrough or basal stop codon readthrough arises as a translational error without any cis  
664 and/or trans facilitator elements and at a very low rate. Programmed translational readthrough  
665 involves cis and/or trans activator elements, and often reaches more than 20% efficiency. The  
666 cis and trans elements described herein constitute first steps in understanding of the molecular  
667 mechanisms involved. Induced translational readthrough involves small molecules that  
668 increase the recruitment of near-cognate tRNAs when the ribosome pauses at a stop codon.

669 (3) Several regulator elements have been reported to modulate stop codon readthrough. The  
670 nucleotide sequence in the vicinity of the stop codon has been reported strongly to influence  
671 the stop codon readthrough rate. RNAs, proteins and small molecules also play a role in stop  
672 codon readthrough efficacy. Given the therapeutic potential of activators of PTC-readthrough,  
673 regulator elements responsible for high efficiency of readthrough deserve in-depth study. It is

674 essential to fill the gaps in our knowledge of the molecular events leading to stop codon  
675 readthrough and its regulation. This should be accomplished by studying the molecular  
676 mechanism of stop codon recognition and the mode of action of readthrough molecules.  
677 (4) None of the therapeutic approaches under development to correct nonsense mutations  
678 responsible for genetic diseases has yet come to fruition. However, the diversity of strategies  
679 explored makes the field of nonsense mutation correction highly dynamic. Step by step,  
680 personalized therapeutic strategies based on stop codon readthrough are taking form. They  
681 should, in the near future, yield treatments for genetic diseases caused by nonsense mutations.

682

### 683 **VIII. ACKNOWLEDGMENTS**

684 M.P. is supported by a grant from la Région Hauts-de-France and l'Université de Lille. F.L. is  
685 an Inserm researcher supported by funding from Vaincre la mucoviscidose, La Ligue Contre  
686 le Cancer and the GIP Cancéropôle Nord Ouest.

687

### 688 **IX. REFERENCES**

- 689 AGRIS, P. F., NARENDRAN, A., SARACHAN, K., VARE, V. Y. P. & ERUYAL, E. (2017). The importance of being  
690 modified: The role of rna modifications in translational fidelity. *Enzymes* **41**, 1-50.
- 691 ALARCON, C. R., GOODARZI, H., LEE, H., LIU, X., TAVAZOIE, S. & TAVAZOIE, S. F. (2015). Hnrnpa2b1 is a  
692 mediator of m(6)a-dependent nuclear rna processing events. *Cell* **162**(6), 1299-1308.
- 693 ANDREEV, D. E., O'CONNOR, P. B., ZHDANOV, A. V., DMITRIEV, R. I., SHATSKY, I. N., PAPKOVSKY, D. B. & BARANOV,  
694 P. V. (2015). Oxygen and glucose deprivation induces widespread alterations in mrna  
695 translation within 20 minutes. *Genome Biology* **16**, 90.
- 696 ARAKAWA, M., SHIOZUKA, M., NAKAYAMA, Y., HARA, T., HAMADA, M., KONDO, S., IKEDA, D., TAKAHASHI, Y.,  
697 SAWA, R., NONOMURA, Y., SHEYKHOESLAMI, K., KONDO, K., KAGA, K., KITAMURA, T., SUZUKI-MIYAGOE,  
698 Y., *et al.* (2003). Negamycin restores dystrophin expression in skeletal and cardiac muscles of  
699 mdx mice. *The Journal of Biochemistry* **134**(5), 751-758.
- 700 ATANASOVA, V. S., JIANG, Q., PRISCO, M., GRUBER, C., PINON HOFBAUER, J., CHEN, M., HAS, C., BRUCKNER-  
701 TUDERMAN, L., MCGRATH, J. A., UITTO, J. & SOUTH, A. P. (2017). Amlexanox enhances premature  
702 termination codon read-through in col7a1 and expression of full length type vii collagen:  
703 Potential therapy for recessive dystrophic epidermolysis bullosa. *Journal of Investigative*  
704 *Dermatology* **137**(9), 1842-1849.
- 705 BANNING, A., SCHIFF, M. & TIKKANEN, R. (2017). Amlexanox provides a potential therapy for nonsense  
706 mutations in the lysosomal storage disorder aspartylglucosaminuria. *Biochimica et Biophysica*  
707 *Acta* **1864**(3), 668-675.
- 708 BARADARAN-HERAVI, A., BALGI, A. D., ZIMMERMAN, C., CHOI, K., SHIDMOOSSAVEE, F. S., TAN, J. S., BERGEAUD, C.,  
709 KRAUSE, A., FLIBOTTE, S., SHIMIZU, Y., ANDERSON, H. J., MOULY, V., JAN, E., PFEIFER, T., JAQUITH, J. B.,

710 *et al.* (2016). Novel small molecules potentiate premature termination codon readthrough by  
711 aminoglycosides. *Nucleic Acids Research* **44**(14), 6583-6598.

712 BEDNAROVA, A., HANNA, M., DURHAM, I., VANCLEAVE, T., ENGLAND, A., CHAUDHURI, A. & KRISHNAN, N. (2017).  
713 Lost in translation: Defects in transfer rna modifications and neurological disorders. *Frontiers*  
714 *in Molecular Neuroscience* **10**, 135.

715 BEIER, H. & GRIMM, M. (2001). Misreading of termination codons in eukaryotes by natural nonsense  
716 suppressor trnas. *Nucleic Acids Research* **29**(23), 4767-4782.

717 BEISSEL, C., NEUMANN, B., UHSE, S., HAMPE, I., KARKI, P. & KREBBER, H. (2019). Translation termination  
718 depends on the sequential ribosomal entry of erf1 and erf3. *Nucleic Acids Research* **47**(9),  
719 4798-4813.

720 BENHABILES, H., GONZALEZ-HILARION, S., AMAND, S., BAILLY, C., PREVOTAT, A., REIX, P., HUBERT, D.,  
721 ADRIAENSSENS, E., REBUFFAT, S., TULASNE, D. & LEJEUNE, F. (2017). Optimized approach for the  
722 identification of highly efficient correctors of nonsense mutations in human diseases. *PLoS*  
723 *One* **12**(11), e0187930.

724 BERIAULT, V., CLEMENT, J. F., LEVESQUE, K., LEBEL, C., YONG, X., CHABOT, B., COHEN, E. A., COCHRANE, A. W.,  
725 RIGBY, W. F. & MOULAND, A. J. (2004). A late role for the association of hnrnp a2 with the hiv-1  
726 hnrnp a2 response elements in genomic rna, gag, and vpr localization. *The Journal of*  
727 *Biological Chemistry* **279**(42), 44141-44153.

728 BEZNOŠKOVA, P., WAGNER, S., JANSEN, M. E., VON DER HAAR, T. & VALASEK, L. S. (2015). Translation initiation  
729 factor eif3 promotes programmed stop codon readthrough. *Nucleic Acids Research* **43**(10),  
730 5099-5111.

731 BEZZERRI, V., API, M., ALLEGRI, M., FABRIZZI, B., COREY, S. J. & CIPOLLI, M. (2020). Nonsense suppression  
732 therapy: New hypothesis for the treatment of inherited bone marrow failure syndromes.  
733 *International Journal of Molecular Sciences* **21**(13), 10.3390/ijms21134672

734 BIDOU, L., HATIN, I., PEREZ, N., ALLAMAND, V., PANTHIER, J. J. & ROUSSET, J. P. (2004). Premature stop  
735 codons involved in muscular dystrophies show a broad spectrum of readthrough efficiencies  
736 in response to gentamicin treatment. *Gene Therapy* **11**(7), 619-627.

737 BLANCHET, S., CORNU, D., HATIN, I., GROSJEAN, H., BERTIN, P. & NAMY, O. (2018). Deciphering the reading of  
738 the genetic code by near-cognate trna. *Proceedings of the National Academy of Sciences of*  
739 *the United States of America* **115**(12), 3018-3023.

740 BONETTI, B., FU, L., MOON, J. & BEDWELL, D. M. (1995). The efficiency of translation termination is  
741 determined by a synergistic interplay between upstream and downstream sequences in  
742 *saccharomyces cerevisiae*. *Journal of Molecular Biology* **251**(3), 334-345.

743 BRASELL, E. J., CHU, L. L., AKPA, M. M., ESHKAR-OREN, I., ALROY, I., CORSINI, R., GILFIX, B. M., YAMANAKA, Y.,  
744 HUERTAS, P. & GOODYER, P. (2019). The novel aminoglycoside, elx-02, permits ctsw138x  
745 translational read-through and restores lysosomal cystine efflux in cystinosis. *PLoS One*  
746 **14**(12), e0223954.

747 BROWN, A., SHAO, S., MURRAY, J., HEGDE, R. S. & RAMAKRISHNAN, V. (2015). Structural basis for stop codon  
748 recognition in eukaryotes. *Nature* **524**(7566), 493-496.

749 BROWN, C. M., STOCKWELL, P. A., TROTMAN, C. N. & TATE, W. P. (1990). Sequence analysis suggests that  
750 tetra-nucleotides signal the termination of protein synthesis in eukaryotes. *Nucleic Acids*  
751 *Research* **18**(21), 6339-6345.

752 BURKE, J. F. & MOGG, A. E. (1985). Suppression of a nonsense mutation in mammalian cells in vivo by  
753 the aminoglycoside antibiotics g-418 and paromomycin. *Nucleic Acids Res* **13**(17), 6265-6272.

754 BUSHBY, K., FINKEL, R., WONG, B., BAROHN, R., CAMPBELL, C., COMI, G. P., CONNOLLY, A. M., DAY, J. W.,  
755 FLANIGAN, K. M., GOEMANS, N., JONES, K. J., MERCURI, E., QUINLIVAN, R., RENFROE, J. B., RUSSMAN, B.,  
756 *et al.* (2014). Ataluren treatment of patients with nonsense mutation dystrophinopathy.  
757 *Muscle Nerve* **50**(4), 477-487.

758 CARDNO, T. S., POOLE, E. S., MATHEW, S. F., GRAVES, R. & TATE, W. P. (2009). A homogeneous cell-based  
759 bicistronic fluorescence assay for high-throughput identification of drugs that perturb viral  
760 gene recoding and read-through of nonsense stop codons. *RNA* **15**(8), 1614-1621.

761 CARTER, A. P., CLEMONS, W. M., BRODERSEN, D. E., MORGAN-WARREN, R. J., WIMBERLY, B. T. & RAMAKRISHNAN,  
762 V. (2000). Functional insights from the structure of the 30s ribosomal subunit and its  
763 interactions with antibiotics. *Nature* **407**(6802), 340-348.

764 CASSAN, M. & ROUSSET, J. P. (2001). Uag readthrough in mammalian cells: Effect of upstream and  
765 downstream stop codon contexts reveal different signals. *BMC Molecular Biology* **2**, 3.

766 CHITTUM, H. S., LANE, W. S., CARLSON, B. A., ROLLER, P. P., LUNG, F. D., LEE, B. J. & HATFIELD, D. L. (1998).  
767 Rabbit beta-globin is extended beyond its uga stop codon by multiple suppressions and  
768 translational reading gaps. *Biochemistry* **37**(31), 10866-10870.

769 CLANCY, J. P., BEBOK, Z., RUIZ, F., KING, C., JONES, J., WALKER, L., GREER, H., HONG, J., WING, L., MACALUSO, M.,  
770 LYRENE, R., SORSCHER, E. J. & BEDWELL, D. M. (2001). Evidence that systemic gentamicin  
771 suppresses premature stop mutations in patients with cystic fibrosis. *American Journal of*  
772 *Respiratory and Critical Care Medicine* **163**(7), 1683-1692.

773 CORREA-CERRO, L. S., WASSIF, C. A., WAYE, J. S., KRAKOWIAK, P. A., COZMA, D., DOBSON, N. R., LEVIN, S. W.,  
774 ANADIOTIS, G., STEINER, R. D., KRAJEWSKA-WALASEK, M., NOWACZYK, M. J. & PORTER, F. D. (2005).  
775 Dhcr7 nonsense mutations and characterisation of mrna nonsense mediated decay in smith-  
776 lemli-opitz syndrome. *Journal of Medical Genetics* **42**(4), 350-357.

777 CRICK, F. H. (1966). Codon-anticodon pairing: The wobble hypothesis. *Journal of Molecular Biology*  
778 **19**(2), 548-555.

779 CUTTING, G. R., KASCH, L. M., ROSENSTEIN, B. J., TSUI, L. C., KAZAZIAN, H. H., JR. & ANTONARAKIS, S. E. (1990).  
780 Two patients with cystic fibrosis, nonsense mutations in each cystic fibrosis gene, and mild  
781 pulmonary disease. *New England Journal of Medicine* **323**(24), 1685-1689.

782 DABROWSKI, M., BUKOWY-BIERYLLO, Z. & ZIETKIEWICZ, E. (2018). Advances in therapeutic use of a drug-  
783 stimulated translational readthrough of premature termination codons. *Mol Med* **24**(1), 25.

784 DEVER, T. E., KINZY, T. G. & PAVITT, G. D. (2016). Mechanism and regulation of protein synthesis in  
785 *saccharomyces cerevisiae*. *Genetics* **203**(1), 65-107.

786 DU, L., DAMOISEAUX, R., NAHAS, S., GAO, K., HU, H., POLLARD, J. M., GOLDSTINE, J., JUNG, M. E., HENNING, S. M.,  
787 BERTONI, C. & GATTI, R. A. (2009). Nonaminoglycoside compounds induce readthrough of  
788 nonsense mutations. *Journal of Experimental Medicine* **206**(10), 2285-2297.

789 DU, L., JUNG, M. E., DAMOISEAUX, R., COMPLETEO, G., FIKE, F., KU, J. M., NAHAS, S., PIAO, C., HU, H. & GATTI, R.  
790 A. (2013). A new series of small molecular weight compounds induce read through of all  
791 three types of nonsense mutations in the atm gene. *Molecular Therapy* **21**(9), 1653-1660.

792 DUECHLER, M., LESZCZYNSKA, G., SOCHACKA, E. & NAWROT, B. (2016). Nucleoside modifications in the  
793 regulation of gene expression: Focus on trna. *Cellular and Molecular Life Sciences* **73**(16),  
794 3075-3095.

795 DUNN, J. G., FOO, C. K., BELLETIER, N. G., GAVIS, E. R. & WEISSMAN, J. S. (2013). Ribosome profiling reveals  
796 pervasive and regulated stop codon readthrough in drosophila melanogaster. *Elife* **2**, e01179.

797 ESWARAPPA, S. M., POTDAR, A. A., KOCH, W. J., FAN, Y., VASU, K., LINDNER, D., WILLARD, B., GRAHAM, L. M.,  
798 DICORLETO, P. E. & FOX, P. L. (2014). Programmed translational readthrough generates  
799 antiangiogenic vegf-ax. *Cell* **157**(7), 1605-1618.

800 FEARON, K., MCCLENDON, V., BONETTI, B. & BEDWELL, D. M. (1994). Premature translation termination  
801 mutations are efficiently suppressed in a highly conserved region of yeast ste6p, a member  
802 of the atp-binding cassette (abc) transporter family. *Journal of Biological Chemistry* **269**(27),  
803 17802-17808.

804 FENG, Y. X., COPELAND, T. D., OROSZLAN, S., REIN, A. & LEVIN, J. G. (1990). Identification of amino acids  
805 inserted during suppression of uaa and uga termination codons at the gag-pol junction of  
806 moloney murine leukemia virus. *Proceedings of the National Academy of Sciences of the*  
807 *United States of America* **87**(22), 8860-8863.

808 FERGUSON, M. W., GERAK, C. A. N., CHOW, C. C. T., RASTELLI, E. J., ELMORE, K. E., STAHL, F., HOSSEINI-  
809 FARAHABADI, S., BARADARAN-HERAVI, A., COLTART, D. M. & ROBERGE, M. (2019). The antimalarial  
810 drug mefloquine enhances tp53 premature termination codon readthrough by  
811 aminoglycoside g418. *PLoS One* **14**(5), e0216423.

812 FINKEL, R. S., FLANIGAN, K. M., WONG, B., BONNEMANN, C., SAMPSON, J., SWEENEY, H. L., REHA, A., NORTHCUTT,  
813 V. J., ELFRING, G., BARTH, J. & PELTZ, S. W. (2013). Phase 2a study of ataluren-mediated  
814 dystrophin production in patients with nonsense mutation duchenne muscular dystrophy.  
815 *PLoS One* **8**(12), e81302.

816 FIRTH, A. E., WILLS, N. M., GESTELAND, R. F. & ATKINS, J. F. (2011). Stimulation of stop codon readthrough:  
817 Frequent presence of an extended 3' rna structural element. *Nucleic Acids Research* **39**(15),  
818 6679-6691.

819 FLOQUET, C., HATIN, I., ROUSSET, J. P. & BIDOU, L. (2012). Statistical analysis of readthrough levels for  
820 nonsense mutations in mammalian cells reveals a major determinant of response to  
821 gentamicin. *PLoS Genetics* **8**(3), e1002608.

822 FORTIN, H., TOMASI, S., DELCROS, J. G., BANSARD, J. Y. & BOUSTIE, J. (2006). *In vivo* antitumor activity of  
823 clitocine, an exocyclic amino nucleoside isolated from *lepista inversa*. *ChemMedChem* **1**(2),  
824 189-196.

825 FREITAG, J., AST, J. & BOLKER, M. (2012). Cryptic peroxisomal targeting via alternative splicing and stop  
826 codon read-through in fungi. *Nature* **485**(7399), 522-525.

827 FRIESEN, W. J., TROTTA, C. R., TOMIZAWA, Y., ZHUO, J., JOHNSON, B., SIERRA, J., ROY, B., WEETALL, M., HEDRICK,  
828 J., SHEEDY, J., TAKASUGI, J., MOON, Y. C., BABU, S., BAIKAZITOV, R., LESZYK, J. D., *et al.* (2017). The  
829 nucleoside analog clitocine is a potent and efficacious readthrough agent. *RNA* **23**(4), 567-  
830 577.

831 GELLER, A. I. & RICH, A. (1980). A uga termination suppression trn<sup>trp</sup> active in rabbit reticulocytes.  
832 *Nature* **283**(5742), 41-46.

833 GOLDMANN, T., OVERLACK, N., MOLLER, F., BELAKHOV, V., VAN WYK, M., BAASOV, T., WOLFRUM, U. & NAGEL-  
834 WOLFRUM, K. (2012). A comparative evaluation of nb30, nb54 and ptc124 in translational  
835 read-through efficacy for treatment of an ush1c nonsense mutation. *EMBO Molecular*  
836 *Medicine* **4**(11), 1186-1199.

837 GOMEZ-GRAU, M., GARRIDO, E., COZAR, M., RODRIGUEZ-SUREDA, V., DOMINGUEZ, C., ARENAS, C., GATTI, R. A.,  
838 CORMAND, B., GRINBERG, D. & VILAGELIU, L. (2015). Evaluation of aminoglycoside and non-  
839 aminoglycoside compounds for stop-codon readthrough therapy in four lysosomal storage  
840 diseases. *PLoS One* **10**(8), e0135873.

841 GONZALEZ-HILARION, S., BEGHYN, T., JIA, J., DEBREUCK, N., BERTE, G., MAMCHAOU, K., MOULY, V., GRUENERT, D.  
842 C., DEPRez, B. & LEJEUNE, F. (2012). Rescue of nonsense mutations by amlexanox in human cells.  
843 *Orphanet Journal of Rare Diseases* **7**, 58.

844 GREENWOOD, G. J. (1959). Neomycin ototoxicity; report of a case. *AMA Arch Otolaryngol* **69**(4), 390-  
845 397.

846 GROSJEAN, H. & WESTHOF, E. (2016). An integrated, structure- and energy-based view of the genetic  
847 code. *Nucleic Acids Research* **44**(17), 8020-8040.

848 GROSS, T., SIEPMANN, A., STURM, D., WINDGASSEN, M., SCARCELLI, J. J., SEEDORF, M., COLE, C. N. & KREBBER, H.  
849 (2007). The dead-box rna helicase dbp5 functions in translation termination. *Science*  
850 **315**(5812), 646-649.

851 GUPTA, P. & LI, Y. R. (2018). Upf proteins: Highly conserved factors involved in nonsense mrna  
852 mediated decay. *Molecular Biology Reports* **45**(1), 39-55.

853 HAAS, M., VLCEK, V., BALABANOV, P., SALMONSON, T., BAKCHINE, S., MARKEY, G., WEISE, M., SCHLOSSER-WEBER,  
854 G., BROHMANN, H., YERRO, C. P., MENDIZABAL, M. R., STOYANOVA-BENINSKA, V. & HILLEGE, H. L.  
855 (2015). European medicines agency review of ataluren for the treatment of ambulant  
856 patients aged 5 years and older with duchenne muscular dystrophy resulting from a  
857 nonsense mutation in the dystrophin gene. *Neuromuscular Disorders* **25**(1), 5-13.

858 HAGERVALL, T. G., ERICSON, J. U., ESBERG, K. B., LI, J. N. & BJORK, G. R. (1990). Role of trna modification in  
859 translational fidelity. *Biochimica et Biophysica Acta* **1050**(1-3), 263-266.

860 HAMADA, K., OMURA, N., TAGUCHI, A., BARADARAN-HERAVI, A., KOTAKE, M., ARAI, M., TAKAYAMA, K., TANIGUCHI,  
861 A., ROBERGE, M. & HAYASHI, Y. (2019). New negamycin-based potent readthrough derivative  
862 effective against tga-type nonsense mutations. *ACS Medicinal Chemistry Letters* **10**(10),  
863 1450-1456.



864 HARGER, J. W. & DINMAN, J. D. (2004). Evidence against a direct role for the upf proteins in  
865 frameshifting or nonsense codon readthrough. *RNA* **10**(11), 1721-1729.

866 HARRELL, L., MELCHER, U. & ATKINS, J. F. (2002). Predominance of six different hexanucleotide recoding  
867 signals 3' of read-through stop codons. *Nucleic Acids Research* **30**(9), 2011-2017.

868 HE, F. & JACOBSON, A. (2015). Nonsense-mediated mrna decay: Degradation of defective transcripts is  
869 only part of the story. *Annual Review of Genetics* **49**, 339-366.

870 HECK, W. E., HINSHAW, H. C. & PARSONS, H. G. (1963). Auditory ototoxicity in tuberculosis patients  
871 treated with a report of the incidence of hearing loss in a series of 1,150 cases. *JAMA* **186**, 18-  
872 20.

873 HOCK, R. & ANDERSON, R. J. (1995). Prevention of drug-induced nephrotoxicity in the intensive care unit.  
874 *Journal of Critical Care* **10**(1), 33-43.

875 HOUCK-LOOMIS, B., DURNEY, M. A., SALGUERO, C., SHANKAR, N., NAGLE, J. M., GOFF, S. P. & D'SOUZA, V. M.  
876 (2011). An equilibrium-dependent retroviral mrna switch regulates translational recoding.  
877 *Nature* **480**(7378), 561-564.

878 HOWARD, M. T., SHIRTS, B. H., PETROS, L. M., FLANIGAN, K. M., GESTELAND, R. F. & ATKINS, J. F. (2000).  
879 Sequence specificity of aminoglycoside-induced stop codon readthrough: Potential  
880 implications for treatment of duchenne muscular dystrophy. *Annals of Neurology* **48**(2), 164-  
881 169.

882 IVANOV, P. V., GEHRING, N. H., KUNZ, J. B., HENTZE, M. W. & KULOZIK, A. E. (2008). Interactions between  
883 upf1, erfs, pabp and the exon junction complex suggest an integrated model for mammalian  
884 nmd pathways. *EMBO Journal* **27**(5), 736-747.

885 JIA, J., WERKMEISTER, E., GONZALEZ-HILARION, S., LEROY, C., GRUENERT, D. C., LAFONT, F., TULASNE, D. & LEJEUNE,  
886 F. (2017). Premature termination codon readthrough in human cells occurs in novel  
887 cytoplasmic foci and requires upf proteins. *Journal of Cell Science* **130**(18), 3009-3022.

888 JUNGREIS, I., CHAN, C. S., WATERHOUSE, R. M., FIELDS, G., LIN, M. F. & KELLIS, M. (2016). Evolutionary  
889 dynamics of abundant stop codon readthrough. *Molecular Biology and Evolution* **33**(12),  
890 3108-3132.

891 KAPUR, M., MONAGHAN, C. E. & ACKERMAN, S. L. (2017). Regulation of mrna translation in neurons-a  
892 matter of life and death. *Neuron* **96**(3), 616-637.

893 KEELING, K. M., SALAS-MARCO, J., OSHEROVICH, L. Z. & BEDWELL, D. M. (2006). Tpa1p is part of an mrnp  
894 complex that influences translation termination, mrna deadenylation, and mrna turnover in  
895 *saccharomyces cerevisiae*. *Molecular and Cellular Biology* **26**(14), 5237-5248.

896 KEREM, B. S., ZIELENSKI, J., MARKIEWICZ, D., BOZON, D., GAZIT, E., YAHAV, J., KENNEDY, D., RIORDAN, J. R.,  
897 COLLINS, F. S., ROMMENS, J. M. & TSUI, L.C. (1990). Identification of mutations in regions  
898 corresponding to the two putative nucleotide (atp)-binding folds of the cystic fibrosis gene.  
899 *Proceedings of the National Academy of Sciences of the United States of America* **87**(21),  
900 8447-8451.

901 KEREM, E., HIRAWAT, S., ARMONI, S., YAAKOV, Y., SHOSEYOV, D., COHEN, M., NISSIM-RAFINIA, M., BLAU, H.,  
902 RIVLIN, J., AVIRAM, M., ELFRING, G. L., NORTH CUTT, V. J., MILLER, L. L., KEREM, B. & WILSCHANSKI, M.  
903 (2008). Effectiveness of ptc124 treatment of cystic fibrosis caused by nonsense mutations: A  
904 prospective phase ii trial. *The Lancet* **372**(9640), 719-727.

905 KEREM, E., KONSTAN, M. W., DE BOECK, K., ACCURSO, F. J., SERMET-GAUDELUS, I., WILSCHANSKI, M., ELBORN, J.  
906 S., MELOTTI, P., BRONSVELD, I., FAJAC, I., MALFROOT, A., ROSENBLUTH, D. B., WALKER, P. A., MCCOLLEY,  
907 S. A., KNOOP, C., *et al.* (2014). Ataluren for the treatment of nonsense-mutation cystic fibrosis:  
908 A randomised, double-blind, placebo-controlled phase 3 trial. *The Lancet Respiratory*  
909 *Medicine* **2**(7), 539-547.

910 KIERZEK, E., MALGOWSKA, M., LISOWIEC, J., TURNER, D. H., GDANIEC, Z. & KIERZEK, R. (2014). The contribution  
911 of pseudouridine to stabilities and structure of rnas. *Nucleic Acids Research* **42**(5), 3492-3501.

912 KLEPPE, A. S. & BORNBERG-BAUER, E. (2018). Robustness by intrinsically disordered c-termini and  
913 translational readthrough. *Nucleic Acids Research* **46**(19), 10184-10194.

914 KONG, R., LASKIN, O. L., KAUSHIK, D., JIN, F., MA, J., MCINTOSH, J., SOUZA, M. & ALMSTEAD, N. (2019).  
915 Ataluren pharmacokinetics in healthy japanese and caucasian subjects. *Clinical Pharmacology*  
916 *in Drug Development* **8**(2), 172-178.

917 KUBO, I., KIM, M., WOOD, W. F. & NAOKI, H. (1986). Clitocine, a new insecticidal nucleoside from the  
918 mushroom *clitocybe inversa*. *Tetrahedron Letters* **27**, 4277-4280.

919 KUROSAKI, T. & MAQUAT, L. E. (2016). Nonsense-mediated mrna decay in humans at a glance. *Journal of*  
920 *Cell Science* **129**(3), 461-467.

921 KUZMIAK, H. A. & MAQUAT, L. E. (2006). Applying nonsense-mediated mrna decay research to the clinic:  
922 Progress and challenges. *Trends in Molecular Medicine* **12**(7), 306-316.

923 LABUNSKYY, V. M., HATFIELD, D. L. & GLADYSHEV, V. N. (2014). Selenoproteins: Molecular pathways and  
924 physiological roles. *Physiological Reviews* **94**(3), 739-777.

925 LEE, H. L. & DOUGHERTY, J. P. (2012). Pharmaceutical therapies to recode nonsense mutations in  
926 inherited diseases. *Pharmacology & Therapeutics* **136**(2), 227-266.

927 LEJEUNE, F. (2017). Nonsense-mediated mrna decay at the crossroads of many cellular pathways. *BMB*  
928 *Reports* **50**, 175-185.

929 LEJEUNE, F. & MAQUAT, L. E. (2005). Mechanistic links between nonsense-mediated mrna decay and  
930 pre-mrna splicing in mammalian cells. *Current Opinion in Cell Biology* **17**(3), 309-315.

931 LEUBITZ, A., FRYDMAN-MAROM, A., SHARPE, N., VAN DUZER, J., CAMPBELL, K. C. M. & VANHOUTTE, F. (2019).  
932 Safety, tolerability, and pharmacokinetics of single ascending doses of elx-02, a potential  
933 treatment for genetic disorders caused by nonsense mutations, in healthy volunteers. *Clinical*  
934 *Pharmacology in Drug Development* **8**(8), 984-994.

935 LEVESQUE, K., HALVORSEN, M., ABRAHAMYAN, L., CHATEL-CHAIX, L., POUPON, V., GORDON, H., DESGROSEILLERS,  
936 L., GATIGNOL, A. & MOULAND, A. J. (2006). Trafficking of hiv-1 rna is mediated by heterogeneous  
937 nuclear ribonucleoprotein a2 expression and impacts on viral assembly. *Traffic* **7**(9), 1177-  
938 1193.

939 LI, Y., WANG, X., LI, C., HU, S., YU, J. & SONG, S. (2014). Transcriptome-wide n(6)-methyladenosine  
940 profiling of rice callus and leaf reveals the presence of tissue-specific competitors involved in  
941 selective mrna modification. *RNA Biology* **11**(9), 1180-1188.

942 LIN, M. F., CARLSON, J. W., CROSBY, M. A., MATTHEWS, B. B., YU, C., PARK, S., WAN, K. H., SCHROEDER, A. J.,  
943 GRAMATES, L. S., ST PIERRE, S. E., ROARK, M., WILEY, K. L., JR., KULATHINAL, R. J., ZHANG, P., MYRICK, K.  
944 V., *et al.* (2007). Revisiting the protein-coding gene catalog of drosophila melanogaster using  
945 12 fly genomes. *Genome Research* **17**(12), 1823-1836.

946 LINDE, L., BOELZ, S., NISSIM-RAFINIA, M., OREN, Y. S., WILSCHANSKI, M., YAACOV, Y., VIRGILIS, D., NEU-YILIK, G.,  
947 KULOZIK, A. E., KEREM, E. & KEREM, B. (2007). Nonsense-mediated mrna decay affects nonsense  
948 transcript levels and governs response of cystic fibrosis patients to gentamicin. *Journal of*  
949 *Clinical Investigation* **117**(3), 683-692.

950 LOENARZ, C., SEKIRNIK, R., THALHAMMER, A., GE, W., SPIVAKOVSKY, E., MACKEEN, M. M., McDONOUGH, M. A.,  
951 COCKMAN, M. E., KESSLER, B. M., RATCLIFFE, P. J., WOLF, A. & SCHOFIELD, C. J. (2014). Hydroxylation  
952 of the eukaryotic ribosomal decoding center affects translational accuracy. *Proceedings of*  
953 *the National Academy of Sciences of the United States of America* **111**(11), 4019-4024.

954 LOUGHRAN, G., CHOU, M. Y., IVANOV, I. P., JUNGREIS, I., KELLIS, M., KIRAN, A. M., BARANOV, P. V. & ATKINS, J. F.  
955 (2014). Evidence of efficient stop codon readthrough in four mammalian genes. *Nucleic Acids*  
956 *Research* **42**(14), 8928-8938.

957 LOUGHRAN, G., JUNGREIS, I., TZANI, I., POWER, M., DMITRIEV, R. I., IVANOV, I. P., KELLIS, M. & ATKINS, J. F.  
958 (2018). Stop codon readthrough generates a c-terminally extended variant of the human  
959 vitamin d receptor with reduced calcitriol response. *The Journal of Biological Chemistry*  
960 **293**(12), 4434-4444.

961 LUECK, J. D., YOON, J. S., PERALES-PUCHALT, A., MACKEY, A. L., INFELD, D. T., BEHLKE, M. A., POPE, M. R.,  
962 WEINER, D. B., SKACH, W. R., MCCRAY, P. B., JR. & AHERN, C. A. (2019). Engineered transfer rnas  
963 for suppression of premature termination codons. *Nature Communications* **10**(1), 822.

964 MALIK, V., RODINO-KLAPAC, L. R., VIOLLET, L., WALL, C., KING, W., AL-DAHAK, R., LEWIS, S., SHILLING, C. J.,  
965 KOTA, J., SERRANO-MUNUERA, C., HAYES, J., MAHAN, J. D., CAMPBELL, K. J., BANWELL, B., DASOUKI, M.,

966 *et al.* (2010). Gentamicin-induced readthrough of stop codons in duchenne muscular  
967 dystrophy. *Annals of Neurology* **67**(6), 771-780.

968 MANUVAKHOVA, M., KEELING, K. & BEDWELL, D. M. (2000). Aminoglycoside antibiotics mediate context-  
969 dependent suppression of termination codons in a mammalian translation system. *RNA* **6**(7),  
970 1044-1055.

971 MARTORELL, L., CORTINA, V., PARRA, R., BARQUINERO, J. & VIDAL, F. (2020). Variable readthrough  
972 responsiveness of nonsense mutations in hemophilia a. *Haematologica* **105**(2), 508-518.

973 MATZ, G. J. (1993). Aminoglycoside cochlear ototoxicity. *Otolaryngologic Clinics of North America*  
974 **26**(5), 705-712.

975 McCAUGHAN, K. K., BROWN, C. M., DALPHIN, M. E., BERRY, M. J. & TATE, W. P. (1995). Translational  
976 termination efficiency in mammals is influenced by the base following the stop codon.  
977 *Proceedings of the National Academy of Sciences of the United States of America* **92**(12),  
978 5431-5435.

979 MERRICK, W. C. (1992). Mechanism and regulation of eukaryotic protein synthesis. *Microbiological*  
980 *Reviews* **56**(2), 291-315.

981 MEYER, K. D., SALETORE, Y., ZUMBO, P., ELEMENTO, O., MASON, C. E. & JAFFREY, S. R. (2012). Comprehensive  
982 analysis of mrna methylation reveals enrichment in 3' utrs and near stop codons. *Cell* **149**(7),  
983 1635-1646.

984 MIKHAILOVA, T., SHUVALOVA, E., IVANOV, A., SUSOROV, D., SHUVALOV, A., KOLOSOV, P. M. & ALKALAEVA, E.  
985 (2017). Rna helicase ddx19 stabilizes ribosomal elongation and termination complexes.  
986 *Nucleic Acids Research* **45**(3), 1307-1318.

987 MINGEOT-LECLERCQ, M. P. & TULKENS, P. M. (1999). Aminoglycosides: Nephrotoxicity. *Antimicrobial*  
988 *Agents and Chemotherapy* **43**(5), 1003-1012.

989 MOOSAJEE, M., TRACEY-WHITE, D., SMART, M., WEETALL, M., TORRIANO, S., KALATZIS, V., DA CRUZ, L., COFFEY, P.,  
990 WEBSTER, A. R. & WELCH, E. (2016). Functional rescue of rep1 following treatment with ptc124  
991 and novel derivative ptc-414 in human choroideremia fibroblasts and the nonsense-  
992 mediated zebrafish model. *Human Molecular Genetics* **25**(16), 3416-3431.

993 MORAIS, P., ADACHI, H. & YU, Y. T. (2020). Suppression of nonsense mutations by new emerging  
994 technologies. *International Journal of Molecular Sciences* **21**(12), 4394).

995 MORI, N., FUNATSU, Y., HIRUTA, K. & GOTO, S. (1985). Analysis of translational fidelity of ribosomes with  
996 protamine messenger rna as a template. *Biochemistry* **24**(5), 1231-1239.

997 MORT, M., IVANOV, D., COOPER, D. N. & CHUZHANOVA, N. A. (2008). A meta-analysis of nonsense  
998 mutations causing human genetic disease. *Human Mutation* **29**(8), 1037-1047.

999 MURAMATSU, T., HECKMANN, K., KITANAKA, C. & KUCHINO, Y. (2001). Molecular mechanism of stop codon  
1000 recognition by erf1: A wobble hypothesis for peptide anticodons. *FEBS Lett* **488**(3), 105-109.

1001 MUTYAM, V., DU, M., XUE, X., KEELING, K. M., WHITE, E. L., BOSTWICK, J. R., RASMUSSEN, L., LIU, B., MAZUR, M.,  
1002 HONG, J. S., FALK LIBBY, E., LIANG, F., SHANG, H., MENSE, M., SUTO, M. J., *et al.* (2016). Discovery of  
1003 clinically approved agents that promote suppression of cystic fibrosis transmembrane  
1004 conductance regulator nonsense mutations. *American Journal of Respiratory and Critical*  
1005 *Care Medicine* **194**(9), 1092-1103.

1006 NAMGOONG, J. H. & BERTONI, C. (2016). Clinical potential of ataluren in the treatment of duchenne  
1007 muscular dystrophy. *Degenerative Neurological and Neuromuscular Disease* **13**(6), 37-48.

1008 NAMY, O., HATIN, I. & ROUSSET, J. P. (2001). Impact of the six nucleotides downstream of the stop codon  
1009 on translation termination. *EMBO reports* **2**(9), 787-793.

1010 NEU-YILIK, G., RAIMONDEAU, E., ELISEEV, B., YERAMALA, L., AMTHOR, B., DENIAUD, A., HUARD, K., KERSCHGENS, K.,  
1011 HENTZE, M. W., SCHAFFITZEL, C. & KULOZIK, A. E. (2017). Dual function of upf3b in early and late  
1012 translation termination. *EMBO Journal* **36**(20), 2968-2986.

1013 NUDELMAN, I., REBIBO-SABBAH, A., CHERNIAVSKY, M., BELAKHOV, V., HAINRICHSON, M., CHEN, F., SCHACHT, J.,  
1014 PILCH, D. S., BEN-YOSEF, T. & BAASOV, T. (2009). Development of novel aminoglycoside (nb54)  
1015 with reduced toxicity and enhanced suppression of disease-causing premature stop  
1016 mutations. *Journal of Medicinal Chemistry* **52**(9), 2836-2845.

1017 NUDELMAN, I., REBIBO-SABBAH, A., SHALLOM-SHEZIFI, D., HAINRICHSON, M., STAHL, I., BEN-YOSEF, T. & BAASOV, T.  
1018 (2006). Redesign of aminoglycosides for treatment of human genetic diseases caused by  
1019 premature stop mutations. *Bioorganic & Medicinal Chemistry Letters* **16**(24), 6310-6315.

1020 OGLE, J. M., CARTER, A. P. & RAMAKRISHNAN, V. (2003). Insights into the decoding mechanism from  
1021 recent ribosome structures. *Trends in Biochemical Sciences* **28**(5), 259-266.

1022 PHILLIPS-JONES, M. K., WATSON, F. J. & MARTIN, R. (1993). The 3' codon context effect on uag suppressor  
1023 trna is different in escherichia coli and human cells. *Journal of Molecular Biology* **233**(1), 1-6.

1024 PIBIRI, I., LENTINI, L., MELFI, R., TUTONE, M., BALDASSANO, S., RICCO GALLUZZO, P., DI LEONARDO, A. & PACE, A.  
1025 (2018). Rescuing the cftr protein function: Introducing 1,3,4-oxadiazoles as translational  
1026 readthrough inducing drugs. *European Journal of Medicinal Chemistry* **159**, 126-142.

1027 PROKHOROVA, I., ALTMAN, R. B., DJUMAGULOV, M., SHRESTHA, J. P., URZHUMTSEV, A., FERGUSON, A., CHANG, C.  
1028 T., YUSUPOV, M., BLANCHARD, S. C. & YUSUPOVA, G. (2017). Aminoglycoside interactions and  
1029 impacts on the eukaryotic ribosome. *Proceedings of the National Academy of Sciences of the*  
1030 *United States of America* **114**(51), E10899-E10908.

1031 RABEA, S. M., BARADARAN-HERAVI, A., BALGI, A. D., KRAUSE, A., HOSSEINI FARAHABADI, S., ROBERGE, M. &  
1032 GRIERSON, D. S. (2019). 2-aminothiazole-4-carboxamides enhance readthrough of premature  
1033 termination codons by aminoglycosides. *ACS Medicinal Chemistry Letters* **10**(5), 726-731.

1034 RAJON, E. & MASEL, J. (2011). Evolution of molecular error rates and the consequences for evolvability.  
1035 *Proceedings of the National Academy of Sciences of the United States of America* **108**(3),  
1036 1082-1087.

1037 REN, G., ZHAO, Y. P., YANG, L. & FU, C. X. (2008). Anti-proliferative effect of clitocine from the mushroom  
1038 *leucopaxillus giganteus* on human cervical cancer hela cells by inducing apoptosis. *Cancer*  
1039 *Letters* **262**(2), 190-200.

1040 ROY, B., FRIESEN, W. J., TOMIZAWA, Y., LESZYK, J. D., ZHUO, J., JOHNSON, B., DAKKA, J., TROTTA, C. R., XUE, X.,  
1041 MUTYAM, V., KEELING, K. M., MOBLEY, J. A., ROWE, S. M., BEDWELL, D. M., WELCH, E. M., *et al.*  
1042 (2016). Ataluren stimulates ribosomal selection of near-cognate trnas to promote nonsense  
1043 suppression. *Proceedings of the National Academy of Sciences of the United States of*  
1044 *America* **113**(44), 12508-12513.

1045 ROY, B., LESZYK, J. D., MANGUS, D. A. & JACOBSON, A. (2015). Nonsense suppression by near-cognate trnas  
1046 employs alternative base pairing at codon positions 1 and 3. *Proceedings of the National*  
1047 *Academy of Sciences of the United States of America* **112**(10), 3038-3043.

1048 SALAS-MARCO, J. & BEDWELL, D. M. (2005). Discrimination between defects in elongation fidelity and  
1049 termination efficiency provides mechanistic insights into translational readthrough. *Journal*  
1050 *of Molecular Biology* **348**(4), 801-815.

1051 SCHUEREN, F., LINGNER, T., GEORGE, R., HOFHUIS, J., DICKEL, C., GARTNER, J. & THOMS, S. (2014). Peroxisomal  
1052 lactate dehydrogenase is generated by translational readthrough in mammals. *Elife* **3**,  
1053 e03640.

1054 SERMET-GAUDELUS, I., BOECK, K. D., CASIMIR, G. J., VERMEULEN, F., LEAL, T., MOGENET, A., ROUSSEL, D., FRITSCH,  
1055 J., HANSENS, L., HIRAWAT, S., MILLER, N. L., CONSTANTINE, S., REHA, A., AJAYI, T., ELFRING, G. L., *et al.*  
1056 (2010). Ataluren (ptc124) induces cystic fibrosis transmembrane conductance regulator  
1057 protein expression and activity in children with nonsense mutation cystic fibrosis. *American*  
1058 *Journal of Respiratory and Critical Care Medicine* **182**(10), 1262-1272.

1059 SERMET-GAUDELUS, I., RENOUIL, M., FAJAC, A., BIDOU, L., PARBAILLE, B., PIERROT, S., DAVY, N., BISMUTH, E.,  
1060 REINERT, P., LENOIR, G., LESURE, J. F., ROUSSET, J. P. & EDELMAN, A. (2007). In vitro prediction of  
1061 stop-codon suppression by intravenous gentamicin in patients with cystic fibrosis: A pilot  
1062 study. *BMC Medicine* **5**, 5.

1063 SHARMA, J., KEELING, K. M. & ROWE, S. M. (2020). Pharmacological approaches for targeting cystic  
1064 fibrosis nonsense mutations. *European Journal of Medicinal Chemistry* **200**, 112436.

1065 SHULMAN, E., BELAKHOV, V., WEI, G., KENDALL, A., MEYRON-HOLTZ, E. G., BEN-SHACHAR, D., SCHACHT, J. &  
1066 BAASOV, T. (2014). Designer aminoglycosides that selectively inhibit cytoplasmic rather than  
1067 mitochondrial ribosomes show decreased ototoxicity: A strategy for the treatment of genetic  
1068 diseases. *The Journal of Biological Chemistry* **289**(4), 2318-2330.

1069 SINGH, A., MANJUNATH, L. E., KUNDU, P., SAHOO, S., DAS, A., SUMA, H. R., FOX, P. L. & ESWARAPPA, S. M.  
1070 (2019). Let-7a-regulated translational readthrough of mammalian ago1 generates a microrna  
1071 pathway inhibitor. *EMBO Journal* **38**(16), e100727.

1072 SINGH, A., URSIC, D. & DAVIES, J. (1979). Phenotypic suppression and misreading *saccharomyces*  
1073 *cerevisiae*. *Nature* **277**(5692), 146-148.

1074 SKUZESKI, J. M., NICHOLS, L. M., GESTELAND, R. F. & ATKINS, J. F. (1991). The signal for a leaky uag stop  
1075 codon in several plant viruses includes the two downstream codons. *Journal of Molecular*  
1076 *Biology* **218**(2), 365-373.

1077 SOGAARD, T. M., JAKOBSEN, C. G. & JUSTESEN, J. (1999). A sensitive assay of translational fidelity  
1078 (readthrough and termination) in eukaryotic cells. *Biochemistry (Mosc)* **64**(12), 1408-1417.

1079 SONG, H., MUGNIER, P., DAS, A. K., WEBB, H. M., EVANS, D. R., TUIITE, M. F., HEMMINGS, B. A. & BARFORD, D.  
1080 (2000). The crystal structure of human eukaryotic release factor erf1--mechanism of stop  
1081 codon recognition and peptidyl-trna hydrolysis. *Cell* **100**(3), 311-321.

1082 SUN, J., YEUNG, C. A., CO, N. N., TSANG, T. Y., YAU, E., LUO, K., WU, P., WA, J. C., FUNG, K. P., KWOK, T. T. & LIU,  
1083 F. (2012). Clitocine reversal of p-glycoprotein associated multi-drug resistance through down-  
1084 regulation of transcription factor nf-kappab in r-hepg2 cell line. *PLoS One* **7**(8), e40720.

1085 SWAN, S. K. (1997). Aminoglycoside nephrotoxicity. *Seminars in Nephrology* **17**(1), 27-33.

1086 TAGUCHI, A., HAMADA, K., KOTAKE, M., SHIOZUKA, M., NAKAMINAMI, H., PILLAIYAR, T., TAKAYAMA, K., YAKUSHIJI,  
1087 F., NOGUCHI, N., USUI, T., MATSUDA, R. & HAYASHI, Y. (2014). Discovery of natural products  
1088 possessing selective eukaryotic readthrough activity: 3-epi-deoxynegamycin and its leucine  
1089 adduct. *ChemMedChem* **9**(10), 2233-2237.

1090 TATE, W. P. & MANNERING, S. A. (1996). Three, four or more: The translational stop signal at length.  
1091 *Molecular Microbiology* **21**(2), 213-219.

1092 TEMPLE, G. F., DOZY, A. M., ROY, K. L. & KAN, Y. W. (1982). Construction of a functional human  
1093 suppressor trna gene: An approach to gene therapy for beta-thalassaemia. *Nature* **296**(5857),  
1094 537-540.

1095 TRZASKA, C., AMAND, S., BAILLY, C., LEROY, C., MARCHAND, V., DUVERNOIS-BERTHET, E., SALIOU, J. M.,  
1096 BENHABILES, H., WERKMEISTER, E., CHASSAT, T., GUILBERT, R., HANNEBIQUE, D., MOURAY, A., COPIN, M.  
1097 C., MOREAU, P. A., *et al.* (2020). 2,6-diaminopurine as a highly potent corrector of uga  
1098 nonsense mutations. *Nature Communications* **11**(1), 1509.

1099 TUTONE, M., PIBIRI, I., LENTINI, L., PACE, A. & ALMERICO, A. M. (2019). Deciphering the nonsense  
1100 readthrough mechanism of action of ataluren: An in silico compared study. *ACS Medicinal*  
1101 *Chemistry Letters* **10**(4), 522-527.

1102 TUTONE, M., PIBIRI, I., PERRIERA, R., CAMPOFELICE, A., CULLETTA, G., MELFI, R., PACE, A., ALMERICO, A. M. &  
1103 LENTINI, L. (2020). Pharmacophore-based design of new chemical scaffolds as translational  
1104 readthrough-inducing drugs (trids). *ACS Medicinal Chemistry Letters* **11**(5), 747-753.

1105 URAKOV, V. N., VALOUEV, I. A., LEWITIN, E. I., PAUSHKIN, S. V., KOSORUKOV, V. S., KUSHNIROV, V. V., SMIRNOV, V.  
1106 N. & TER-AVANESYAN, M. D. (2001). Itt1p, a novel protein inhibiting translation termination in  
1107 *saccharomyces cerevisiae*. *BMC Molecular Biology* **2**, 9.

1108 WAGNER, K. R., HAMED, S., HADLEY, D. W., GROPMAN, A. L., BURSTEIN, A. H., ESCOLAR, D. M., HOFFMAN, E. P. &  
1109 FISCHBECK, K. H. (2001). Gentamicin treatment of duchenne and becker muscular dystrophy  
1110 due to nonsense mutations. *Annals of Neurology* **49**(6), 706-711.

1111 WANG, D., BELAKHOV, V., KANDASAMY, J., BAASOV, T., LI, S. C., LI, Y. T., BEDWELL, D. M. & KEELING, K. M.  
1112 (2012). The designer aminoglycoside nb84 significantly reduces glycosaminoglycan  
1113 accumulation associated with mps i-h in the idua-w392x mouse. *Molecular Genetics and*  
1114 *Metabolism* **105**(1), 116-125.

1115 WANG, W., CZAPLINSKI, K., RAO, Y. & PELTZ, S. W. (2001). The role of upf proteins in modulating the  
1116 translation read-through of nonsense-containing transcripts. *EMBO Journal* **20**(4), 880-890.

1117 WANGEN, J. R. & GREEN, R. (2020). Stop codon context influences genome-wide stimulation of  
1118 termination codon readthrough by aminoglycosides. *Elife* **9**, e52611.

- 1119 WELCH, E. M., BARTON, E. R., ZHUO, J., TOMIZAWA, Y., FRIESEN, W. J., TRIFILLIS, P., PAUSHKIN, S., PATEL, M.,  
 1120 TROTTA, C. R., HWANG, S., WILDE, R. G., KARP, G., TAKASUGI, J., CHEN, G., JONES, S., *et al.* (2007).  
 1121 Ptc124 targets genetic disorders caused by nonsense mutations. *Nature* **447**(7140), 87-91.  
 1122 WILDE, R. G., KENNEDY, P. D., ALMSTEAD, N. G., WELCH, E. M., TAKASUGI, J. J. & FRIESEN, W. J. (2007).  
 1123 Nucleoside compounds and their use for treating cancer and diseases associated with somatic  
 1124 mutations. In *USPTO (Ed.)*, vol. 7291603B2 (ed. U. S. Patent). PTC Therapeutics, Inc, USA.
- 1125 WILHELM, J. M., JESSOP, J. J. & PETTITT, S. E. (1978). Aminoglycoside antibiotics and eukaryotic protein  
 1126 synthesis: Stimulation of errors in the translation of natural messengers in extracts of  
 1127 cultured human cells. *Biochemistry* **17**(7), 1149-1153.
- 1128 WILLS, N. M., GESTELAND, R. F. & ATKINS, J. F. (1991). Evidence that a downstream pseudoknot is  
 1129 required for translational read-through of the moloney murine leukemia virus gag stop  
 1130 codon. *Proceedings of the National Academy of Sciences of the United States of America*  
 1131 **88**(16), 6991-6995.
- 1132 WITTENSTEIN, A., CASPI, M., DAVID, Y., SHORER, Y., NADAR-PONNIAH, P. T. & ROSIN-ARBESFELD, R. (2019).  
 1133 Serum starvation enhances nonsense mutation readthrough. *Journal of Molecular Medicine*  
 1134 (*Berl*) **97**(12), 1695-1710.
- 1135 WU, G., HUANG, C. & YU, Y. T. (2015). Pseudouridine in mrna: Incorporation, detection, and recoding.  
 1136 *Methods in Enzymology* **560**, 187-217.
- 1137 WU, W. J., SHA, S. H., MCLAREN, J. D., KAWAMOTO, K., RAPHAEL, Y. & SCHACHT, J. (2001). Aminoglycoside  
 1138 ototoxicity in adult cba, c57bl and balb mice and the sprague-dawley rat. *Hearing Research*  
 1139 **158**(1-2), 165-178.
- 1140 XUE, X., MUTYAM, V., TANG, L., BISWAS, S., DU, M., JACKSON, L. A., DAI, Y., BELAKHOV, V., SHALEV, M., CHEN, F.,  
 1141 SCHACHT, J., R, J. B., BAASOV, T., HONG, J., BEDWELL, D. M., *et al.* (2014). Synthetic  
 1142 aminoglycosides efficiently suppress cystic fibrosis transmembrane conductance regulator  
 1143 nonsense mutations and are enhanced by ivacaftor. *American Journal of Respiratory Cell and*  
 1144 *Molecular Biology* **50**(4), 805-816.
- 1145 XUE, X., MUTYAM, V., THAKERAR, A., MOBLEY, J., BRIDGES, R. J., ROWE, S. M., KEELING, K. M. & BEDWELL, D. M.  
 1146 (2017). Identification of the amino acids inserted during suppression of cftr nonsense  
 1147 mutations and determination of their functional consequences. *Human Molecular Genetics*  
 1148 **26**(16), 3116-3129.
- 1149 YAMAGUCHI, Y., HAYASHI, A., CAMPAGNONI, C. W., KIMURA, A., INUZUKA, T. & BABA, H. (2012). L-mpz, a novel  
 1150 isoform of myelin p0, is produced by stop codon readthrough. *The Journal of Biological*  
 1151 *Chemistry* **287**(21), 17765-17776.
- 1152 YESMIN, F., BHUIYAN, R. H., OHMI, Y., OHKAWA, Y., TAJIMA, O., OKAJIMA, T. & FURUKAWA, K. (2020).  
 1153 Aminoglycosides are efficient reagents to induce readthrough of premature termination  
 1154 codon in mutant b4galnt1 genes found in families of hereditary spastic paraplegia. *The*  
 1155 *Journal of Biochemistry* **168**(2), 103-112.
- 1156 ZINGMAN, L. V., PARK, S., OLSON, T. M., ALEKSEEV, A. E. & TERZIC, A. (2007). Aminoglycoside-induced  
 1157 translational read-through in disease: Overcoming nonsense mutations by pharmacogenetic  
 1158 therapy. *Clinical Pharmacology & Therapeutics* **81**(1), 99-103.

1159

## 1160 Figure Legends

1161 **Fig. 1.** Comparison of translation termination and stop codon readthrough mechanisms. (A)

1162 Translation termination. When the ribosome reaches a stop codon, in most cases the

1163 translation termination complex is recruited. When the A site of the ribosome covers a stop

1164 codon, eRF3 and ABCE1 interact with the ribosome. ABCE1 recruits the Dbp5/DDX19-eRF1  
1165 complex, likely with the help of PABPC1. Dbp5/DDX19 then hydrolyses ATP to ADP,  
1166 promoting its own departure. The energy supplied by eRF3-driven GTP hydrolysis favours a  
1167 change in the conformational structure of eRF1, with subsequent dissociation of the  
1168 synthesised peptide. ABCE1-driven ATP hydrolysis supplies energy for the release of the  
1169 ribosome, which is then recycled. (B) Stop codon readthrough. At a very low rate or under  
1170 certain conditions (drugs, eIF3, regulatory elements), a near-cognate tRNA, rather than the  
1171 translation termination complex, is recruited when the ribosome reaches a stop codon. The  
1172 elongation factor EF-Tu allows polymerization of the peptide, with GTP hydrolysis leading to  
1173 continuation of translation to the next stop codon. ABCE1, ATP-binding cassette sub-family  
1174 E member 1; DDX19, DEAD-box helicase 19B; EF-Tu, elongation factor thermo unstable;  
1175 eIF3, eukaryotic initiation factor 3; eRF, eukaryotic release factor; GGQ, glycine-glycine-  
1176 glutamine motif; mRNA, messenger RNA; PABP, polyA-binding protein; tRNA, transfer  
1177 RNA.

1178

1179 **Fig. 2.** Different types of stop codon readthrough. (A) Non-programmed translational  
1180 readthrough. This type of readthrough occurs at a basal level without any regulatory elements  
1181 and without the presence of readthrough molecules. This readthrough happens at premature  
1182 termination codons (PTCs) and physiological stop codons at a very low rate. (B) Programmed  
1183 translational readthrough. Some mRNAs are subject to readthrough of the physiological stop  
1184 codon, due to the presence of regulatory elements. This type of readthrough is more efficient  
1185 than that shown in A. (C) Induced translational readthrough. The presence of molecules  
1186 (aminoglycosides or non-aminoglycosides) promotes activation of readthrough. This type of  
1187 readthrough is more efficient than that shown in A. DDX19, DEAD-box helicase 19B; eRF,

1188 eukaryotic release factor; GGQ, glycine-glycine-glutamine motif; mRNA, messenger RNA;  
1189 PABP, polyA-binding protein; tRNA, transfer RNA; UTR, untranslated region.

1190

1191 **Fig. 3.** Strategies used to screen molecules for readthrough-promoting activity. (A) A  
1192 premature termination codon (PTC) is introduced into a cDNA encoding an enzyme or a  
1193 fluorescent protein. Measurement of the enzymatic activity or fluorescence related to this  
1194 protein will indicate that readthrough has occurred. (B) Use of two cDNAs encoding an  
1195 enzymatic activity or a fluorescence separated by a stop codon. The product of the first cDNA  
1196 is used to normalize the signal. Measurement of the product of the second cDNA will indicate  
1197 that readthrough has occurred. (C) Screening system with an intron introduced into a cDNA to  
1198 promote a splicing event. The PTC is introduced more than 55 nucleotides upstream of the  
1199 intronic sequence to activate nonsense-mediated mRNA decay (NMD) of the corresponding  
1200 mRNA.

1201

1202 Table 1. Aminoglycoside molecules with known readthrough activity.

1203 Table 2. Non-aminoglycoside molecules with known readthrough activity.

1204 Table 3. Readthrough potentiator molecules.

1205

1206

1207

1208