Deciphering the molecular mechanism of stop codon readthrough

Martine Palma and Fabrice Lejeune*

*Univ. Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, UMR9020 – UMR 1277 -
CANTHER – Cancer Heterogeneity, Plasticity and Resistance to Therapies, F-59000 Lille,
France*

*Author for correspondence (E-mail: fabrice.lejeune@inserm.fr; Tel.: 33-320871059).

ABSTRACT

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

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

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	I. INTRODUCTION
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,

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

II. TRANSLATION TERMINATION

Translation is a process that promotes amino acid polymerization leading to a peptide sequence based on an mRNA sequence. The ribosome carries out translation with the help of cofactors, ensuring accurate decoding of the open reading frame (ORF) (Dever, Kinzy & Pavitt, 2016; Merrick, 1992; Kapur, Monaghan & Ackerman, 2017). Misincorporation of an amino acid occurs at a very low rate. For example, misincorporation of a lysine instead of an arginine during translation of protamine mRNA has been estimated at only 0.06% to 0.2% (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^{[Ser]Sec}, 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

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

III. DIFFERENT TYPES OF STOP CODON READTHROUGH

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

(1) Non-programmed translational readthrough

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

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

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

(2) Programmed translational readthrough

The second type of readthrough is called programmed translational readthrough (Fig. 2B). It targets specific mRNAs (Loughran *et al.*, 2018; Freitag, Ast & Bolker, 2012; Dunn *et al.*, 2013) and is a proteome-expanding mechanism allowing synthesis of specific protein isoforms with particular functions. It is thus a way to synthesize two isoforms of a protein 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

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

(3) Induced translational readthrough

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

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

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

IV. PARAMETERS INFLUENCING STOP CODON READTHROUGH

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

(1) Cis elements activating stop codon readthrough

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

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

(2) Trans elements activating readthrough

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

(a) Proteins and RNAs

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

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

As readthrough opposes translation termination and *vice versa*, it is not surprising that loss of 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

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

(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.

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.*, 2012). It seems, however, that this is not an absolute rule, as some readthrough activators
482 with no NMD-inhibiting action promote greater synthesis of full-length proteins than do dual-
483 action molecules. For instance, the readthrough activators *Lepista flaccida* extract H7 and 2,6-
484 diaminopurine, which do not inhibit NMD, correct UGA and UAA (extract H7) or UGA only
485 (2,6-diaminopurine) more effectively than dual-action G418 (Benhabiles *et al.*, 2017; Trzaska
486 *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

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

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

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

VI. NEW DEVELOPMENTS AND THERAPEUTIC TARGETS

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

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

Studies aiming to increase the effectiveness of readthrough compounds have identified some molecules that potentiate the readthrough activity of aminoglycosides (Table 3). Interestingly, these molecules do not promote readthrough by themselves. For example, the phthalimide derivative CDX5-1 increases about 180-fold the readthrough efficiency of G418 in both yeast and human cells (Baradaran-Heravi *et al.*, 2016). More recently discovered potentiators of aminoglycosides include the antimalarial mefloquine (Ferguson *et al.*, 2019) and 2-aminothiazole-4-carboxamides (Rabea *et al.*, 2019). How these molecules improve the readthrough efficiency of aminoglycosides is unclear, but by making it possible to administer lower doses of aminoglycosides, they should reduce aminoglycoside-linked toxic effects. As the use of screening systems to identify molecules with readthrough activity takes time and money, new targeted approaches are being or could be developed to promote efficient readthrough. One such approach is based on the mechanism of action of 2,6-diaminopurine, recently shown to promote readthrough of UGA premature stop codons by interfering with the

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

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

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

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

VII. CONCLUSIONS

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

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

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

essential to fill the gaps in our knowledge of the molecular events leading to stop codon readthrough and its regulation. This should be accomplished by studying the molecular mechanism of stop codon recognition and the mode of action of readthrough molecules.

(4) None of the therapeutic approaches under development to correct nonsense mutations responsible for genetic diseases has yet come to fruition. However, the diversity of strategies explored makes the field of nonsense mutation correction highly dynamic. Step by step, personalized therapeutic strategies based on stop codon readthrough are taking form. They should, in the near future, yield treatments for genetic diseases caused by nonsense mutations.

VIII. ACKNOWLEDGMENTS

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

IX. REFERENCES

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

- et al. (2016). Novel small molecules potentiate premature termination codon readthrough by aminoglycosides. *Nucleic Acids Research* **44**(14), 6583-6598.
- BEDNAROVA, A., HANNA, M., DURHAM, I., VANCLEAVE, T., ENGLAND, A., CHAUDHURI, A. & KRISHNAN, N. (2017). Lost in translation: Defects in transfer rna modifications and neurological disorders. *Frontiers in Molecular Neuroscience* **10**, 135.
- BEIER, H. & GRIMM, M. (2001). Misreading of termination codons in eukaryotes by natural nonsense suppressor trnas. *Nucleic Acids Research* **29**(23), 4767-4782.
- BEISSEL, C., NEUMANN, B., UHSE, S., HAMPE, I., KARKI, P. & KREBBER, H. (2019). Translation termination depends on the sequential ribosomal entry of erf1 and erf3. *Nucleic Acids Research* **47**(9), 4798-4813.
- BENHABILES, H., GONZALEZ-HILARION, S., AMAND, S., BAILLY, C., PREVOTAT, A., REIX, P., HUBERT, D., ADRIAENSSENS, E., REBUFFAT, S., TULASNE, D. & LEJEUNE, F. (2017). Optimized approach for the identification of highly efficient correctors of nonsense mutations in human diseases. *PLoS One* **12**(11), e0187930.
- BERIAULT, V., CLEMENT, J. F., LEVESQUE, K., LEBEL, C., YONG, X., CHABOT, B., COHEN, E. A., COCHRANE, A. W., RIGBY, W. F. & MOULAND, A. J. (2004). A late role for the association of hnrnp a2 with the hiv-1 hnrnp a2 response elements in genomic rna, gag, and vpr localization. *The Journal of Biological Chemistry* **279**(42), 44141-44153.
- BEZNOSKOVA, P., WAGNER, S., JANSEN, M. E., VON DER HAAR, T. & VALASEK, L. S. (2015). Translation initiation factor eif3 promotes programmed stop codon readthrough. *Nucleic Acids Research* **43**(10), 5099-5111.
- BEZZERRI, V., API, M., ALLEGRI, M., FABRIZZI, B., COREY, S. J. & CIPOLLI, M. (2020). Nonsense suppression therapy: New hypothesis for the treatment of inherited bone marrow failure syndromes. *International Journal of Molecular Sciences* **21**(13), 10.3390/ijms21134672
- BIDOU, L., HATIN, I., PEREZ, N., ALLAMAND, V., PANTHIER, J. J. & ROUSSET, J. P. (2004). Premature stop codons involved in muscular dystrophies show a broad spectrum of readthrough efficiencies in response to gentamicin treatment. *Gene Therapy* **11**(7), 619-627.
- BLANCHET, S., CORNU, D., HATIN, I., GROSJEAN, H., BERTIN, P. & NAMY, O. (2018). Deciphering the reading of the genetic code by near-cognate trna. *Proceedings of the National Academy of Sciences of the United States of America* **115**(12), 3018-3023.
- BONETTI, B., FU, L., MOON, J. & BEDWELL, D. M. (1995). The efficiency of translation termination is determined by a synergistic interplay between upstream and downstream sequences in *saccharomyces cerevisiae*. *Journal of Molecular Biology* **251**(3), 334-345.
- BRASELL, E. J., CHU, L. L., AKPA, M. M., ESHKAR-OREN, I., ALROY, I., CORSINI, R., GILFIX, B. M., YAMANAKA, Y., HUERTAS, P. & GOODYER, P. (2019). The novel aminoglycoside, elx-02, permits ctsw138x translational read-through and restores lysosomal cystine efflux in cystinosis. *PLoS One* **14**(12), e0223954.
- BROWN, A., SHAO, S., MURRAY, J., HEGDE, R. S. & RAMAKRISHNAN, V. (2015). Structural basis for stop codon recognition in eukaryotes. *Nature* **524**(7566), 493-496.
- BROWN, C. M., STOCKWELL, P. A., TROTMAN, C. N. & TATE, W. P. (1990). Sequence analysis suggests that tetra-nucleotides signal the termination of protein synthesis in eukaryotes. *Nucleic Acids Research* **18**(21), 6339-6345.
- BURKE, J. F. & MOGG, A. E. (1985). Suppression of a nonsense mutation in mammalian cells in vivo by the aminoglycoside antibiotics g-418 and paromomycin. *Nucleic Acids Res* **13**(17), 6265-6272.
- BUSHBY, K., FINKEL, R., WONG, B., BAROHN, R., CAMPBELL, C., COMI, G. P., CONNOLLY, A. M., DAY, J. W., FLANIGAN, K. M., GOEMANS, N., JONES, K. J., MERCURI, E., QUINLIVAN, R., RENFROE, J. B., RUSSMAN, B., et al. (2014). Ataluren treatment of patients with nonsense mutation dystrophinopathy. *Muscle Nerve* **50**(4), 477-487.
- CARDNO, T. S., POOLE, E. S., MATHEW, S. F., GRAVES, R. & TATE, W. P. (2009). A homogeneous cell-based bicistronic fluorescence assay for high-throughput identification of drugs that perturb viral gene recoding and read-through of nonsense stop codons. *RNA* **15**(8), 1614-1621.

- CARTER, A. P., CLEMONS, W. M., BRODERSEN, D. E., MORGAN-WARREN, R. J., WIMBERLY, B. T. & RAMAKRISHNAN, V. (2000). Functional insights from the structure of the 30s ribosomal subunit and its interactions with antibiotics. *Nature* **407**(6802), 340-348.
- CASSAN, M. & ROUSSET, J. P. (2001). Uag readthrough in mammalian cells: Effect of upstream and downstream stop codon contexts reveal different signals. *BMC Molecular Biology* **2**, 3.
- CHITTUM, H. S., LANE, W. S., CARLSON, B. A., ROLLER, P. P., LUNG, F. D., LEE, B. J. & HATFIELD, D. L. (1998). Rabbit beta-globin is extended beyond its uga stop codon by multiple suppressions and translational reading gaps. *Biochemistry* **37**(31), 10866-10870.
- CLANCY, J. P., BEBOK, Z., RUIZ, F., KING, C., JONES, J., WALKER, L., GREER, H., HONG, J., WING, L., MACALUSO, M., LYRENE, R., SORSCHER, E. J. & BEDWELL, D. M. (2001). Evidence that systemic gentamicin suppresses premature stop mutations in patients with cystic fibrosis. *American Journal of Respiratory and Critical Care Medicine* **163**(7), 1683-1692.
- CORREA-CERRO, L. S., WASSIF, C. A., WAYE, J. S., KRAKOWIAK, P. A., COZMA, D., DOBSON, N. R., LEVIN, S. W., ANADIOTIS, G., STEINER, R. D., KRAJEWSKA-WALASEK, M., NOWACZYK, M. J. & PORTER, F. D. (2005). Dhcr7 nonsense mutations and characterisation of mrna nonsense mediated decay in smith-lemli-opitz syndrome. *Journal of Medical Genetics* **42**(4), 350-357.
- CRICK, F. H. (1966). Codon-anticodon pairing: The wobble hypothesis. *Journal of Molecular Biology* **19**(2), 548-555.
- CUTTING, G. R., KASCH, L. M., ROSENSTEIN, B. J., TSUI, L. C., KAZAZIAN, H. H., JR. & ANTONARAKIS, S. E. (1990). Two patients with cystic fibrosis, nonsense mutations in each cystic fibrosis gene, and mild pulmonary disease. *New England Journal of Medicine* **323**(24), 1685-1689.
- DABROWSKI, M., BUKOWY-BIERYLLO, Z. & ZIETKIEWICZ, E. (2018). Advances in therapeutic use of a drug-stimulated translational readthrough of premature termination codons. *Mol Med* **24**(1), 25.
- DEVER, T. E., KINZY, T. G. & PAVITT, G. D. (2016). Mechanism and regulation of protein synthesis in *saccharomyces cerevisiae*. *Genetics* **203**(1), 65-107.
- DU, L., DAMOISEAUX, R., NAHAS, S., GAO, K., HU, H., POLLARD, J. M., GOLDSTINE, J., JUNG, M. E., HENNING, S. M., BERTONI, C. & GATTI, R. A. (2009). Nonaminoglycoside compounds induce readthrough of nonsense mutations. *Journal of Experimental Medicine* **206**(10), 2285-2297.
- DU, L., JUNG, M. E., DAMOISEAUX, R., COMPLETO, G., FIKE, F., KU, J. M., NAHAS, S., PIAO, C., HU, H. & GATTI, R. A. (2013). A new series of small molecular weight compounds induce read through of all three types of nonsense mutations in the atm gene. *Molecular Therapy* **21**(9), 1653-1660.
- DUECHLER, M., LESZCZYNSKA, G., SOCHACKA, E. & NAWROT, B. (2016). Nucleoside modifications in the regulation of gene expression: Focus on trna. *Cellular and Molecular Life Sciences* **73**(16), 3075-3095.
- DUNN, J. G., FOO, C. K., BELLETIER, N. G., GAVIS, E. R. & WEISSMAN, J. S. (2013). Ribosome profiling reveals pervasive and regulated stop codon readthrough in *drosophila melanogaster*. *Elife* **2**, e01179.
- ESWARAPPA, S. M., POTDAR, A. A., KOCH, W. J., FAN, Y., VASU, K., LINDNER, D., WILLARD, B., GRAHAM, L. M., DICORLETO, P. E. & FOX, P. L. (2014). Programmed translational readthrough generates antiangiogenic vegf-ax. *Cell* **157**(7), 1605-1618.
- FEARON, K., MCCLENDON, V., BONETTI, B. & BEDWELL, D. M. (1994). Premature translation termination mutations are efficiently suppressed in a highly conserved region of yeast ste6p, a member of the atp-binding cassette (abc) transporter family. *Journal of Biological Chemistry* **269**(27), 17802-17808.
- FENG, Y. X., COPELAND, T. D., OROSZLAN, S., REIN, A. & LEVIN, J. G. (1990). Identification of amino acids inserted during suppression of uaa and uga termination codons at the gag-pol junction of moloney murine leukemia virus. *Proceedings of the National Academy of Sciences of the United States of America* **87**(22), 8860-8863.
- FERGUSON, M. W., GERAKE, C. A. N., CHOW, C. C. T., RASTELLI, E. J., ELMORE, K. E., STAHL, F., HOSSEINI-FARAHABADI, S., BARADARAN-HERAVI, A., COLTART, D. M. & ROBERGE, M. (2019). The antimalarial drug mefloquine enhances tp53 premature termination codon readthrough by aminoglycoside g418. *PLoS One* **14**(5), e0216423.

- FINKEL, R. S., FLANIGAN, K. M., WONG, B., BONNEMANN, C., SAMPSON, J., SWEENEY, H. L., REHA, A., NORTHCUTT, V. J., ELFRING, G., BARTH, J. & PELTZ, S. W. (2013). Phase 2a study of ataluren-mediated dystrophin production in patients with nonsense mutation duchenne muscular dystrophy. *PLoS One* **8**(12), e81302.
- FIRTH, A. E., WILLS, N. M., GESTELAND, R. F. & ATKINS, J. F. (2011). Stimulation of stop codon readthrough: Frequent presence of an extended 3' rna structural element. *Nucleic Acids Research* **39**(15), 6679-6691.
- FLOQUET, C., HATIN, I., ROUSSET, J. P. & BIDOU, L. (2012). Statistical analysis of readthrough levels for nonsense mutations in mammalian cells reveals a major determinant of response to gentamicin. *PLoS Genetics* **8**(3), e1002608.
- FORTIN, H., TOMASI, S., DELCROS, J. G., BANSARD, J. Y. & BOUSTIE, J. (2006). *In vivo* antitumor activity of clitocine, an exocyclic amino nucleoside isolated from *lepista inversa*. *ChemMedChem* **1**(2), 189-196.
- FREITAG, J., AST, J. & BOLKER, M. (2012). Cryptic peroxisomal targeting via alternative splicing and stop codon read-through in fungi. *Nature* **485**(7399), 522-525.
- FRIESEN, W. J., TROTTE, C. R., TOMIZAWA, Y., ZHUO, J., JOHNSON, B., SIERRA, J., ROY, B., WEETALL, M., HEDRICK, J., SHEEDY, J., TAKASUGI, J., MOON, Y. C., BABU, S., BAIKAZITOV, R., LESZYK, J. D., *et al.* (2017). The nucleoside analog clitocine is a potent and efficacious readthrough agent. *RNA* **23**(4), 567-577.
- GELLER, A. I. & RICH, A. (1980). A uga termination suppression trnatrp active in rabbit reticulocytes. *Nature* **283**(5742), 41-46.
- GOLDMANN, T., OVERLACK, N., MOLLER, F., BELAKHOV, V., VAN WYK, M., BAASOV, T., WOLFRUM, U. & NAGEL-WOLFRUM, K. (2012). A comparative evaluation of nb30, nb54 and ptc124 in translational read-through efficacy for treatment of an ush1c nonsense mutation. *EMBO Molecular Medicine* **4**(11), 1186-1199.
- GOMEZ-GRAU, M., GARRIDO, E., COZAR, M., RODRIGUEZ-SUREDA, V., DOMINGUEZ, C., ARENAS, C., GATTI, R. A., CORMAND, B., GRINBERG, D. & VILAGELIU, L. (2015). Evaluation of aminoglycoside and non-aminoglycoside compounds for stop-codon readthrough therapy in four lysosomal storage diseases. *PLoS One* **10**(8), e0135873.
- GONZALEZ-HILARION, S., BEGHYN, T., JIA, J., DEBREUCK, N., BERGE, G., MAMCHAOU, K., MOULY, V., GRUENERT, D. C., DEPREZ, B. & LEJEUNE, F. (2012). Rescue of nonsense mutations by amlexanox in human cells. *Orphanet Journal of Rare Diseases* **7**, 58.
- GREENWOOD, G. J. (1959). Neomycin ototoxicity; report of a case. *AMA Arch Otolaryngol* **69**(4), 390-397.
- GROSJEAN, H. & WESTHOF, E. (2016). An integrated, structure- and energy-based view of the genetic code. *Nucleic Acids Research* **44**(17), 8020-8040.
- GROSS, T., SIEPMANN, A., STURM, D., WINDGASSEN, M., SCARCELLI, J. J., SEEDORF, M., COLE, C. N. & KREBBER, H. (2007). The dead-box rna helicase dbp5 functions in translation termination. *Science* **315**(5812), 646-649.
- GUPTA, P. & LI, Y. R. (2018). Upf proteins: Highly conserved factors involved in nonsense mrna mediated decay. *Molecular Biology Reports* **45**(1), 39-55.
- HAAS, M., VLCEK, V., BALABANOV, P., SALMONSON, T., BAKCHINE, S., MARKEY, G., WEISE, M., SCHLOSSER-WEBER, G., BROHMANN, H., YERRO, C. P., MENDIZABAL, M. R., STOYANOVA-BENINSKA, V. & HILLEGE, H. L. (2015). European medicines agency review of ataluren for the treatment of ambulant patients aged 5 years and older with duchenne muscular dystrophy resulting from a nonsense mutation in the dystrophin gene. *Neuromuscular Disorders* **25**(1), 5-13.
- HAGERVALL, T. G., ERICSON, J. U., ESBERG, K. B., LI, J. N. & BJORK, G. R. (1990). Role of trna modification in translational fidelity. *Biochimica et Biophysica Acta* **1050**(1-3), 263-266.
- HAMADA, K., OMURA, N., TAGUCHI, A., BARADARAN-HERAVI, A., KOTAKE, M., ARAI, M., TAKAYAMA, K., TANIGUCHI, A., ROBERGE, M. & HAYASHI, Y. (2019). New negamycin-based potent readthrough derivative effective against tga-type nonsense mutations. *ACS Medicinal Chemistry Letters* **10**(10), 1450-1456.

- HARGER, J. W. & DINMAN, J. D. (2004). Evidence against a direct role for the upf proteins in frameshifting or nonsense codon readthrough. *RNA* **10**(11), 1721-1729.
- HARRELL, L., MELCHER, U. & ATKINS, J. F. (2002). Predominance of six different hexanucleotide recoding signals 3' of read-through stop codons. *Nucleic Acids Research* **30**(9), 2011-2017.
- HE, F. & JACOBSON, A. (2015). Nonsense-mediated mrna decay: Degradation of defective transcripts is only part of the story. *Annual Review of Genetics* **49**, 339-366.
- HECK, W. E., HINSHAW, H. C. & PARSONS, H. G. (1963). Auditory ototoxicity in tuberculosis patients treated with a report of the incidence of hearing loss in a series of 1,150 cases. *JAMA* **186**, 18-20.
- HOCK, R. & ANDERSON, R. J. (1995). Prevention of drug-induced nephrotoxicity in the intensive care unit. *Journal of Critical Care* **10**(1), 33-43.
- HOUCK-LOOMIS, B., DURNEY, M. A., SALGUERO, C., SHANKAR, N., NAGLE, J. M., GOFF, S. P. & D'SOUZA, V. M. (2011). An equilibrium-dependent retroviral mrna switch regulates translational recoding. *Nature* **480**(7378), 561-564.
- HOWARD, M. T., SHIRTS, B. H., PETROS, L. M., FLANIGAN, K. M., GESTELAND, R. F. & ATKINS, J. F. (2000). Sequence specificity of aminoglycoside-induced stop condon readthrough: Potential implications for treatment of duchenne muscular dystrophy. *Annals of Neurology* **48**(2), 164-169.
- IVANOV, P. V., GEHRING, N. H., KUNZ, J. B., HENTZE, M. W. & KULOZIK, A. E. (2008). Interactions between upf1, erfs, pabp and the exon junction complex suggest an integrated model for mammalian nmd pathways. *EMBO Journal* **27**(5), 736-747.
- JIA, J., WERKMEISTER, E., GONZALEZ-HILARION, S., LEROY, C., GRUENERT, D. C., LAFONT, F., TULASNE, D. & LEJEUNE, F. (2017). Premature termination codon readthrough in human cells occurs in novel cytoplasmic foci and requires upf proteins. *Journal of Cell Science* **130**(18), 3009-3022.
- JUNGREIS, I., CHAN, C. S., WATERHOUSE, R. M., FIELDS, G., LIN, M. F. & KELLIS, M. (2016). Evolutionary dynamics of abundant stop codon readthrough. *Molecular Biology and Evolution* **33**(12), 3108-3132.
- KAPUR, M., MONAGHAN, C. E. & ACKERMAN, S. L. (2017). Regulation of mrna translation in neurons-a matter of life and death. *Neuron* **96**(3), 616-637.
- KEELING, K. M., SALAS-MARCO, J., OSHEROVICH, L. Z. & BEDWELL, D. M. (2006). Tpa1p is part of an mrnp complex that influences translation termination, mrna deadenylation, and mrna turnover in *saccharomyces cerevisiae*. *Molecular and Cellular Biology* **26**(14), 5237-5248.
- KEREM, B. S., ZIELENSKI, J., MARKIEWICZ, D., BOZON, D., GAZIT, E., YAHAV, J., KENNEDY, D., RIORDAN, J. R., COLLINS, F. S., ROMMENS, J. M. & TSUI, L.C. (1990). Identification of mutations in regions corresponding to the two putative nucleotide (atp)-binding folds of the cystic fibrosis gene. *Proceedings of the National Academy of Sciences of the United States of America* **87**(21), 8447-8451.
- KEREM, E., HIRAWAT, S., ARMONI, S., YAAKOV, Y., SHOSEYOV, D., COHEN, M., NISSIM-RAFINIA, M., BLAU, H., RIVLIN, J., AVIRAM, M., ELFRING, G. L., NORTH CUTT, V. J., MILLER, L. L., KEREM, B. & WILSCHANSKI, M. (2008). Effectiveness of ptc124 treatment of cystic fibrosis caused by nonsense mutations: A prospective phase ii trial. *The Lancet* **372**(9640), 719-727.
- KEREM, E., KONSTAN, M. W., DE BOECK, K., ACCURSO, F. J., SERMET-GAUDELUS, I., WILSCHANSKI, M., ELBORN, J. S., MELOTTI, P., BRONSVELD, I., FAJAC, I., MALFROOT, A., ROSENBLUTH, D. B., WALKER, P. A., MCCOLLEY, S. A., KNOOP, C., *et al.* (2014). Ataluren for the treatment of nonsense-mutation cystic fibrosis: A randomised, double-blind, placebo-controlled phase 3 trial. *The Lancet Respiratory Medicine* **2**(7), 539-547.
- KIERZEK, E., MALGOWSKA, M., LISOWIEC, J., TURNER, D. H., GDANIEC, Z. & KIERZEK, R. (2014). The contribution of pseudouridine to stabilities and structure of rnas. *Nucleic Acids Research* **42**(5), 3492-3501.
- KLEPPE, A. S. & BORNBERG-BAUER, E. (2018). Robustness by intrinsically disordered c-termini and translational readthrough. *Nucleic Acids Research* **46**(19), 10184-10194.

- KONG, R., LASKIN, O. L., KAUSHIK, D., JIN, F., MA, J., MCINTOSH, J., SOUZA, M. & ALMSTEAD, N. (2019). Ataluren pharmacokinetics in healthy japanese and caucasian subjects. *Clinical Pharmacology in Drug Development* **8**(2), 172-178.
- KUBO, I., KIM, M., WOOD, W. F. & NAOKI, H. (1986). Clitocine, a new insecticidal nucleoside from the mushroom *clitocybe inversa*. *Tetrahedron Letters* **27**, 4277-4280.
- KUROSAKI, T. & MAQUAT, L. E. (2016). Nonsense-mediated mrna decay in humans at a glance. *Journal of Cell Science* **129**(3), 461-467.
- KUZMIAK, H. A. & MAQUAT, L. E. (2006). Applying nonsense-mediated mrna decay research to the clinic: Progress and challenges. *Trends in Molecular Medicine* **12**(7), 306-316.
- LABUNSKYY, V. M., HATFIELD, D. L. & GLADYSHEV, V. N. (2014). Selenoproteins: Molecular pathways and physiological roles. *Physiological Reviews* **94**(3), 739-777.
- LEE, H. L. & DOUGHERTY, J. P. (2012). Pharmaceutical therapies to recode nonsense mutations in inherited diseases. *Pharmacology & Therapeutics* **136**(2), 227-266.
- LEJEUNE, F. (2017). Nonsense-mediated mrna decay at the crossroads of many cellular pathways. *BMB Reports* **50**, 175-185.
- LEJEUNE, F. & MAQUAT, L. E. (2005). Mechanistic links between nonsense-mediated mrna decay and pre-mrna splicing in mammalian cells. *Current Opinion in Cell Biology* **17**(3), 309-315.
- LEUBITZ, A., FRYDMAN-MAROM, A., SHARPE, N., VAN DUZER, J., CAMPBELL, K. C. M. & VANHOUTTE, F. (2019). Safety, tolerability, and pharmacokinetics of single ascending doses of elx-02, a potential treatment for genetic disorders caused by nonsense mutations, in healthy volunteers. *Clinical Pharmacology in Drug Development* **8**(8), 984-994.
- LEVESQUE, K., HALVORSEN, M., ABRAHAMIAN, L., CHATEL-CHAIX, L., POUPON, V., GORDON, H., DESGROSEILLERS, L., GATIGNOL, A. & MOULAND, A. J. (2006). Trafficking of hiv-1 rna is mediated by heterogeneous nuclear ribonucleoprotein a2 expression and impacts on viral assembly. *Traffic* **7**(9), 1177-1193.
- LI, Y., WANG, X., LI, C., HU, S., YU, J. & SONG, S. (2014). Transcriptome-wide n(6)-methyladenosine profiling of rice callus and leaf reveals the presence of tissue-specific competitors involved in selective mrna modification. *RNA Biology* **11**(9), 1180-1188.
- LIN, M. F., CARLSON, J. W., CROSBY, M. A., MATTHEWS, B. B., YU, C., PARK, S., WAN, K. H., SCHROEDER, A. J., GRAMATES, L. S., ST PIERRE, S. E., ROARK, M., WILEY, K. L., JR., KULATHINAL, R. J., ZHANG, P., MYRICK, K. V., *et al.* (2007). Revisiting the protein-coding gene catalog of drosophila melanogaster using 12 fly genomes. *Genome Research* **17**(12), 1823-1836.
- LINDE, L., BOELZ, S., NISSIM-RAFINIA, M., OREN, Y. S., WILSCHANSKI, M., YAACOV, Y., VIRGILIS, D., NEU-YILIK, G., KULOZIK, A. E., KEREM, E. & KEREM, B. (2007). Nonsense-mediated mrna decay affects nonsense transcript levels and governs response of cystic fibrosis patients to gentamicin. *Journal of Clinical Investigation* **117**(3), 683-692.
- LOENARZ, C., SEKIRNIK, R., THALHAMMER, A., GE, W., SPIVAKOVSKY, E., MACKEEN, M. M., McDONOUGH, M. A., COCKMAN, M. E., KESSLER, B. M., RATCLIFFE, P. J., WOLF, A. & SCHOFIELD, C. J. (2014). Hydroxylation of the eukaryotic ribosomal decoding center affects translational accuracy. *Proceedings of the National Academy of Sciences of the United States of America* **111**(11), 4019-4024.
- LOUGHRAN, G., CHOU, M. Y., IVANOV, I. P., JUNGREIS, I., KELLIS, M., KIRAN, A. M., BARANOV, P. V. & ATKINS, J. F. (2014). Evidence of efficient stop codon readthrough in four mammalian genes. *Nucleic Acids Research* **42**(14), 8928-8938.
- LOUGHRAN, G., JUNGREIS, I., TZANI, I., POWER, M., DMITRIEV, R. I., IVANOV, I. P., KELLIS, M. & ATKINS, J. F. (2018). Stop codon readthrough generates a c-terminally extended variant of the human vitamin d receptor with reduced calcitriol response. *The Journal of Biological Chemistry* **293**(12), 4434-4444.
- LUECK, J. D., YOON, J. S., PERALES-PUCHALT, A., MACKEY, A. L., INFELD, D. T., BEHLKE, M. A., POPE, M. R., WEINER, D. B., SKACH, W. R., MCCRAY, P. B., JR. & AHERN, C. A. (2019). Engineered transfer rnas for suppression of premature termination codons. *Nature Communications* **10**(1), 822.
- MALIK, V., RODINO-KLAPAC, L. R., VIOLLET, L., WALL, C., KING, W., AL-DAHAK, R., LEWIS, S., SHILLING, C. J., KOTA, J., SERRANO-MUNUERA, C., HAYES, J., MAHAN, J. D., CAMPBELL, K. J., BANWELL, B., DASOUKI, M.,

- et al. (2010). Gentamicin-induced readthrough of stop codons in duchenne muscular dystrophy. *Annals of Neurology* **67**(6), 771-780.
- MANUVAKHOVA, M., KEELING, K. & BEDWELL, D. M. (2000). Aminoglycoside antibiotics mediate context-dependent suppression of termination codons in a mammalian translation system. *RNA* **6**(7), 1044-1055.
- MARTORELL, L., CORTINA, V., PARRA, R., BARQUINERO, J. & VIDAL, F. (2020). Variable readthrough responsiveness of nonsense mutations in hemophilia a. *Haematologica* **105**(2), 508-518.
- MATZ, G. J. (1993). Aminoglycoside cochlear ototoxicity. *Otolaryngologic Clinics of North America* **26**(5), 705-712.
- MCCAUGHAN, K. K., BROWN, C. M., DALPHIN, M. E., BERRY, M. J. & TATE, W. P. (1995). Translational termination efficiency in mammals is influenced by the base following the stop codon. *Proceedings of the National Academy of Sciences of the United States of America* **92**(12), 5431-5435.
- MERRICK, W. C. (1992). Mechanism and regulation of eukaryotic protein synthesis. *Microbiological Reviews* **56**(2), 291-315.
- MEYER, K. D., SALETORRE, Y., ZUMBO, P., ELEMENTO, O., MASON, C. E. & JAFFREY, S. R. (2012). Comprehensive analysis of mrna methylation reveals enrichment in 3' utrs and near stop codons. *Cell* **149**(7), 1635-1646.
- MIKHAILOVA, T., SHUVALOVA, E., IVANOV, A., SUSOROV, D., SHUVALOV, A., KOLOSOV, P. M. & ALKALAEVA, E. (2017). Rna helicase ddx19 stabilizes ribosomal elongation and termination complexes. *Nucleic Acids Research* **45**(3), 1307-1318.
- MINGEOT-LECLERCQ, M. P. & TULKENS, P. M. (1999). Aminoglycosides: Nephrotoxicity. *Antimicrobial Agents and Chemotherapy* **43**(5), 1003-1012.
- MOOSAJEE, M., TRACEY-WHITE, D., SMART, M., WEETALL, M., TORRIANO, S., KALATZIS, V., DA CRUZ, L., COFFEY, P., WEBSTER, A. R. & WELCH, E. (2016). Functional rescue of rep1 following treatment with ptc124 and novel derivative ptc-414 in human choroideremia fibroblasts and the nonsense-mediated zebrafish model. *Human Molecular Genetics* **25**(16), 3416-3431.
- MORAIS, P., ADACHI, H. & YU, Y. T. (2020). Suppression of nonsense mutations by new emerging technologies. *International Journal of Molecular Sciences* **21**(12), 4394.
- MORI, N., FUNATSU, Y., HIRUTA, K. & GOTO, S. (1985). Analysis of translational fidelity of ribosomes with protamine messenger rna as a template. *Biochemistry* **24**(5), 1231-1239.
- MORT, M., IVANOV, D., COOPER, D. N. & CHUZHANOVA, N. A. (2008). A meta-analysis of nonsense mutations causing human genetic disease. *Human Mutation* **29**(8), 1037-1047.
- MURAMATSU, T., HECKMANN, K., KITANAKA, C. & KUCHINO, Y. (2001). Molecular mechanism of stop codon recognition by erf1: A wobble hypothesis for peptide anticodons. *FEBS Lett* **488**(3), 105-109.
- MUTYAM, V., DU, M., XUE, X., KEELING, K. M., WHITE, E. L., BOSTWICK, J. R., RASMUSSEN, L., LIU, B., MAZUR, M., HONG, J. S., FALK LIBBY, E., LIANG, F., SHANG, H., MENSE, M., SUTO, M. J., et al. (2016). Discovery of clinically approved agents that promote suppression of cystic fibrosis transmembrane conductance regulator nonsense mutations. *American Journal of Respiratory and Critical Care Medicine* **194**(9), 1092-1103.
- NAMGOONG, J. H. & BERTONI, C. (2016). Clinical potential of ataluren in the treatment of duchenne muscular dystrophy. *Degenerative Neurological and Neuromuscular Disease* **13**(6), 37-48.
- NAMY, O., HATIN, I. & ROUSSET, J. P. (2001). Impact of the six nucleotides downstream of the stop codon on translation termination. *EMBO reports* **2**(9), 787-793.
- NEU-YILIK, G., RAIMONDEAU, E., ELISEEV, B., YERAMALA, L., AMTHOR, B., DENIAUD, A., HUARD, K., KERSCHGENS, K., HENTZE, M. W., SCHAFFITZEL, C. & KULOZIK, A. E. (2017). Dual function of upf3b in early and late translation termination. *EMBO Journal* **36**(20), 2968-2986.
- NUDELMAN, I., REBIBO-SABBAH, A., CHERNIAVSKY, M., BELAKHOV, V., HAINRICHSON, M., CHEN, F., SCHACHT, J., PILCH, D. S., BEN-YOSEF, T. & BAASOV, T. (2009). Development of novel aminoglycoside (nb54) with reduced toxicity and enhanced suppression of disease-causing premature stop mutations. *Journal of Medicinal Chemistry* **52**(9), 2836-2845.

- NUDELMAN, I., REBIBO-SABBAH, A., SHALLOM-SHEZIFI, D., HAINRICHSON, M., STAHL, I., BEN-YOSEF, T. & BAASOV, T. (2006). Redesign of aminoglycosides for treatment of human genetic diseases caused by premature stop mutations. *Bioorganic & Medicinal Chemistry Letters* **16**(24), 6310-6315.
- OGLE, J. M., CARTER, A. P. & RAMAKRISHNAN, V. (2003). Insights into the decoding mechanism from recent ribosome structures. *Trends in Biochemical Sciences* **28**(5), 259-266.
- PHILLIPS-JONES, M. K., WATSON, F. J. & MARTIN, R. (1993). The 3' codon context effect on uag suppressor trna is different in escherichia coli and human cells. *Journal of Molecular Biology* **233**(1), 1-6.
- PIBIRI, I., LENTINI, L., MELFI, R., TUTONE, M., BALDASSANO, S., RICCO GALLUZZO, P., DI LEONARDO, A. & PACE, A. (2018). Rescuing the cftr protein function: Introducing 1,3,4-oxadiazoles as translational readthrough inducing drugs. *European Journal of Medicinal Chemistry* **159**, 126-142.
- PROKHOROVA, I., ALTMAN, R. B., DJUMAGULOV, M., SHRESTHA, J. P., URZHUMTSEV, A., FERGUSON, A., CHANG, C. T., YUSUPOV, M., BLANCHARD, S. C. & YUSUPOVA, G. (2017). Aminoglycoside interactions and impacts on the eukaryotic ribosome. *Proceedings of the National Academy of Sciences of the United States of America* **114**(51), E10899-E10908.
- RABEA, S. M., BARADARAN-HERAVI, A., BALGI, A. D., KRAUSE, A., HOSSEINI FARAHABADI, S., ROBERGE, M. & GRIERSON, D. S. (2019). 2-aminothiazole-4-carboxamides enhance readthrough of premature termination codons by aminoglycosides. *ACS Medicinal Chemistry Letters* **10**(5), 726-731.
- RAJON, E. & MASEL, J. (2011). Evolution of molecular error rates and the consequences for evolvability. *Proceedings of the National Academy of Sciences of the United States of America* **108**(3), 1082-1087.
- REN, G., ZHAO, Y. P., YANG, L. & FU, C. X. (2008). Anti-proliferative effect of clitocine from the mushroom *leucopaxillus giganteus* on human cervical cancer hela cells by inducing apoptosis. *Cancer Letters* **262**(2), 190-200.
- ROY, B., FRIESEN, W. J., TOMIZAWA, Y., LESZYK, J. D., ZHUO, J., JOHNSON, B., DAKKA, J., TROTTA, C. R., XUE, X., MUTYAM, V., KEELING, K. M., MOBLEY, J. A., ROWE, S. M., BEDWELL, D. M., WELCH, E. M., *et al.* (2016). Ataluren stimulates ribosomal selection of near-cognate trnas to promote nonsense suppression. *Proceedings of the National Academy of Sciences of the United States of America* **113**(44), 12508-12513.
- ROY, B., LESZYK, J. D., MANGUS, D. A. & JACOBSON, A. (2015). Nonsense suppression by near-cognate trnas employs alternative base pairing at codon positions 1 and 3. *Proceedings of the National Academy of Sciences of the United States of America* **112**(10), 3038-3043.
- SALAS-MARCO, J. & BEDWELL, D. M. (2005). Discrimination between defects in elongation fidelity and termination efficiency provides mechanistic insights into translational readthrough. *Journal of Molecular Biology* **348**(4), 801-815.
- SCHUEREN, F., LINGNER, T., GEORGE, R., HOFHUIS, J., DICKEL, C., GARTNER, J. & THOMS, S. (2014). Peroxisomal lactate dehydrogenase is generated by translational readthrough in mammals. *Elife* **3**, e03640.
- SERMET-GAUDELUS, I., BOECK, K. D., CASIMIR, G. J., VERMEULEN, F., LEAL, T., MOGENET, A., ROUSSEL, D., FRITSCH, J., HANSSENS, L., HIRAWAT, S., MILLER, N. L., CONSTANTINE, S., REHA, A., AJAYI, T., ELFRING, G. L., *et al.* (2010). Ataluren (ptc124) induces cystic fibrosis transmembrane conductance regulator protein expression and activity in children with nonsense mutation cystic fibrosis. *American Journal of Respiratory and Critical Care Medicine* **182**(10), 1262-1272.
- SERMET-GAUDELUS, I., RENOUIL, M., FAJAC, A., BIDOU, L., PARBAILLE, B., PIERROT, S., DAVY, N., BISMUTH, E., REINERT, P., LENOIR, G., LESURE, J. F., ROUSSET, J. P. & EDELMAN, A. (2007). In vitro prediction of stop-codon suppression by intravenous gentamicin in patients with cystic fibrosis: A pilot study. *BMC Medicine* **5**, 5.
- SHARMA, J., KEELING, K. M. & ROWE, S. M. (2020). Pharmacological approaches for targeting cystic fibrosis nonsense mutations. *European Journal of Medicinal Chemistry* **200**, 112436.
- SHULMAN, E., BELAKHOV, V., WEI, G., KENDALL, A., MEYRON-HOLTZ, E. G., BEN-SHACHAR, D., SCHACHT, J. & BAASOV, T. (2014). Designer aminoglycosides that selectively inhibit cytoplasmic rather than mitochondrial ribosomes show decreased ototoxicity: A strategy for the treatment of genetic diseases. *The Journal of Biological Chemistry* **289**(4), 2318-2330.

- SINGH, A., MANJUNATH, L. E., KUNDU, P., SAHOO, S., DAS, A., SUMA, H. R., FOX, P. L. & ESWARAPPA, S. M. (2019). Let-7a-regulated translational readthrough of mammalian ago1 generates a microrna pathway inhibitor. *EMBO Journal* **38**(16), e100727.
- SINGH, A., URSIC, D. & DAVIES, J. (1979). Phenotypic suppression and misreading *saccharomyces cerevisiae*. *Nature* **277**(5692), 146-148.
- SKUZESKI, J. M., NICHOLS, L. M., GESTELAND, R. F. & ATKINS, J. F. (1991). The signal for a leaky uag stop codon in several plant viruses includes the two downstream codons. *Journal of Molecular Biology* **218**(2), 365-373.
- SOGAARD, T. M., JAKOBSEN, C. G. & JUSTESSEN, J. (1999). A sensitive assay of translational fidelity (readthrough and termination) in eukaryotic cells. *Biochemistry (Mosc)* **64**(12), 1408-1417.
- SONG, H., MUGNIER, P., DAS, A. K., WEBB, H. M., EVANS, D. R., TUITE, M. F., HEMMINGS, B. A. & BARFORD, D. (2000). The crystal structure of human eukaryotic release factor erf1--mechanism of stop codon recognition and peptidyl-trna hydrolysis. *Cell* **100**(3), 311-321.
- 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, F. (2012). Clitocine reversal of p-glycoprotein associated multi-drug resistance through down-regulation of transcription factor nf-kappab in r-hepg2 cell line. *PLoS One* **7**(8), e40720.
- SWAN, S. K. (1997). Aminoglycoside nephrotoxicity. *Seminars in Nephrology* **17**(1), 27-33.
- TAGUCHI, A., HAMADA, K., KOTAKE, M., SHIOZUKA, M., NAKAMINAMI, H., PILLAIYAR, T., TAKAYAMA, K., YAKUSHIJI, F., NOGUCHI, N., USUI, T., MATSUDA, R. & HAYASHI, Y. (2014). Discovery of natural products possessing selective eukaryotic readthrough activity: 3-epi-deoxynegamycin and its leucine adduct. *ChemMedChem* **9**(10), 2233-2237.
- TATE, W. P. & MANNERING, S. A. (1996). Three, four or more: The translational stop signal at length. *Molecular Microbiology* **21**(2), 213-219.
- TEMPLE, G. F., DOZY, A. M., ROY, K. L. & KAN, Y. W. (1982). Construction of a functional human suppressor trna gene: An approach to gene therapy for beta-thalassaemia. *Nature* **296**(5857), 537-540.
- TRZASKA, C., AMAND, S., BAILLY, C., LEROY, C., MARCHAND, V., DUVERNOIS-BERTHET, E., SALIOU, J. M., BENHABILES, H., WERKMEISTER, E., CHASSAT, T., GUILBERT, R., HANNEBIQUE, D., MOURAY, A., COPIN, M. C., MOREAU, P. A., *et al.* (2020). 2,6-diaminopurine as a highly potent corrector of uga nonsense mutations. *Nature Communications* **11**(1), 1509.
- TUTONE, M., PIBIRI, I., LENTINI, L., PACE, A. & ALMERICO, A. M. (2019). Deciphering the nonsense readthrough mechanism of action of ataluren: An in silico compared study. *ACS Medicinal Chemistry Letters* **10**(4), 522-527.
- TUTONE, M., PIBIRI, I., PERRIERA, R., CAMPOFELICE, A., CULLETTA, G., MELFI, R., PACE, A., ALMERICO, A. M. & LENTINI, L. (2020). Pharmacophore-based design of new chemical scaffolds as translational readthrough-inducing drugs (trids). *ACS Medicinal Chemistry Letters* **11**(5), 747-753.
- URAKOV, V. N., VALOUEV, I. A., LEWITIN, E. I., PAUSHKIN, S. V., KOSORUKOV, V. S., KUSHNIROV, V. V., SMIRNOV, V. N. & TER-AVANESYAN, M. D. (2001). Itt1p, a novel protein inhibiting translation termination in *saccharomyces cerevisiae*. *BMC Molecular Biology* **2**, 9.
- WAGNER, K. R., HAMED, S., HADLEY, D. W., GROPMAN, A. L., BURSTEIN, A. H., ESCOLAR, D. M., HOFFMAN, E. P. & FISCHBECK, K. H. (2001). Gentamicin treatment of duchenne and becker muscular dystrophy due to nonsense mutations. *Annals of Neurology* **49**(6), 706-711.
- WANG, D., BELAKHOV, V., KANDASAMY, J., BAASOV, T., LI, S. C., LI, Y. T., BEDWELL, D. M. & KEELING, K. M. (2012). The designer aminoglycoside nb84 significantly reduces glycosaminoglycan accumulation associated with mps i-h in the idua-w392x mouse. *Molecular Genetics and Metabolism* **105**(1), 116-125.
- WANG, W., CZAPLINSKI, K., RAO, Y. & PELTZ, S. W. (2001). The role of upf proteins in modulating the translation read-through of nonsense-containing transcripts. *EMBO Journal* **20**(4), 880-890.
- WANGEN, J. R. & GREEN, R. (2020). Stop codon context influences genome-wide stimulation of termination codon readthrough by aminoglycosides. *Elife* **9**, e52611.

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

Figure Legends

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

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

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

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

Table 1. Aminoglycoside molecules with known readthrough activity.

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

Table 3. Readthrough potentiator molecules.