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Opinion

Slippy-Sloppy translation: a tale of programmed and induced-ribosomal frameshifting

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Programmed ribosomal frameshifting (PRF) is a key mechanism that viruses use to generate essential proteins for replication, and as a means of regulating gene expression. PRF generally involves recoding signals or frameshift stimulators to elevate the occurrence of frameshifting at shift-prone 'slippery' sequences. Given its essential role in viral replication, targeting PRF was envisioned as an attractive tool to block viral infection. However, in contrast to controlled-PRF mechanisms, recent studies have shown that ribosomes of many human cancer cell types are prone to frameshifting upon amino acid shortage; thus, these cells are deemed to be sloppy. The resulting products of a sloppy frameshift at the 'hungry' codons are aberrant proteins the degradation and display of which at the cell surface can trigger T cell activation. In this review, we address recent discoveries in ribosomal frameshifting and their functional consequences for the proteome in human cancer cells.

Highlights

Programmed ribosomal frameshifting (PRF) is a common mechanism in viruses whereby translating ribosomes shift coding reading frames at specific mRNA locations, producing multiple proteins essential for virus maturation.

Targeting PRF in viruses can be used to control virus replication, as recently suggested for severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2).

As opposed to PRF, ribosomal frameshifting can also be triggered by amino acid availability in yeast and human cancers.

Cancer-induced deregulation of the mitogen-activated protein kinase (MAPK)/mammalian target of rapamycin (mTOR) pathway promotes ribosomal frameshifting upon acute shortage of tryptophan.

The aberrant proteins produced by deregulated translation at the tryptophan codons can be presented at the cell surface to provoke a specific T cell recognition.

Insights of translation

Translation is the process responsible for the production of thousands of proteins from mRNA [1]. During initiation involving 80S eukaryotic ribosomes, the small ribosomal subunit (40S) is loaded with an initiator methionine-charged tRNA and binds the 5′-untranslated region (UTR) of an mRNA to scan and identify a start codon (AUG). Subsequently, the large subunit of the ribosome (60S) binds to the 40S subunit to create a fully functional ribosome that will elongate through an open reading frame (ORF) [2]. As the ribosome progresses through the ORF, codons are translated into their designated amino acid to produce a polypeptide chain. Last, when a stop codon is encountered (UAA, UAG, or UGA), release factors terminate the translation, releasing a fully produced protein.

Translation is a tightly regulated process. As such, several diverse regulatory mechanisms exist to ensure translational fidelity to avoid dysregulation that may be detrimental for the cell [3,4]. In addition to increasing and decreasing protein production, cells have evolved a multitude of mechanisms, such as alternative translation start sites or **PRF** (see Glossary) to diversify gene expression. Nonetheless, despite tight regulation, under stress or inadequate growth conditions, **ribosomal frameshifting** and stop codon read-through have been shown to augment the proteome landscape. For example, in 1972, ribosomal frameshifting was reported by the discovery of low levels of active product encoded by mutants with frameshifting mutations at any of a diverse number of locations within the encoding gene [5]. This was the first study indicating that, even though translation is a tightly controlled mechanism, in some circumstances, translation errors can occur that lead to the generation of new proteins. These new proteins will not only impact protein function, but may also affect immune recognition. As discussed later, once processed into short peptides of 8–14 amino acids long, their display at the cell surface of cells (as part of the immunopeptidome) may trigger the immune reaction. Therefore, the contribution

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of ribosomes to cancer progression in the context of the immunopeptidome has become in recent years a subject of intensive research field.

Ribosome heterogeneity and its implication in protein diversity

For many decades, ribosomes were only considered to be protein-producing machines; however, recent studies have indicated that the functionality of the ribosome is more complex [6,7]. For example, post-translational modifications of rRNAs and ribosomal proteins have been found to give rise to many more functions of the ribosome than initially thought, evoking a new and upcoming field of translation regulation. Ribosomal protein expression and tRNA modification have emerged over the past decade to be crucial for cancer cell survival [8,9]. A large-scale experiment using RNA-seq data revealed an unanticipated plasticity of ribosomal protein expression across normal and malignant human cells. As an example, *RPL39L*, a ribosomal protein paralog gene, was found upregulated specifically in hepatocellular carcinoma as well in some breast and lung carcinomas [10]. This suggested heterogeneity of ribosomal proteins in cancer, which might drive specific protein translation to favor tumorigenesis.

Although circulating cancer cells disseminate through the body, only a small proportion of these cells become metastatic. Surprisingly, ribosomal protein L15 (RPL15) has been described to have a role in metastasis in circulating tumor cells (CTCs) by driving the translation of core ribosomal proteins [11]. Clinically, RPL15 shows high expression in CTCs and was associated with poor prognosis in patients with breast cancer, showing the relevance of ribosome heterogeneity in clinical outcome. Another RPL protein, RPL24, was also found to be essential for tumorigenesis in mice overexpressing the C-Myc oncogene [12]. In addition to ribosomal proteins, tRNA modifications were also described to promote resistance to targeted therapy. The U_{34} enzymes, catalyzer of uridine$_{34}$ modifications, are essential for protein synthesis rewiring and resistance to targeted therapy in melanoma carrying *BRAF*^{V600E} mutations [13]. Together, these findings highlight the importance of modifications in the ribosome protein composition of rRNA and tRNA in cancer.

In addition to cancer onset, progression, and metastasis, ribosome function can also affect disease outcome by modulating the immunopeptidome. A substantial fraction of the immunopeptidome is derived from shorted live-proteins (SLiPs) as well as rapidly degraded nascent polypeptides (DRiPs) [14]. These DRiPs were shown to originate from proteins that did not achieve full functional integration into the proteome, and provided a potential explanation of the observation that virus-infected cells were recognized by antigen-specific CD8$^+$ cytotoxic T lymphocytes early after infection and before source protein could be detected [15,16]. Mechanistically, DRiPs are highly connected to ribosomal diversity, whereby specific ribosome subunit expression drastically influence DRiP generation. As an example, RPL6 and RPL28 have opposite effects on peptide generation by influenza A virus even though they are structurally positioned adjacent on the ribosome [17]. Hereby, RPL6 depletion decreases ubiquitin-dependent peptide presentation, while RPL28 depletion increases peptide generation [17]. Furthermore, 40S ribosomal protein S28 depletion increases total peptide supply by increasing DRiP synthesis from non-canonical translation of untranslated regions and non-AUG start codons, therefore sensitizing tumors cells for T cell targeting [17]. Interestingly, DRiPs were described as being identified from alternative mRNA isoforms as well as due to ribosomal frameshifting [18,19]. Additionally, ribosomal frameshifting was shown to be influenced by ribosome heterogeneity, because it is altered by RPL10 mutations in different species [20,21]. Thus, understanding the mechanisms behind ribosomal frameshifting may represent a new way of stimulating the immune system.

Glossary

GCN2: a serine/threonine protein kinase known to bind uncharged tRNAs as a result of an amino acid shortage. GCN2 is activated by autophosphorylation and triggers a cell survival program. To do so, GCN2 inactivates eukaryotic initiation factor 2α (eIF2A), resulting in repression of general protein synthesis. Additionally, the activation of GCN2 leads to expression of the transcription factor ATF4, which induces the expression of survival genes, such as *ASNS*. Recently, it was shown that ribosome collision-induced ZAKα kinase activates GCN2 independent of uncharged tRNAs to sustain cellular survival.

Indoleamine 2,3-dioxygenase 1 (IDO-1): enzyme responsible for the first step of tryptophan catabolism to produce kynurenine. IDO-1 is a rate-limiting enzyme that, for a long time, was considered a therapeutic target due to its involvement in the generation of kynurenine, an immunosuppressive metabolite of activated T cells.

p38/JNK: belongs to the MAPK pathway family. This pathway is responsive to stress stimuli, such as UV radiation, cytokines, as well as ribosome collisions upon amino acid shortage. Once activated by phosphorylation, JNK induces apoptosis by activating the well-established apoptotic factors BAX and BIM, as well as death signaling via c-Jun, FOS, or active p53 transcription. Prolonged and severe ribotoxic stress leads to p38/JNK activation and apoptosis via ZAKα.

Programmed ribosomal frameshifting (PRF): a translational recoding phenomenon by which organisms, such as viruses, produce proteins from overlapping alternative reading frames. These rare events are supported by specific mRNA signals. In many cases, the mRNA comprises a slippery region followed by a strong secondary structure, such as pseudoknot, which slows down ribosomes. This mechanism is highly documented in HIV-1 retrovirus, in which a chimeric protein is produced (Gag-Pol) from the same mRNA (gag) due to a PRF signal at the end of the canonical ORF from Gag mRNA.

Ribosomal frameshifting: translational errors arising from stalled ribosomes, for example, due to amino acid depletion. What exactly triggers ribosomal frameshifting

during tryptophan shortage is still unknown, but is likely linked to ribosome collisions.

Ribosomal frameshifting

Ribosomal frameshifting, also termed translational frameshifting, is one form of translational recoding by which the ribosome either shifts one nucleotide forward (in the direction on the 3′ end) or one nucleotide backward (in the direction of the 5′ end), generating different proteins from the same mRNA [22,23]. The first evidence of ribosomal frameshifting was discovered when the addition of serine tRNA to an *Escherichia coli* cell-free protein synthesis system caused –1 frameshifting events of a viral reporter gene [24]. In addition to bacteria, yeast have also shown the capacity to frameshift. Here, ribosomal frameshifting was detected in the retrotransposon Ty, where a sequence of 14 nucleotides was sufficient to support the generation of frameshifted products [25]. Interestingly, any alteration within a core sequence of seven of these 14 nucleotides reduced frameshifting, supporting the notion that information embedded in the mRNA sequence is the driver of this ribosomal frameshifting event [26]. Further studies of ribosomal frameshifting in yeast and humans pinpointed the general role of specific mRNA structures in mediating ribosomal frameshifts [22]. Nonetheless, exceptions to this rule exist. For example, in a bacteria model, ribosomal frameshifting occurred despite the lack of any mRNA secondary structure in the region [27]. Here, a specific mRNA sequence, called Shine-Dalgarno, which causes translational pausing due to its interaction with rRNAs, led to ribosomal frameshifting [28].

Programmed ribosomal frameshifting in viral decoding

Many RNA viruses use ribosomal frameshifting to produce essential viral proteins, a mechanism named PRF. PRF is often initiated by an RNA secondary structure, such as a stem-loop or a pseudoknot, which decreases the speed of elongating ribosomes (Figure 1, Key figure) [23]. Upstream of this structure, a specific RNA motif, comprising X XXY YYZ, where X can be any nucleotide, Y is A or U, and Z cannot be G, called a slippery sequence, allows elongating ribosomes to ‘slip’ and change frame, mainly moving one nucleotide forward (+1PRF) or backward (–1PRF) (Figure 1). Of note, –2 frameshifting, although less reported, is also used by other viruses to access a conserved alternative ORF [29,30].

In HIV-1, a structural viral protein, called Gag, is translated from a viral RNA that contains a –1PRF motif near the end of the ORF [31]. The –1PRF event produces a chimeric protein (Gag-Pol) required for RNA-dependent RNA polymerase activity. The occurrence of PRF in viruses was highlighted by the recent discovery of structural ribosomal frameshifting in the mRNA of severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) [32]. As in HIV-1, the ribosome encounters a stimulatory pseudoknot RNA fold preceded by a slippery site, resulting in a –1PRF. Given that PRF is essential for virus replication, merafloxacin, a drug that prevents ribosomal frameshifting, was described as an antiviral strategy for SARS-CoV-2 [33,34]. Moreover, disturbing ribosomal frameshifting in viruses was envisioned as a therapeutic opportunity. For example, several compounds were screened to inhibit or enhance the frameshifting-stimulating RNA sequence to imbalance the Gag:Pol ratio, therefore reducing viral replication in HIV-1 [35,36].

Given that frameshifting is used for gene regulation in viruses, infected cells have also developed external factors to inhibit PRF as a defense mechanism. A negative regulator of PRF, called Shiftless (SFL), was found to be produced by host cells to stop translation of these viral proteins [37]. Mechanistically, following HIV-1 viral infection, recipient cells produce diverse cytokines, such as interferon-gamma (IFNγ), to counteract stress. IFNγ induces the expression of SFL, which, in turn, inhibits –1PRF by causing premature termination by directly interacting with the translating ribosome [37]. SFL, also known as C19orf66, not only inhibits ribosomal frameshifting of HIV-1 viral RNA, but can also interrupt virus replication of other viruses, such as Dengue Virus (DENV) or Zika virus, by interacting with mRNA-binding proteins that positively affect viral replication [38,39].

Key figure

Mechanisms underlying programmed ribosomal frameshifting (PRF) in viruses

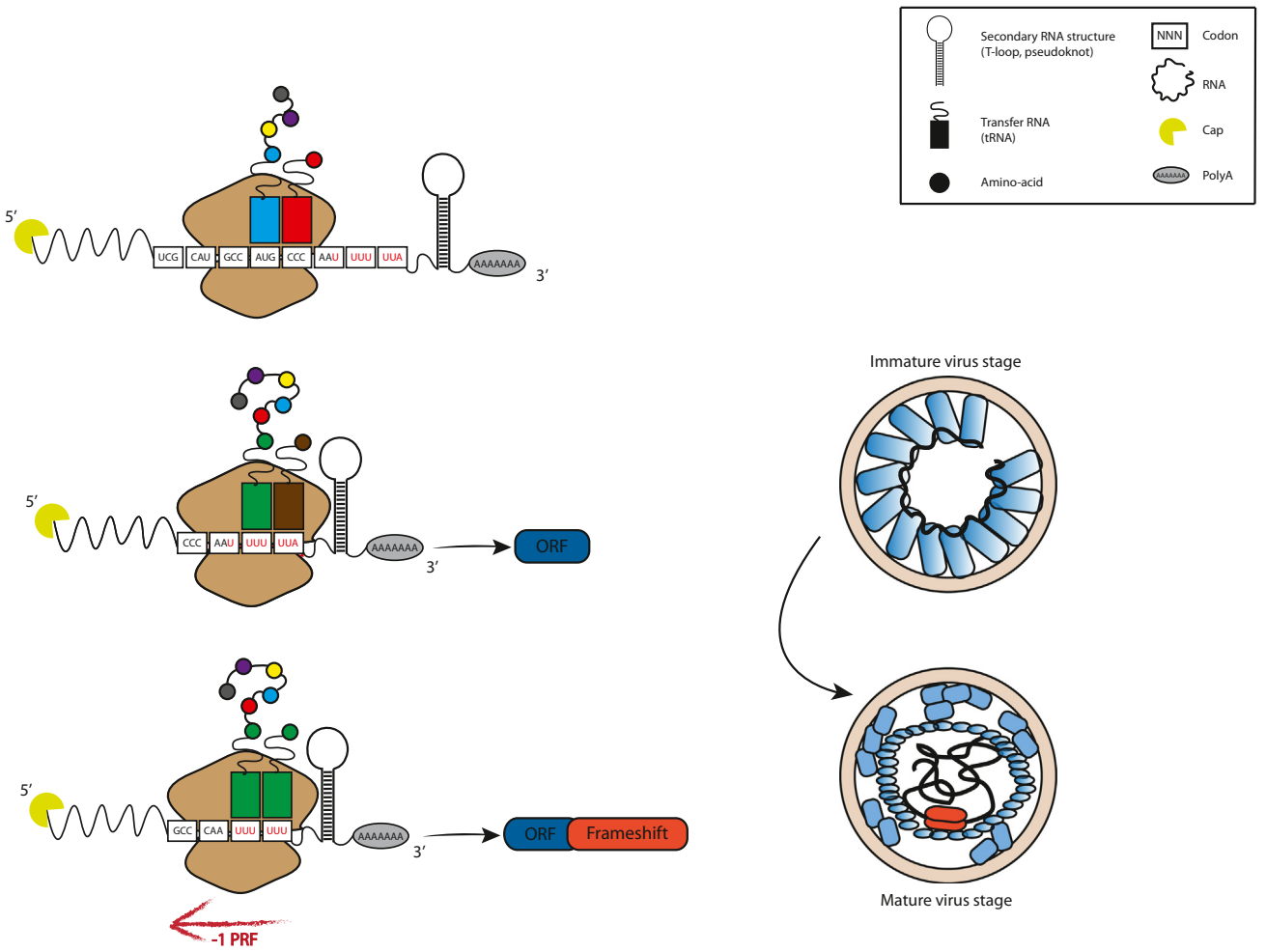

Figure 1. The viral mRNA comprises a strong secondary RNA structure, such as a T-loop or a pseudoknot, preceded by a slippery sequence, depicted in red. The slippery sequence generally comprises a seven-nucleotide motif of the form X XXY YYZ, where X denotes any nucleotide, Y denotes A or U, and Z is A, U, or C. When the ribosome encounters the secondary structure, the ribosome stalls and the slippery sequence allows ribosomal frameshifting (–1PRF), producing an alternative protein. Here, the canonical protein is required for viral structure maintenance, while the frameshift product is required for RNA polymerase activity. In this example, ribosomal frameshifting is essential for virus maturation. Abbreviation: ORF, open reading frame.

Programmed ribosomal frameshifting in higher eukaryotes

The aforementioned examples indicate that PRF is not only observed to be essential for viral gene regulation, but also has the ability to be regulated by external factors. To strengthen this point, studies of antizyme protein, found from yeast to mammals, reveal the effect of polyamine levels on frameshifting regulation [40]. Here, ribosomes frameshift close to the ORF stop codon to produce the main part of the antizyme by a +1PRF. Remarkably, when the intracellular level of polyamines is elevated, numerous ribosome frameshifting events occur, producing an antizyme

that will regulate polyamines levels by degrading ornithine decarboxylase (ODC), a key enzyme in polyamine synthesis [41,42].

Inspired by PRF in viruses, Belew and colleagues computationally analyzed the transcriptome of higher eukaryotes for the occurrence of PRF [43]. They found that a PRF-like motif was present in ~10% of cellular mRNAs even though they are not always conserved between species; however, only a few of these PRFs have been functionally confirmed. In human mRNA, CCR5, the HIV-1 co-receptor, was reported to be subjected to –1PRF directed by a mRNA pseudoknot and stimulated by miRNAs [44]. Although this example was recently challenged, other functional mammalian frameshifts were also reported [45]. Another example comes from adenomatous polyposis coli (*APC*), a tumor suppressor gene in which ribosomal frameshifting was identified at an A-rich slippery region in the coding sequence [46]. A final example comes from the gene encoding PEG10. This gene contains two overlapping ORFs, but the production of ORF2 is only possible by a –1PRF during ORF1 translation [47,48]. Biologically, the resulting –1PRF encodes a protease that was found to be highly expressed during the early stages of development to regulate PEG10 functionality [47]. Thus, similar to viruses, PRF in cells demonstrates the ability to allow for gene regulation.

Ribosomal frameshifting by external factors

PRF is not the only mechanism identified to result in frameshifting events. Unsurprisingly, gene expression levels can influence frameshifting. In *E. coli,* it was shown that +1 frameshifting at a rare arginine codon in tandem was driven by mRNA expression levels, whereby mRNA levels positively influenced ribosomal frameshifting [49].

In addition to expression levels, tRNA availability compared with its respective anticodon can be a rate-limiting factor during translation. Indeed, because of a longer time required for the matching charged tRNA to be available, rare codons can affect protein production and quality [50,51]. In *E. coli*, for example, it was reported that rare codons for arginine induce stalling, leading to ribosomal frameshifting [52].

Furthermore, amino acid availability is also critical to ensure ribosome progression. In the T4 bacteriophage, it was shown that a shortage of tryptophan tRNA can induce ribosomal frameshifting during the translation of $r_{II}B$ [53]. Such ribosomal frameshifting is likely to induce the production of truncated polypeptides that will eventually be eliminated by the cells by dedicated mechanisms such as endoplasmic reticulum-associated protein degradation (ERAD) [54]. However, for cells to overcome ribosome stalling or truncated mRNAs, dedicated rescue pathways have evolved.

In eukaryotic cells, the ribosome-associated protein quality control (RQC) pathway resolves issues that can occur during the translation process, such as eliminating nascent polypeptides, recycling stalled ribosomes, and inhibiting translation initiation [3,55]. Interestingly, several studies have reported that RQC also has the ability to prevent ribosomal frameshifting (Box 1). Nonetheless, despite these tightly controlled regulatory mechanisms in healthy eukaryotic cells, a different outcome was observed in cancer cells when they encountered amino acid shortages.

Amino acid-induced ribosomal frameshifting

Tryptophan, encoded by the UGG codon, is an essential amino acid; therefore, it is exclusively obtained from the diet. Interestingly, tryptophan catabolism regulates antitumor immunity activity [56]. Secretion of IFNγ by cancer-infiltrating T cells induces the production of **indoleamine 2,3 dioxygenase 1 (IDO1)** in targeted cancer cells. High levels of IDO1 are associated with reduced

Box 1. How does ribosome quality control affect ribosomal frameshifting?

Given that stalled ribosomes can be detrimental for cells, ribosomal quality control (RQC) proteins are responsible for resolving this issue. For example, collided ribosomes are recognized by ZNF598, an E3 ubiquitin ligase that ubiquitinylates ribosomal proteins, such as RPS10, to relieve stalling [74,75]. Furthermore, ZNF598 knockdown has been shown to stimulate ribosomal frameshifting when the ribosomes are stalled at a poly(A) sequence [74].

More components of the RQC complex were identified to be involved in the resolution of stalled ribosomes; for example, RACK1 is involved in the recognition and resolution of stalled ribosomes; N4BP2 degrades the stalled mRNA; NEMF and listerin participate in degrading nascent polypeptide chains; and GIGYF2 inhibits general translation [76,77]. More recently, EDF1 was shown to have a major role in facilitating RQC pathways and its deletion caused an increase in ribosomal frameshifting in a stalled-sequence reporter model [78–81].

Interestingly, when a large amount of ribosomes stall, prolonged ribosome collisions activate a molecular cascade that induces apoptosis [67]. ZAKα, a protein kinase, is a member of the stress response pathway (SAPK) that is self-activated by phosphorylation. When the level of ribosome collisions is low, ZAKα activates **GCN2**, which, in turn, promotes a cell survival program by repressing translation initiation and inducing the expression of several cell survival genes [67,82]. However, when colliding ribosomes accumulate, ZAKα activates the **p38/JNK** pathway, triggering cell death.

antitumor immunity as it metabolizes tryptophan into kynurenine via the kynurenine pathway [57]. Kynurenine diffuses to T cells, binds and activates the transcriptional repressor aryl hydrocarbon receptor (AHR) to suppress T cell activity; therefore, the blockade of IDO-1 was envisioned as an alternative therapeutic approach [58]. However, prolonged activation of IDO1 by IFNγ results in severe tryptophan shortage, with broad consequences for mRNA translation in the targeted cancer cells [19]. While ribosome profiling indicates both ribosome accumulation at the start codon and stalling at the tryptophan codon, downstream mRNA translation and sustained protein production in the absence of tryptophan, although decreased, was still observed [19]. While sustained protein production in the absence of tryptophan was initially reported in melanoma cell lines, further experiments have shown this phenomenon to occur in many other cancer cell types [59]. The question that arises is how ribosomes overcome tryptophan shortage at the tryptophan codon.

Using reporter vectors assays and mass spectrometry, it was shown that ribosomes in cancer cells frameshift similar to PRF (Figure 2) [59]. However, bioinformatics analyses showed that frameshifting as a result of amino acid shortage is not associated with a specific mRNA structure or a specific mRNA motif [19]. Thus, the widespread characteristic of cancer cells using ribosomal frameshifting to sustain protein production in the absence of essential amino acids, reported for the first time, was deemed ‘sloppiness’ [59].

In cancer cells, oncogenic mutations often deregulate mRNA translation, which has a critical role in tumor progression [60]. Which deregulated pathways could result in sloppiness? Sloppiness measurements combined with mutational analysis of over 30 cancer cell lines indicate a causal link with oncogenic mutations in the mitogen-activated protein kinase (MAPK) pathway [59]. In particular, cell lines harboring oncogenic mutations in EGFR, RAS, and RAF display high levels of sloppiness. The link between the MAPK pathway and sloppiness was further reinforced using ectopic expression and drug inhibition of key oncogenes, such as H-RAS^{G12V} and BRAF^{V600E}.

MAPK as a driver of ribosomal frameshifting

The phosphorylation of the 40S-ribosomal protein subunit RPS6 is a key downstream event induced by activated MAPK pathway, in which ERK1/2 activates the ribosomal S6 kinase (S6K1) [61]. In cancer cells, RPS6 phosphorylation inhibition abolished tryptophan depletion-induced frameshifting, supporting the role of RPS6 phosphorylation in driving oncogene-dependent sloppiness [59]. RPS6 is a well-established ribosomal protein where its phosphorylation is essential for: optimal translation, ribosome biogenesis, cell size regulation, synaptic plasticity, T-cells fitness, and

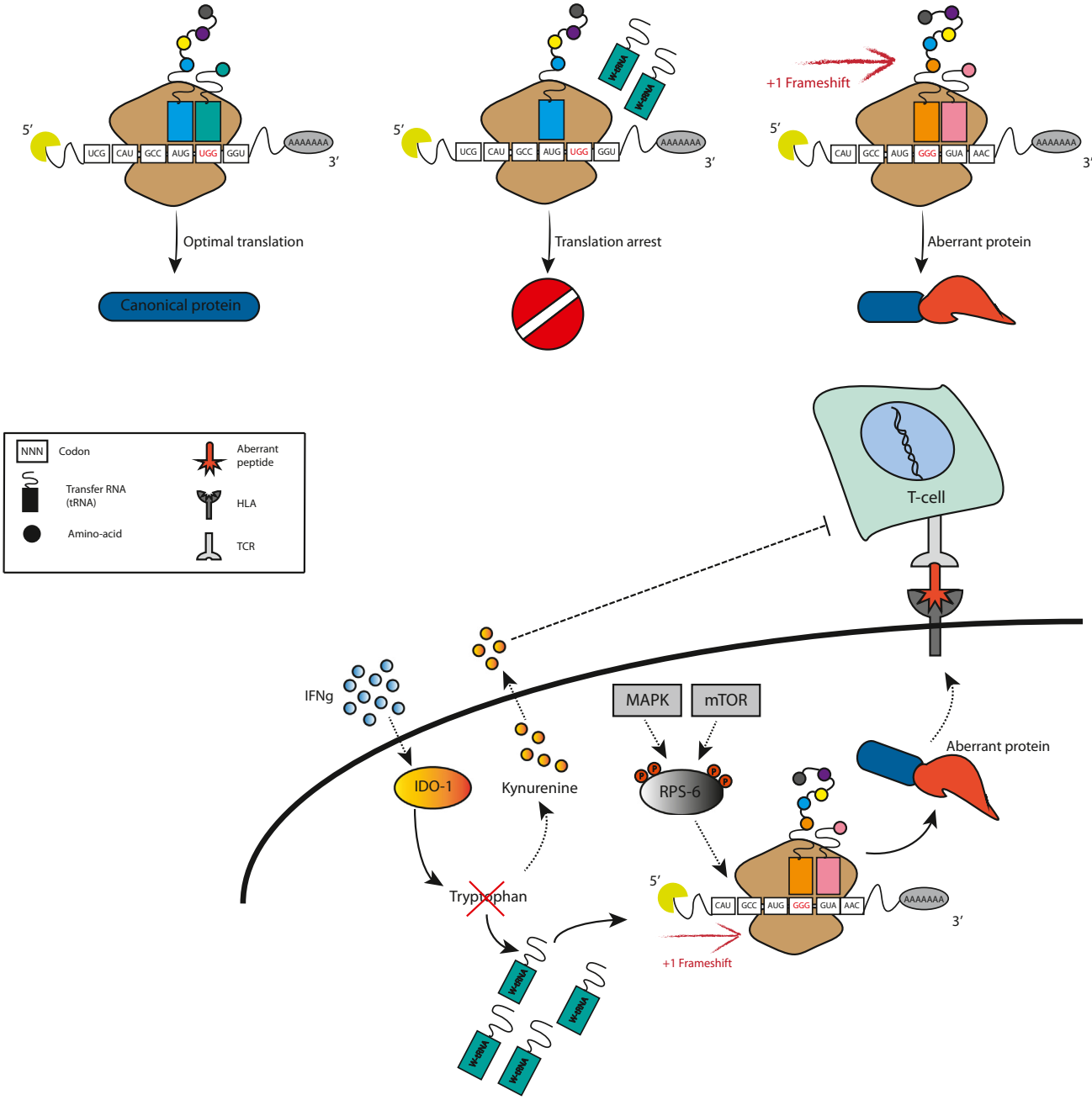

Figure 2. Mechanisms underlying ribosomal frameshifting in cancer cells upon tryptophan shortage. Tryptophan shortage results in uncharged W-tRNA, inducing ribosome stalling at tryptophan codons (UGG, depicted in red). In sloppy cancer cells, ribosomal frameshifting is used to sustain protein synthesis, creating aberrant protein. Oncogenic pathways, such as mitogen-activated protein kinase (MAPK) or mammalian target of rapamycin (mTOR), stimulate ribosomal frameshifting by phosphorylating the ribosomal protein RPS6. Aberrant proteins resulting from ribosomal frameshifting are then processed, displayed at the cell surface, and can be recognized by dedicated T cells. Abbreviations: HLA, human leukocyte antigen; IDO-1, indoleamine 2,3-dioxygenase 1; IFN, interferon; TCR, T cell receptor.

more [62–65]. However, unexpectedly, mouse-embryonic fibroblasts (MEF) of phospho-deficient RPS6 mice increased protein synthesis, suggesting a conflicting role of RPS6 in the regulation of protein synthesis [62]. In addition, phosphorylation of RPS6 differentially affects mRNA translation based

on ORF length [66]. Indeed, RPS6 phosphorylation promotes the translation of mRNAs with a short coding sequence more strongly than the one with a long coding sequence. In accordance with the last point, whereas knock out of RPS6 did affect in-frame protein translation of the frameshifting reporter used in melanoma cells, chemical-RPS6 kinase inhibition abrogated frameshifting upon tryptophan shortage without affecting in-frame protein synthesis, suggesting additional functions of RPS6 [59].

Efficient translation can be envisioned as a fine balance between speed and efficiency. Therefore, for viruses and cancer cells, the intrinsic need of the cells to divide rapidly might come at the expense of frame maintenance, creating sloppy behavior. Indeed, sloppiness is a cancer-specific occurrence; however, not all cancer cell lines have the ability to be sloppy [59]. The activation of the MAPK pathway is not always sufficient to induce sloppiness given that some cancer cell lines carrying diverse RAS mutations do not undergo frameshifting. This suggests that oncogenic pathways are not the only factor required for sloppiness to occur. For example, ribosomal heterogeneity, translation speed, additional somatic mutations, and tissue context may also influence sloppiness. While PRF is a consequence of local slippery mRNA sequences, sloppiness is induced by amino acid shortage in cancer cells. Although the outcome of both processes is ribosomal frameshifting, PRF is an accurate process designed to generate downstream essential proteins, whereas sloppiness is likely to result in the expression of either nonfunctional truncated or aberrant proteins. To what extent is the importance of ribosomal frameshifting to cancer cells facing microenvironmental stress? It remains unclear whether inhibition of ribosomal frameshifting influences cancer cell viability. However, in non-transformed cells, prolonged ribosome stalling and collisions induce cell death through the activation of the c-Jun N-terminal kinase (JNK) pathway [67].

Functional consequences of sloppiness in cancer cells

How can aberrant proteins emerging from sloppiness be exploited further? First, aberrant proteins can be processed into peptides that can be presented to immune cells by human leukocyte antigen (HLA) class I molecules [19]. Upon initial examination of the immunopeptidome from a melanoma cell line treated with IFNγ, 81 aberrant epitopes resulting from sloppy mRNA translation were identified. Two aberrant epitopes were shown to be immunogenic by activating naïve $CD8^+$ T cells from healthy donors [19]. In addition, one of the major caveats of traditional cancer treatment is acquired resistance. Encouragingly, resistant cancer cells retained the sloppiness phenotype, suggesting the possibility of using immunotherapy directed against aberrant epitopes [59]. This finding opens new opportunities for treating resistant cancers with targeted immunotherapy (Figure 2).

Perspectives

Ribosomal frameshifting due to tryptophan depletion opens a new area of research in which aberrant peptides could ultimately be used for immunotherapy. To expand the landscape of the immunopeptidome of tumors cells, in particular those that present a low number of immunogenic peptides, all possible approaches need to be used. Therefore, the effect of other amino acid depletions must be investigated. Interestingly, tyrosine depletion was shown to drive +1 ribosomal frameshifting in a reporter assay [19]. However, in contrast to tryptophan, tyrosine is encoded by two codons (UAU and UAC), reducing the chance of generating a sufficiently long aberrant peptide with HLA-presentation capacities. Moreover, tryptophan depletion is a common event that occurs in cancer cells upon IFNγ exposure. Another type of amino acid depletion may be more challenging to induce *in vivo*. However, asparaginase, the asparagine-depleting enzyme, is broadly used for treating acute lymphoblastic leukemia [68]. Identifying whether asparaginase induces frameshifting at hungry codons could lead to

the discovery of new aberrant peptides. Similarly, others enzymes that trigger amino acid depletion within tumors have been developed and might induce cancer-specific ribosomal frameshifting [69].

Even though sloppiness behavior is associated with MAPK deregulation, the exact mechanism by which the ribosome changes frame needs further investigation. Upon tryptophan depletion, not only +1 ribosomal frameshifting, but also −1 ribosomal frameshifting was detected, suggesting that ribosome collisions influence the trailing ribosome differently [19]. Ribosome collisions were reported to alter frameshifting in bacterial mRNAs, whereby bacteria missing the ribosomal bL9 were more prone to collide and to frameshift [70]. Once again, this study shows that ribosome heterogeneity is likely to be fundamental for the ribosome to frameshift. Beyond RPS6, understanding which ribosomal subunits or ribosomal modifications are necessary for ribosomal frameshifting will permit manipulation of the number of aberrant peptides generated by cancer cells. Interestingly, RPS6 is localized at the interface between the small and the large subunit of the ribosome, suggesting a role in maintaining the rigidity of the ribosome [71]. Other ribosomal proteins located either near the exit tunnel or at the interface between the large and the small subunits of the ribosome could also influence ribosomal frameshifting. In addition, IFN is known to induce modifications, such as phosphorylation, at some ribosomal proteins, which could drive the ribosome to frameshift [72]. Investigation of ribosomal and tRNA modifications represents a new field for uncovering ribosomal frameshifting mechanisms. Therefore, comparing the ribosomal subunits enriched for sloppy cancer cells will give new insights into amino acid-induced ribosomal frameshifting.

Finally, only a small but significant proportion of ribosomes frameshift at hungry codons. Recently, it was shown that, in addition to ribosomal frameshifting, in-frame protein translation continued by codon reassignment [73]. Here, the cells wrongly incorporate phenylalanine instead of tryptophan, generating aberrant proteins. This study adds another layer of ribosomal errors to amino acid shortages that could eventually be used for immunotherapy.

Concluding remarks

Here, we have summarized new insights into ribosomal frameshifting upon amino acid limitation in cancer cells. While slippery regions can give rise to PRF events to express key cellular proteins and to regulate gene expression, sloppy protein production is a consequence of deregulated mRNA translation in cancer. How sloppiness can affect cancer survival and whether aberrant proteins, the products of sloppiness, can be exploited for targeted cancer therapy remain to be further investigated (see Outstanding questions).

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Author contributions

J.C and R.A conceptualized the idea, and drafted and wrote the manuscript. J.C designed the figures. K.M and R.N corrected and helped to finalize the manuscript. R.A provided financial support.

Declaration of interests

The authors declare that they have no competing interests.

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Outstanding questions

What is the exact benefit to cancer cells using ribosomal frameshifting? For example, does ribosomal frameshifting help to better survive stress produced by tumor expansion?

Is ribosomal frameshifting in cancer cells specific to tryptophan, or can it be induced by the shortage of other amino acids. If so, what is their biological relevance?

Sloppiness behavior is associated with a deregulated MAPK/mammalian target of rapamycin (mTOR) pathway. However, not all cancer cell lines with a deregulated MAPK/mTOR pathway are sloppy. Which additional factors dictate sloppiness?

RPS6 phosphorylation appears to be an essential event for ribosomal frameshifting upon tryptophan shortage. The mechanism by which phosphorylated RPS6 promotes sloppiness is unclear. For example, does it drive sloppiness by affecting translation initiation or by regulating ribosome function at collision sites?

Ribosomal heterogeneity is an important factor in determining cancer outcome by influencing metastasis or response to treatment. Is sloppiness influenced by the protein make-up of ribosomes in different cell types?

Ribosomal collision was shown to cause ribosomal frameshifting. Is sloppiness a result of colliding ribosomes? If so, which of the colliding ribosomes is responsible for the frameshifting product? Can ribosome collision explain why both +1 and −1 ribosomal frameshifting are detected in cancer cells?

So far, ribosomal frameshifting upon tryptophan shortage has only been detected in cancer cells. It is relevant to know whether some healthy cells frameshift upon shortages. For example, highly dividing healthy cells might be prone to frameshift.

Immunopeptidomics experiments allow the detection of aberrant peptides arising from ribosomal frameshifting at tryptophan codons. From a therapeutic perspective, it is of relevance to know whether

there are aberrant peptides commonly presented at the cell surface of cancer cells following tryptophan depletion. Such peptides could be prioritized as targets for immunotherapy.

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