Review

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Protein synthesis control in cancer: selectivity and therapeutic targeting

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Abstract

Translational control of mRNAs is a point of convergence for many oncogenic signals through which cancer cells tune protein expression in tumorigenesis. Cancer cells rely on translational control to appropriately adapt to limited resources while maintaining cell growth and survival, which creates a selective therapeutic window compared to non-transformed cells. In this review, we first discuss how cancer cells modulate the translational machinery to rapidly and selectively synthesize proteins in response to internal oncogenic demands and external factors in the tumor microenvironment. We highlight the clinical potential of compounds that target different translation factors as anti-cancer therapies. Next, we detail how RNA sequence and structural elements interface with the translational machinery and RNA-binding proteins to coordinate the translation of specific pro-survival and pro-growth programs. Finally, we provide an overview of the current and emerging technologies that can be used to illuminate the mechanisms of selective translational control in cancer cells as well as within the microenvironment.

Keywords cancer; protein synthesis; translation and protein quality; translation inhibitors; translational control

Subject Categories Cancer; Translation & Protein Quality

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Introduction

Cancer cells continually alter gene expression programs to adapt, grow, and survive in non-physiological environments. It is now evident in the field that cancer cells can adapt to different stress conditions triggered by internal or external stimuli through the regulation of gene expression at the translational level (Truitt & Ruggero, 2016). Importantly, they can selectively synthesize proteins urgently needed on a rapid timescale (Shamir *et al*, 2016). While transcriptional regulation remains a major focus of cancer biologists, genome-wide analyses have uncovered discrepancies between RNA abundance and corresponding protein levels, highlighting how quantification of RNA expression alone is insufficient to capture the actual protein levels in the cell (Liu *et al*, 2016; Buccitelli & Selbach, 2020).

Translation is a complex, multi-step process which requires a multitude of factors—ribosomes, tRNAs, amino acids, and translation factors—working in concert to mediate protein synthesis. The process of translation is divided into different steps: initiation, elongation, termination, and ribosome recycling (Sonenberg & Hinnebusch, 2009; Jackson *et al*, 2010; Dever & Green, 2012; Robichaud *et al*, 2019). In this review, we will mainly focus on the initiation step, which is the rate-limiting step controlling translation. Cancer cells tightly control this step, which impinges on selective translational control of specific mRNA networks. The untranslated regions (UTRs), which are the non-coding regions of the mRNA flanking the coding sequence, are essential for the regulation of translation (Hinnebusch *et al*, 2016; Leppek *et al*, 2018). In particular, 5′UTRs contain several RNA sequence elements and secondary structures that provide a platform for *trans* element binding in order to modulate protein synthesis (Hinnebusch *et al*, 2016; Schuster & Hsieh, 2019). We will explore the different elements found in the 5′UTRs and 3′UTRs that cancer cells use to regulate gene expression and how these RNA regulons coordinate the expression of functionally related genes to steer many hallmarks of cancer development.

In this review, as a part of the Cancer Review Series 2021, we will highlight the important emerging concept that translation is selectively regulated to tailor a proteome in support of cancer initiation, progression, and metastasis. We will first discuss how cancer cells hijack different translation factors to drive translation of specific transcripts to maintain cancer cell fitness. Next, we will focus on how oncogenic pathways use *trans* and *cis* elements on specific transcripts to alter protein expression. Acting in concert, these factors promote expression of the mediators of nearly all hallmarks of cancer from "classical" hallmarks such as sustaining proliferation and control of cell survival to the "emerging" hallmark of avoiding immune destruction (Hanahan & Weinberg, 2011). Finally, we discuss current and developing technologies to study translational control in cancer. Through the application of these new techniques, we will continue to

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uncover the manifold ways cancer cells rely on translational control and how to exploit that unique vulnerability therapeutically.

Translation machinery and translational specificity

For many decades, translation factors were considered housekeeping proteins without any selectivity in promoting protein synthesis. However, more recent studies from several groups revealed that translation factors are hijacked by many oncogenes to drive transcript-specific translation in order to maintain cancer cell fitness. In this section, we will focus on how different components of the translation machinery are involved in selective, pro-oncogenic gene regulation and how they can be targeted therapeutically.

The eukaryotic initiation factor 4F (eIF4F) complex is the major node that oncogenic signaling pathways target to regulate gene expression at the translation level. The complex consists of the major cap-binding protein, eIF4E, the scaffold protein, eIF4G, and the RNA helicase, eIF4A. Each component of the complex has been shown to be deregulated in different cancer types. Among them, eIF4E has emerged as a crucial nexus of translational control that is hyperactivated downstream of several oncogenic pathways (Fig 1A). While Myc promotes the transcription of eIF4E (Rosenwald *et al*, 1993; Jones *et al*, 1996), oncogenic Ras activates the phosphorylation of eIF4E by regulating the MAPK-interacting serine/threonine kinase 1 (MNK1) (Waskiewicz *et al*, 1997; Furic *et al*, 2010), resulting in eIF4E hyperactivation. Moreover, eIF4E is also regulated via the mTOR pathway through eIF4E-binding protein (4EBPs) suppressors, which inhibit eIF4E activity (Haghighat *et al*, 1995; Hsieh *et al*, 2012; Pourdehnad *et al*, 2013). All these oncogenic pathways converge to modulate eIF4E activity and highlight the importance of eIF4E in regulation of the cancer translatome. eIF4E-dependent translation was shown to be essential in regulating selective translation involved in many diverse aspects of cancer from metabolism (Cunningham *et al*, 2014) to invasion (Robichaud *et al*, 2015). For example, tumor cells selectively exploit eIF4E to translate mRNAs needed to overcome an anti-tumor immune response (Xu *et al*, 2019) or to make the tumor microenvironment more favorable for tumor growth (Bartish *et al*, 2020). One of the most surprising discoveries over the last several years is that, contrary to previous beliefs, eIF4E expression is not a limiting factor for overall protein synthesis. Reducing eIF4E levels by 50% does not perturb normal development and global protein synthesis; however, reduced eIF4E remarkably suppresses oncogenic transformation (Truitt *et al*, 2015). These findings uncovered that an excess amount of eIF4E is pro-oncogenic, and importantly, specific eIF4E-dependent translational control in cancer cells represents a new therapeutic vulnerability. Therefore, there is a growing interest in generating compounds that inhibit the activity of eIF4E (Fig 1B). Inhibitors that block the ability of eIF4E to recruit the pre-initiation complex, such as the compounds 4EGI-1 (Moerke *et al*, 2007), 4E1RCat (Cencic *et al*, 2011b), and 4E2RCat (Cencic *et al*, 2011a), have displayed anti-tumor effects in pre-clinical trials (Chen *et al*, 2012). Moreover, MNK1 inhibitors, such as cercosporamide and tomivosertib (also named eFT508), which block eIF4E phosphorylation, and hence the activity of eIF4E, suppress tumor progression and metastasis in both xenograft and genetically engineered mouse models (Konicek *et al*, 2011; Xu *et al*, 2019). Notably, eFT508 is currently in Phase II clinical trials (NCT03616834, NCT04622007).

Although eIF4E has emerged as a high priority therapeutic target, the genetic interacting partners that act in concert with eIF4E-dependent translational control to maintain cancer cell fitness are not well characterized. Our lab recently performed a genome-wide CRISPRi screening to identify synthetic lethal partners of eIF4E, which uncovered more than 600 genetic interactions that sustain eIF4E oncogenic activity (Kuzuoglu-Ozturk *et al*, 2021). Each interaction represents a potential target for combination therapy that can selectively target cancer cells at the post-transcriptional level. Moreover, the screen unveiled novel functional connections between eIF4E and unexpected cellular processes, such as mitochondrial protein homeostasis. Specifically, cancer cells rely on selective eIF4E-dependent translation to manage mitochondrial proteotoxic stress through increased translation of a master autophagy regulator and transcription factor, Tfeb, promoting cancer cell survival. These findings illustrate how the activity of a translation factor can orchestrate a wide variety of specific cellular processes genome wide to overcome oncogenic stress.

In addition to eIF4E, the DEAD-box helicase eIF4A, a member of the eIF4F complex, is a key nexus in the regulation of pro-cancerous signaling (Fig 1A). eIF4A unwinds secondary structures located in the 5′ untranslated regions (UTRs) to facilitate the scanning of the 43S ribosome complex for start codon recognition, and therefore, it is thought to be critical for the translation of mRNAs with long and complex 5′UTRs (Svitkin *et al*, 2001). There are two paralogs of eIF4A, eIF4A1 and eIF4A2, which are 90% homologous at the amino acid level (Nielsen & Trachsel, 1988). Interestingly, eIF4A1, a direct transcriptional target of Myc (Lin *et al*, 2008), is often overexpressed in a range of malignancies and has been shown to mediate selective translation of several oncogenes (Ji *et al*, 2003; Liang *et al*, 2014b; Modelska *et al*, 2015). In addition, a recent study showed that a decrease in eIF4A1 dosage suppresses lymphomagenesis in the Eμ-Myc mouse model (Sénéchal *et al*, 2021). On the other hand, the overall role of eIF4A2 in translation and cancer is poorly understood. Ongoing research in cancer biology is mainly focused on eIF4A1-dependent selective translation. Recent advancements employing ribosome profiling for transcriptome-wide measurements of translational efficiency uncovered that mRNAs containing polypurine and GC-rich sequence motifs in their 5′UTRs are specifically more sensitive to eIF4A1 activity (Rubio *et al*, 2014; Wolfe *et al*, 2014). Importantly, many oncogenes have complex 5′UTRs and have been shown to be dependent on eIF4A1 for efficient translation (Rubio *et al*, 2014; Steinhardt *et al*, 2014; Wolfe *et al*, 2014; Kong *et al*, 2019). Following these observations, numerous eIF4A inhibitors have been developed and have demonstrated potent anti-tumorigenic effects in different pre-clinical cancer models (Fig 1B). These data suggest that eIF4A1 is a very valuable target for cancer therapy and indeed one eIF4A inhibitor, eFT226 (known as zotatifin), is already in Phase I/II clinical trials (NCT04092673). The availability of eIF4A inhibitors has made it possible to investigate its targets. Specific mRNA targets of eIF4A1 have been identified through extensive studies in a variety of blood and solid tumor cancer models. These mRNAs vary from known oncogenes such as Myc and Mdm2 to key regulators of proliferation such as the cell cycle kinases Cdk6/Cdk10 (Table 1). Detailed information about the spectrum of eIF4A inhibitors can be found in reviews focused on this topic (Voss *et al*, 2017; Pal *et al*, 2019).

The structural component of the eIF4F complex, the scaffold protein eIF4GI, is upregulated in many different cancer types and is

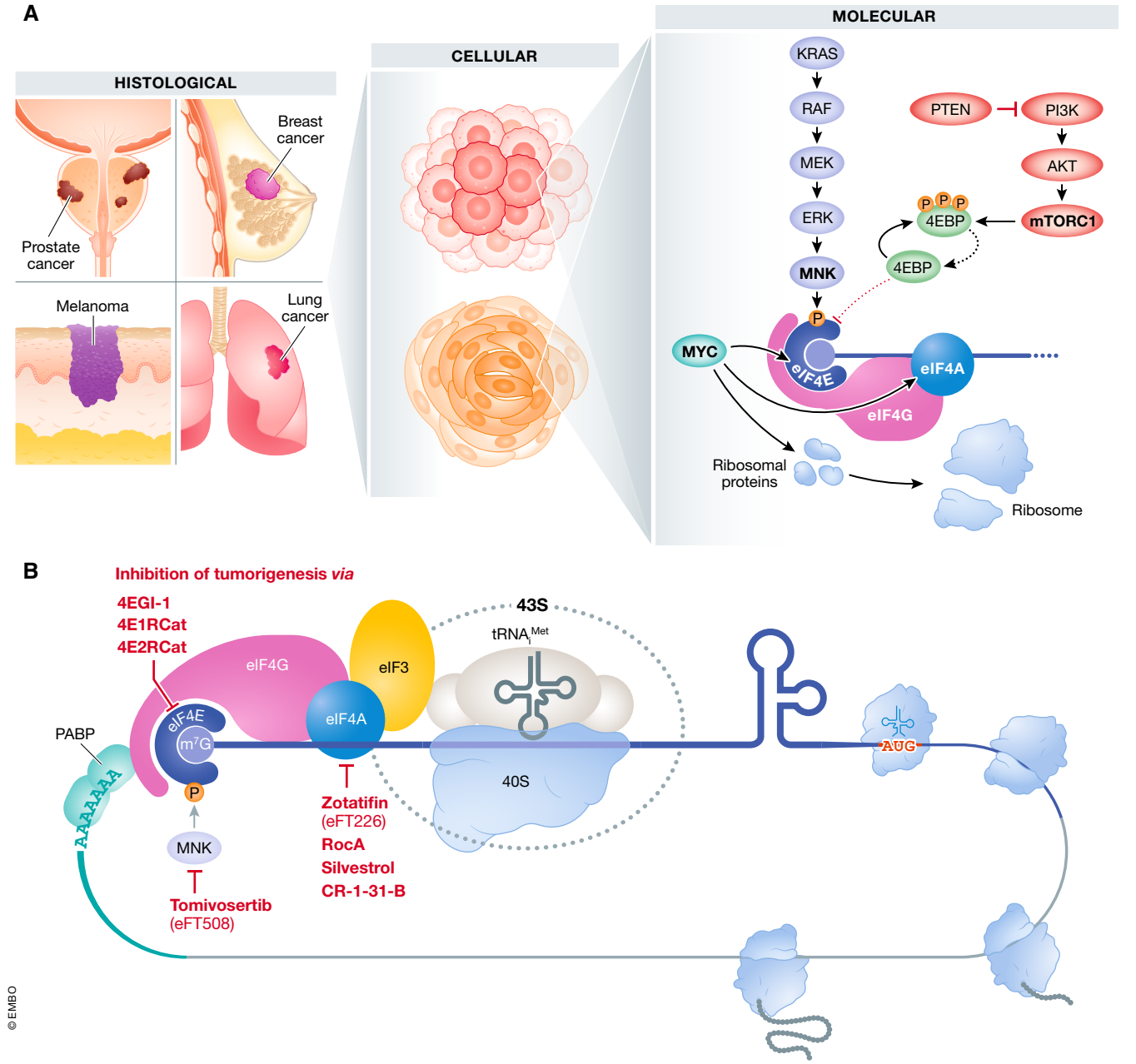

Figure 1. Oncogenic regulation and therapeutic targeting of the eIF4F complex.

(A) In various cancer types, oncogenic pathways regulate the expression and activity of the translation machinery, converging on the eIF4F complex. The Myc oncogene promotes transcription of eIF4E, eIF4A, and several ribosomal proteins. MAPK-interacting serine/threonine kinase MNK1, activated downstream of the RAS/ERK pathway, phosphorylates eIF4E which is crucial for the activity of the protein. In addition, mTORC1, which acts downstream of PI3K/AKT, phosphorylates 4E-binding proteins, 4EBPs, which in turn release eIF4E to promote translation. Unphosphorylated 4EBPs compete with eIF4G to bind to eIF4E and inhibit translation. (B) Compounds targeting eIF4F complex inhibit cancer cell proliferation and tumorigenesis *in vitro* and *in vivo*. Tomivosertib (eFT508) inhibits MNK1, which regulates the activity of eIF4E through phosphorylation, and is showing promising results in clinical trials. 4EGI-1, 4E1RCat, and 4E2RCat target the eIF4E-eIF4G interaction to block cap-dependent translation. Rocaglate derivatives, Zotatifin (eFT226), Rocaglamide (RocA), Silvestrol, and CR-1-31-B, inhibit the activity of eIF4A by clamping the protein to polypurine stretches of the RNA. Among them, Zotatifin is already in Phase II clinical trials.

associated with increased metastases and higher tumor stage in prostate cancer and ovarian cancer, respectively (Braunstein *et al*, 2007; Comtesse *et al*, 2007; Attar-Schneider *et al*, 2014; Li *et al*, 2016; Jaiswal *et al*, 2018; Valle *et al*, 2021). There are three members of the eIF4G protein family: eIF4GI (highest expression), eIF4GII (lowest expression), and DAP5 (known as eIF4G2, p97, and NAT1) (Parra *et al*, 2018). The majority of studies have focused on the role of eIF4GI and DAP5 in mediating selective translational control in cancer,

Table 1. Transcripts dependent on eIF4A for efficient translation.

Cancer model	eIF4A inhibitor	Specific mRNAs	Experimental method	Notes	Reference
T-cell acute lymphoblastic leukemia	Silvestrol	Genome-wide analysis identified 281 downregulated mRNAs including *MYC*, *NOTCH1*, *MYB*, *CDK6*, *MDM2*, *CCND3*, *BCL2* and *ETS1*	Ribosome Profiling	eIF4A1 helicase activity is essential to resolve G-quadruplex structures at the 5′UTR of the target mRNAs	Wolfe *et al* (2014)
Triple-negative breast cancer cell line MDA-MB-231	Silvestrol	Genome-wide analysis identified 284 downregulated mRNAs including *CCND1*, *ARF6*, *BCL2*, *ROCK1*, and *CDK6*	Ribosome Profiling	Silvestrol sensitivity is dependent on 5′UTR complexity	Rubio *et al* (2014)
Diffuse large B-cell lymphomas	Silvestrol	*CARD11*, *BCL10*, *MALT1*	Targeted approach		Steinhardt *et al* (2014)
$BRAF^{V600E}$ melanoma	Silvestrol	Genome-wide analysis: *CREBBP*, *HPRT*, *MLL3*, *NCOA6*, *ARID5B*, and *RICTOR*	Polysome Profiling	Combination with BRAF and MEK inhibitors inhibits the emergence of melanoma persister cells	Shen *et al* (2019)
PDAC	Silvestrol	*ARF6*	Targeted approach	KRAS promotes translation of ARF6 by suppressing Pdcd4, an inhibitor of eIF4A	Hashimoto *et al* (2019)
Breast cancer stem cells (BCSC)	Rocaglamide A (RocA)	*NANOG*, *OCT4*, and drug transporters	Targeted approach	RocA treatment reduces self-renewal ability of BCSC and induces apoptosis	Sridharan *et al* (2019)
Prostatic ductal adenocarcinoma (PDAC)	CR-1-31-B	mRNAs involved in redox and central carbon mechanism: *CDK4/6*	Polysome Profiling	CR-1-31-B suppresses PDA growth *in vitro* and *in vivo*	Chan *et al* (2019)
ER+ breast cancer, KRAS-mutant NSCLC	CR-1-31-B	mRNAs involved in the inhibition of apoptosis (*MCL1* and *BCL2*) and in the regulation of cell cycle progression (CCND1, CCND3, CCNE1, and CDK6)	Targeted approach	Combination of CDK4/5 inhibitor palbociclib with CR-1-31-B suppresses growth of the cells *in vitro* and *in vivo*	Kong *et al* (2019)
Breast Cancer Cells	Zotatifin (eFT226)	Receptor Tyrosine Kinases: *ERBB2*, *FGFR1*, and *FGFR2*	Targeted Approach	Combination with PI3K/AKT inhibitors with Zotatifin suppresses cancer cell growth *in vitro* and *in vivo*	Gerson-Gurwitz *et al* (2021)

while the function of eIF4GII remains largely unstudied. eIF4GI acts as a specific translation factor by modulating the stoichiometry of the eIF4F complex, and it also promotes selective translation through its ability to engage with internal ribosome entry sites (IRES) located in the 5′UTRs of key proangiogenic, hypoxia, and survival mRNAs (Braunstein *et al*, 2007; Silvera *et al*, 2009). eIF4GI can directly bind to the IRES elements in the 5′UTR of these mRNAs independently of eIF4E and drive cap-independent translation by recruiting additional initiation factors and the ribosome to initiate translation. Similarly, DAP5 can also promote cap-independent translation of mRNAs important for invasion, metastasis, and apoptosis, such as *BCL2*, *APAF1*, *cIAP1*, *CDK1*, and a (Hundsdoerfer *et al*, 2005; Marash *et al*, 2008; Weingarten-Gabbay *et al*, 2014). Moreover, eIF4GI can also regulate translation of a subset of mRNAs important for survival and DNA damage response pathway in breast cancer (Badura *et al*, 2012). Additionally, a recent paper also showed that eIF4GI regulates expression of specific immunoregulatory proteins in non-small cell lung cancer and
may represent a therapeutic vulnerability for this cancer type (Valle *et al*, 2021).

The majority of prior research on translation initiation has focused on the eIF4F complex, consisting of only three proteins. However, exciting new research is drawing focus to other key components of the translation initiation machinery, in addition to the eIF4F complex. A multi-protein complex eIF3, the largest initiation factor with 13 subunits, has been implicated in controlling the translation of mRNAs important for cellular proliferation (Fig 2A). eIF3 binds to 40S ribosomal subunit and promotes the binding of methionyl-tRNAi and mRNA (Hershey, 2015). To date, evidence shows that overexpression of six individual subunits (3a, 3b, 3c, 3h, 3i, and 3m), while the repression of two others (3e and 3f), can cause malignant transformation (Hershey, 2015). Recent studies have uncovered the role of specific eIF3 subunits in selective translation and their function in different diseases, including cancer (Wolf *et al*, 2020; Fujii *et al*, 2021). Transcriptome-wide assessment of eIF3 RNA binding with PAR-CLIP showed that eIF3 binds the 5′UTRs of specific mRNAs associated with cancer-related pathways, such as cell cycle control, differentiation, and apoptosis (Lee *et al*, 2015). Importantly, eIF3 binding to the oncogene *JUN* versus the tumor suppressor *BTG1* mRNAs was found to have an opposite effect on translation (Lee *et al*, 2015). Additionally, eIF3d can bind the mRNA cap, in particular the cap of *JUN* mRNA, where it is essential for the assembly of the translation initiation complex independently of eIF4F (Lee *et al*, 2016). Expanding the role of eIF3d in selective translation, a recent paper demonstrated that Myc promotes the specific translation of the *SF3A3* mRNA through an eIF3d-mediated mechanism, which, in turn, regulates splicing and metabolic reprogramming that underlie Myc-driven tumorigenesis (Cieśla *et al*, 2021). Additionally, a study showed that eIF3e promotes synthesis of the mitochondrial electron transport chain proteins through

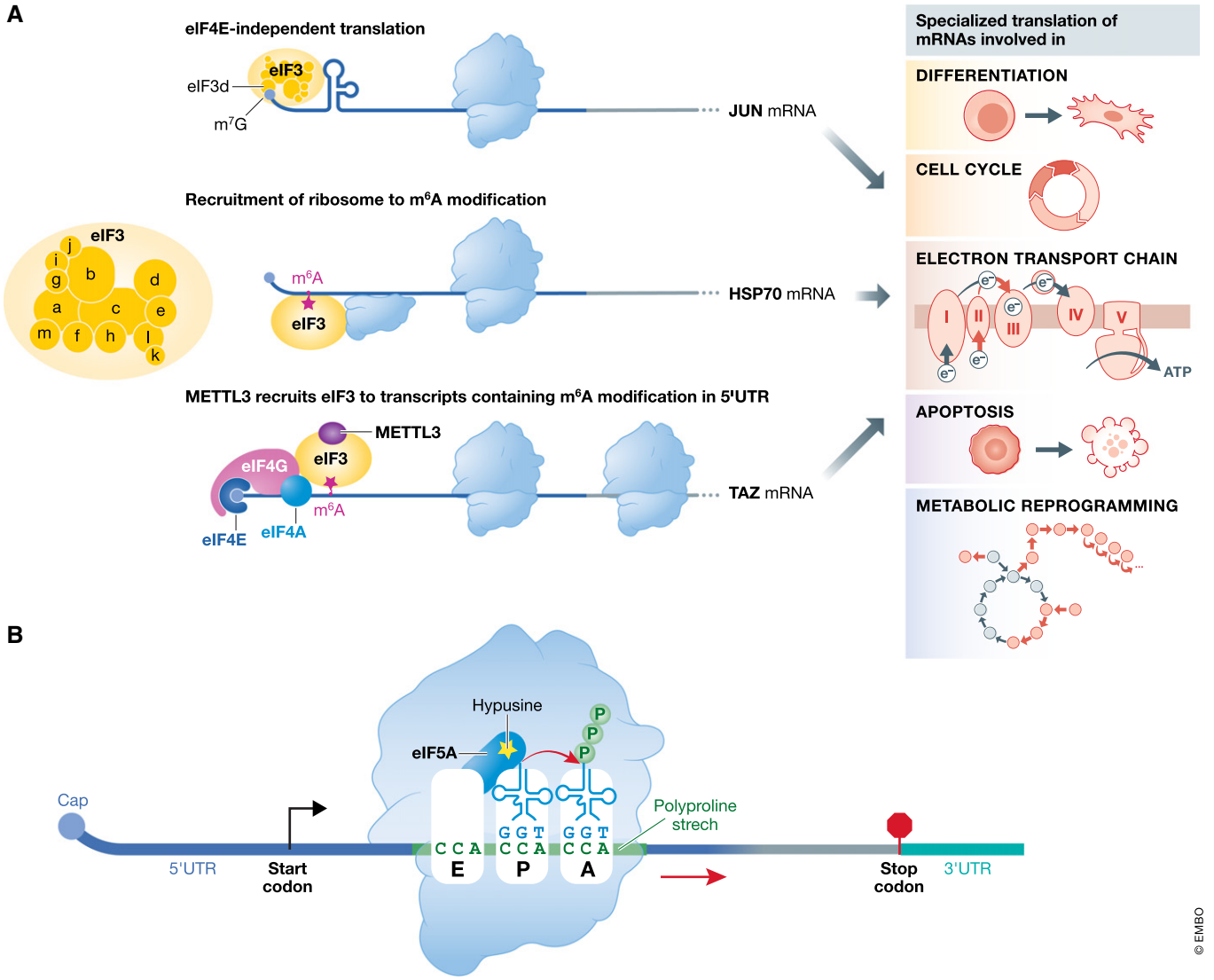

Figure 2. Roles of eIF3 and eIF5A in selective translational control.

(A) The eIF3 complex with 13 subunits regulates specialized translation of mRNAs encoding proteins involved in differentiation, electron transport chain (ETC), cell cycle, apoptosis, and metabolic reprogramming. Different mechanisms of eIF3-dependent translation are shown. eIF3 can directly bind to the cap via its 3D subunit and drive cap-independent translation (*JUN* mRNA). eIF3 can recruit the ribosome to m^{6}A modification containing mRNA for translation of specific mRNAs (*HSP70* mRNA). In addition, METTL3 can recruit eIF3 to transcripts containing m^{6}A modification in their 5′UTR and promote translation (*TAZ* mRNA). (B) eIF5A plays a role as ribosomal pause relief factor. eIF5A promotes peptide bond formation when the ribosome is stalled on a polyproline stretch. The unique post-translational modification in eIF5A, hypusination, is required for the activity of the protein.

their 5′UTRs in the MCF7 breast cancer cell line (Shah *et al*, 2016). Although the current understanding of the role of individual subunits of the eIF3 complex is limited, these initiation factors are emerging as key players in directing the expression of the cancer-specific proteome.

A growing body of evidence highlights the importance of the interaction between the eIF3 complex and the N^6-methyladenosine (m^{6}A) machinery to specify selective translation (Fig 2A). Several excellent recent reviews provided an in-depth perspective on the role of m^{6}A and RNA modifications more broadly in the translational control of cancer (Barbieri & Kouzarides, 2020; He & He, 2021). An important study demonstrated that eIF3 can directly bind to m^{6}A-modified bases in the 5′UTR of select mRNA to recruit the 43S complex and initiate cap-independent translation (Meyer *et al*, 2015). This mechanism is critical for maintaining translation of specific m^{6}A-containing mRNAs, such as *HSP70*, under stress conditions by bypassing cap-binding proteins (Meyer *et al*, 2015). eIF3 was also shown to interact with the m^{6}A "reader" protein YTHDF1 to promote the delivery of m^{6}A-containing mRNAs to the translational machinery, providing a mechanism to selectively increase translation efficiency (Wang *et al*, 2015). In addition, METTL3, an m^{6}A "writer", was shown to promote the translation of a subset of

target mRNAs, including several oncogenes such as *EGFR* and *TAZ*, by recruiting eIF3 to the translation initiation complex, independently of its methyltransferase activity or m^6A "reader" proteins, YTHDF1 or 2 (Lin *et al*, 2016). In fact, METTL3 interacts specifically with the eIF3h subunit to promote mRNA looping, enhancing ribosome recycling and the formation of densely packed polyribosomes, which together boost the translation of specific oncogenic mRNAs important for lung cancer (Choe *et al*, 2018). METTL3 can also interact with eIF3b to mediate translation of the *YAP* mRNA in lung cancer cells (Jin *et al*, 2019). Overall, the eIF3 complex is a multifaceted component of the translational machinery that integrates upstream oncogenic signals along with RNA structure and epigenetic modifications to exquisitely regulate selective post-transcriptional gene expression.

In addition to initiation factors, exciting studies are highlighting the possible roles of elongation factors in translation specificity and cancer etiology (Knight *et al*, 2020). However, precisely how elongation factors impact cancer-specific translation is poorly understood. One of the emerging elongation factors mediating selective translation is eIF5A. This protein was originally defined as an initiation factor; however, recent studies show its main role in translation elongation as a ribosomal pause relief factor (Fig 2B). In humans, there are two eIF5A isoforms, eIF5A1 and eIF5A2, both of which contain the amino acid hypusine formed by a post-translational modification unique to a specific lysine residue in eIF5A. This modified amino acid is essential for the activity of eIF5A; therefore, it has attracted attention as a therapeutic target (Mathews & Hershey, 2015). While eIF5A1 is ubiquitously expressed in most cells and tissues, eIF5A2 is specifically expressed in the testes and brain (Jenkins *et al*, 2001; Clement *et al*, 2003). Interestingly, eIF5A2 is more broadly expressed in cancers of different tissues of origins (Caraglia *et al*, 2013; Wang *et al*, 2013; Mathews & Hershey, 2015; Wu *et al*, 2020a). While the role of eIF5A1 in cancer is still puzzling and requires further investigation, the majority of mechanistic studies on specificity of translation elongation have focused on eIF5A1. Therefore, we will refer to eIF5A1/2 jointly as eIF5A in this section. eIF5A is required to promote peptide bond formation when the ribosome is stalled (Gregio *et al*, 2009; Saini *et al*, 2009; Gutierrez *et al*, 2013; Pelechano & Alepuz, 2017; Schuller *et al*, 2017). Moreover, eIF5A depletion was shown to promote translation initiation at upstream near-cognate start codons in yeast (Ivanov *et al*, 2018). An interesting recent study showed that a similar mechanism is conserved in human cells and, surprisingly, eIF5A regulates start codon selection of the *MYC* mRNA in cancer cells (Manjunath *et al*, 2019). In particular, loss of eIF5A promotes expression of an N-terminally extended c-Myc protein, demonstrating a novel translational regulation mechanism for *MYC* (Manjunath *et al*, 2019). Moreover, eIF5A may more generally regulate selective translation of oncogenes containing proline stretches or tripeptides (Met-Phe-Phe), which require eIF5A activity to prevent ribosome stalling (Saini *et al*, 2009; Gutierrez *et al*, 2013). In this way, eIF5A may be a critical factor for maintenance of cancer cell fitness by releasing stalled ribosomes to support the increased metabolic burden of oncogenic transformation. A recent study demonstrated that eIF5A promotes translation of specific mitochondrial transcripts involved in the tricarboxylic acid (TCA) cycle and oxidative phosphorylation, opening a new avenue of research into eIF5A function (Puleston *et al*, 2019). Further studies are required to elucidate the role of eIF5A in transcript-specific translation that promotes cancer survival, in particular the connection between eIF5A and mitochondrial function. These initial studies into the functions of eIF5A in cancer demonstrate that eIF5A is an intriguing novel nexus of translational control and its role in *MYC* translation may represent a potential cancer cell selective therapeutic target.

Non-coding RNAs (ncRNAs) also play a vital role as part of the translation machinery. The process of translation relies upon many different types of ncRNAs, such as ribosomal RNAs (rRNAs), transfer RNAs (tRNAs), microRNAs (miRNAs), and long ncRNAs (lncRNAs), whose functions and contributions to cancer are the subject of excellent reviews (Anastasiadou *et al*, 2018; Goodall & Wickramasinghe, 2021). Here, we will focus on how the availability of the decoding components of the translation machinery, tRNAs, regulates selective translation (Fig 3). As the decoders of the genetic code, tRNAs recognize specific triplet codons on the mRNA, mediating proper ribosomal incorporation of specific amino acids into the growing polypeptide. The human genome contains a total of 61 distinct sense codons, many of which encode the same amino acid. These 61 codons are recognized by a total of 49 tRNAs with unique anti-codon sequences. tRNAs are found to be upregulated in different cancer types, which has historically been thought to promote global protein synthesis. Major oncogenic signals like the MAPK-ERK and PI3K/mTOR pathways as well as Myc co-opt the tRNA synthesis machinery, in particular RNA polymerase III activity, to modulate tRNA expression levels in support of cancer cell proliferation (Felton-Edkins *et al*, 2003; Gomez-Roman *et al*, 2003; Woiwode *et al*, 2008; Kantidakis *et al*, 2010). However, emerging evidence suggests that cancer cells selectively increase expression of specific tRNAs to enhance translation elongation efficiency for distinct subsets of mRNAs based on their codon composition (Dittmar *et al*, 2006; Pavon-Eternod *et al*, 2009; Goodarzi *et al*, 2015). In breast cancer, upregulation of $tRNA^{Glu}_{UUC}$ and $tRNA^{Arg}_{CCG}$ promote metastasis by enhancing expression of direct target genes, such as *EXOSC2* and *GRIPAP1*, in a codon-specific manner (Goodarzi *et al*, 2016) (Fig 3). Altered expression of specific tRNAs within the tumor microenvironment can also be a crucial driver of cancer progression. Increased levels of initiator methionine tRNA, particularly in stromal fibroblasts, are sufficient to promote tumor invasion, migration, and metastasis, specifically through altered translation of extracellular matrix components and dysregulated integrin signaling (Birch *et al*, 2016; Clarke *et al*, 2016). Moreover, cancer-specific tRNA signatures can be crucial for the fate of the cells as they can coordinate the selective expression of different cellular programs, such as proliferation or differentiation (Gingold *et al*, 2014; Aharon-Hefetz *et al*, 2020). In addition, tumor cells also mistranslate with higher frequency compared to non-transformed cells and this translational error can promote tumor growth (Santos *et al*, 2018). Together these studies demonstrate that tRNAs are key regulatory components that shape the oncogenic translatome.

The relationship between tRNA availability and codon usage can also precisely tune the expression of specific cancer-promoting genes. The effect of codon bias in cancer has mainly focused on the translation efficiency of different Ras oncogene isoforms. Two members of the family, *KRAS* and *HRAS*, have predominantly rare or common codons, respectively. This difference modulates their protein expression levels, although they share 85% identity at amino

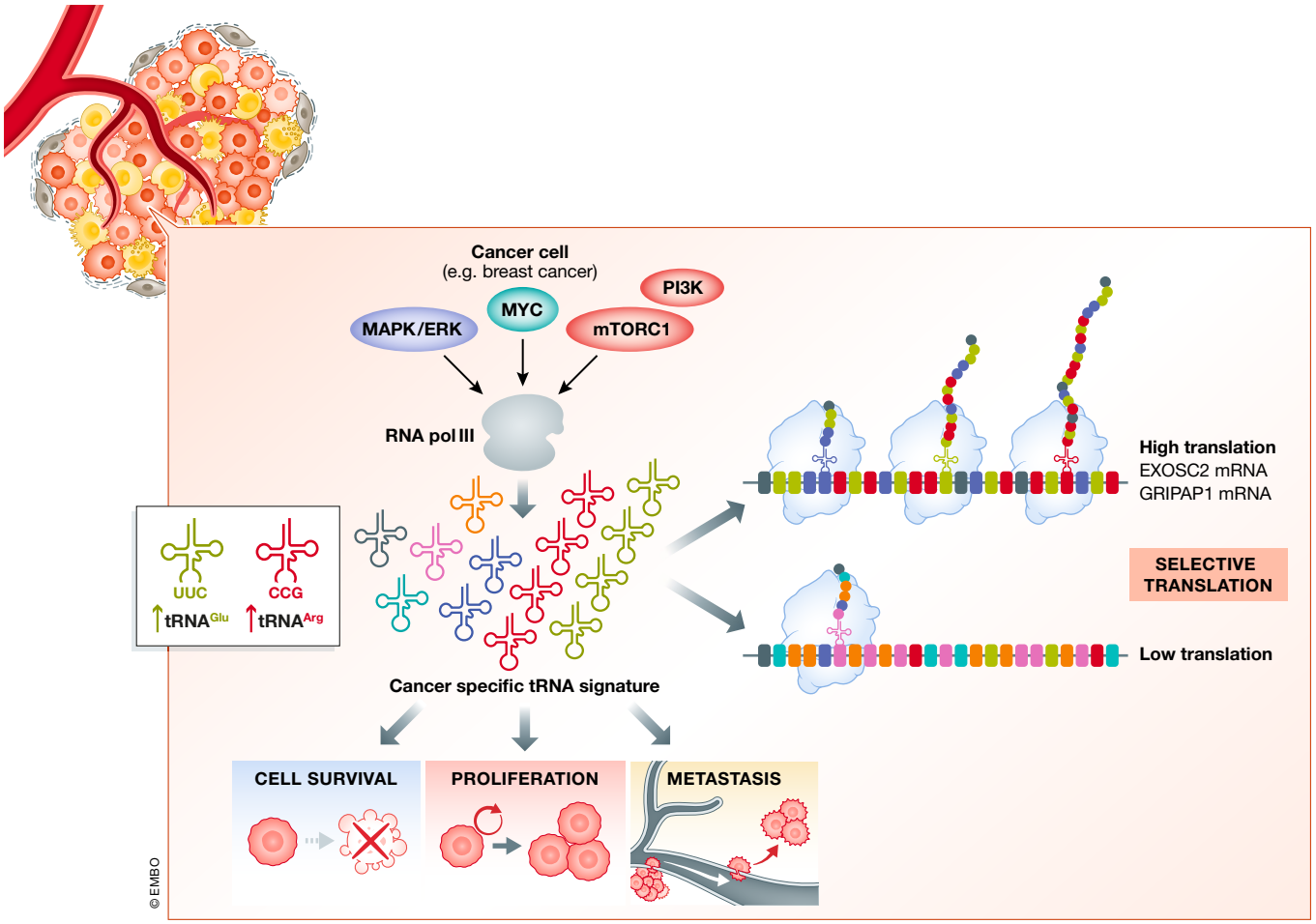

Figure 3. Codon usage and tRNAs in cancer.

Cancer-specific repertoire of tRNAs, the decoding components of the translation machinery, promote selective translation to maintain cancer cell fitness. RNA polymerase III, which is regulated by three major oncogenic pathways, MAPK/ERK, MYC, and PI3K/mTOR, can specifically alter the abundance and availability of specific tRNAs in cancer cells to promote translation elongation efficiency for specific subsets of mRNAs based on their codon composition. As an example, breast cancer cells express high levels of $tRNA^{Glu}_{UUC}$ and $tRNA^{Arg}_{CCG}$ which enhance translation of *EXOSC2* and *GRIPAP1* mRNAs in a codon-specific manner to promote tumorigenesis.

acid level (Lampson *et al*, 2013). Changing rare codons to common codons in *KRAS* not only increased the expression of K-Ras but also enhanced its tumorigenic capacity (Lampson *et al*, 2013; Pershing *et al*, 2015). *KRAS* codon usage was also shown to affect transcription rate and protein conformation (Fu *et al*, 2018). Moreover, a recent study revealed that cancer mutational bias in codons within a family of oncogenes, such as Ras, may be related to how well the coding sequence is selectively and efficiently translated in proliferating cells compared to primary, non-transformed cells (Benisty *et al*, 2020). The paradigm of Ras mRNA translation illustrates that neither mRNA transcript levels nor amino acid composition are sufficient predictors of oncogenic protein expression. The tunable and differential translation of Ras isoforms shows the centrality of translation in the process of oncogenic transformation and sustained tumorigenesis. In addition, the MAPK pathway can enhance expression of transcripts with rare codons, such as *KRAS* (Peterson *et al*, 2020), showing the complex, intertwined nature of translational regulation. Moreover, translational efficiency modulated by codon usage is not limited to RAS family members as similar codon usage patterns have also been reported for different oncogene protein families such as AKT, RAF, and FGFR (Benisty *et al*, 2020). From the classical initiation factors of the eIF4F complex to elongation factors like eIF5A to tRNA contributions, we are only beginning to appreciate how every facet of translational initiation works in concert to mediate the precise tuning of the cancer proteome to support cancer growth.

Specific regulation of mRNA transcripts to mediate expression of the cancer proteome

The translational machinery works in concert with the mRNA sequence and structure to tailor the composition of the pro-tumorigenic proteome. Many oncogenic signaling programs converge on translational control, usurping functional RNA regulatory elements in mRNA to specify the translation of pro-proliferative, pro-survival, and anti-apoptotic genetic programs to overcome the

cellular stresses of oncogenic transformation, aberrant proliferation, and adaptation to the tumor microenvironment (Xu & Ruggero, 2019). In this way, cancer cells rely on altered translational activity, creating an addiction that distinguishes cancer cells from non-transformed cells. Selective translation of cancer-promoting mRNAs depends upon both *cis*- and *trans*-regulatory factors. In *trans*, the translational machinery in conjunction with RNA-binding proteins (RBPs) precisely control the translation of specific mRNAs, often regulating the expression of functionally linked groups of transcripts (for in-depth reviews see (Pereira *et al*, 2017; Harvey *et al*, 2018; Qin *et al*, 2020)). Acting in *cis*, mRNA sequence features, such as RNA motif elements and alternative translation initiation sites (ATIS), as well as RNA structure integrate the upstream oncogenic signals to mediate the specificity and efficiency of translation.

Sequence features/motifs

Key features of mRNAs contribute to their selective translation downstream of oncogenic signaling programs. Sequence-specific RNA elements in the untranslated regions of genes have been functionally associated with altered translational efficiency in response to pro-tumorigenic signaling (Fig 4A). One of the first identified examples is the 5′-terminal oligopyrimidine tract (TOP) motif, which regulates the translation efficiency of mRNAs encoding core components of the translational machinery, including ribosomal proteins (Levy *et al*, 1991) and translation factors (Iadevaia *et al*, 2008). The 5′ TOP motif is characterized by an invariable C residue proximal to the cap followed by an uninterrupted stretch of 4–15 pyrimidines (Perry, 2005). Critically, mTOR signaling regulates the translation of TOP motif-containing mRNAs, enabling the coordinated expression of protein synthesis to control cell growth (Jefferies *et al*, 1994; Tang *et al*, 2001; Hsieh *et al*, 2012; Thoreen *et al*, 2012). In this context, mTOR activity integrates a wide variety of stress conditions or altered nutrient states common in cancer cells, such as hypoxia or amino acid starvation, to orchestrate a selective cellular response that promotes cancer cell survival (Tang *et al*, 2001; Miloslavski *et al*, 2014). Recent work has implicated additional oncogenic pathways in regulating 5′TOP-specific translational control. In particular, loss of the tumor suppressor Arf promotes the translation of 5′TOP-containing mRNAs, implicating other pathways that act alongside mTOR signaling to promote expression of pro-growth protein repertoire (Cottrell *et al*, 2020).

Genome-wide analysis of the oncogenic mTOR translation program has uncovered a broader landscape of "TOP-like" sequence motifs. Ribosome profiling of hyperactive mTOR-dependent translation in prostate cancer cells identified an enriched "TOP-like" sequence, namely the pyrimidine-rich translational element (PRTE), which is characterized by an invariant uridine at position 6 flanked by pyrimidines and, unlike the TOP motif, is located at variable positions within the 5′UTR (Hsieh *et al*, 2012). PRTE-containing genes are enriched for key regulators of metastasis, such as *YBX1* and *MTA1*, that coordinate cancer cell invasion downstream of oncogenic mTOR signaling. Additionally, the PRTE motif is necessary for the efficient translation of phosphoribosyl pyrophosphate synthetase 2 (*PRPS2)*, a rate-limiting nucleotide biosynthesis enzyme that maintains a sufficient nucleotide pool to promote MYC-induced tumorigenesis (Cunningham *et al*, 2014). The TOP and PRTE motifs demonstrate the vital role of RNA sequence elements in integrating upstream oncogenic signals from mTOR to Myc to precisely respond to the unique metabolic demands of cancer cells.

Another recently identified RNA sequence element confers an even greater specificity to the translational regulation. Transcripts possessing the cytosine-enriched regulator of translation (CERT) motif are sensitive to the expression level of the cap-binding protein, eIF4E, during early tumorigenesis. The CERT motif mediates the selective translation initiation of mRNA networks that are essential for cellular transformation (Truitt *et al*, 2015). In particular, the CERT motif was shown to modulate the expression of key target genes of the antioxidant response, particularly ferritin and glutathione, to counteract reactive oxygen species and aid cancer cell survival (Truitt *et al*, 2015). Subsequent research has implicated CERT-dependent translation as a pivotal response to cellular stress states, such as neuronal injury and regrowth as well as hyperactive mTOR signaling in epilepsy (Rozenbaum *et al*, 2018; Kim *et al*, 2019). Moreover, the CERT motif was found to be enriched in 4EBP1 target genes in prostate cancer cells, which is concordant with its initial identification in transcripts sensitive to eIF4E dosage (Jin *et al*, 2020). Intriguingly, recent work uncovered enrichment of the CERT sequence in the 5′UTR of genes dependent upon the DEAD-box helicase DDX3 for efficient translation initiation (Calviello *et al*, 2021). These data suggest an intriguing relationship between RNA structures and sequences in selective translation initiation. Notably, DDX3 can mediate the selective translation of a mRNA network that contributes to tumor development (Oh *et al*, 2016). Overall, the CERT RNA element is emerging as a nexus of translational control, orchestrating a timely response to cellular stressors, particularly in cancer formation and progression.

Another type of *cis*-regulatory element is the translation initiator of short 5′ UTR (TISU) regulatory motif that promotes accurate,

Figure 4. RNA sequence elements regulate selective translation.

(A) RNA sequence *cis*-regulatory elements in both the 5′UTR and 3′UTR of mRNAs play a role in specifying transcripts for translation downstream of certain oncogenic signals. For example, both the TOP and PRTE motifs mediate the translation of transcripts sensitive to mTOR activity. These RNA sequence elements function as part of a coordinated mechanism to regulate the translation of specific pro-oncogenic programs, such as EMT (GRE), metabolic dysregulation (PRTE), and response to oxidative stress (CERT). Consensus sequences of each motif are shown. (B) RNA sequence elements interface with RNA-binding proteins (RBPs) to regulate the translation of specific transcripts. A well-studied example is the RBP LARP1's modulation of the translation of 5′TOP motif containing transcripts. When mTORC1 is inactive, LARP1 binds the TOP motif to block eIF4F binding to the mRNA cap. However, active mTORC1 phosphorylates and physically binds LARP1 to allow eIF4F to access the cap and promote translation initiation. (C) RNA sequence features in the 5′UTR can alter translation of the main ORF (mORF). Under homeostatic conditions, the translation machinery engages upstream ORFs (uORFs) to diminish mORF translation. However, under oncogenic stress, uORF translation is suppressed to promote the translation of the mORF, which often encodes oncogenes or pro-survival factors. In a similar way, cancer cell signaling can promote translation initiation at alternative start codons that are in-frame with the main ORF to generate N-terminally extended proteins. These alternative proteoforms possess different functions from the canonical protein as has been well described for the tumor suppressor PTEN.

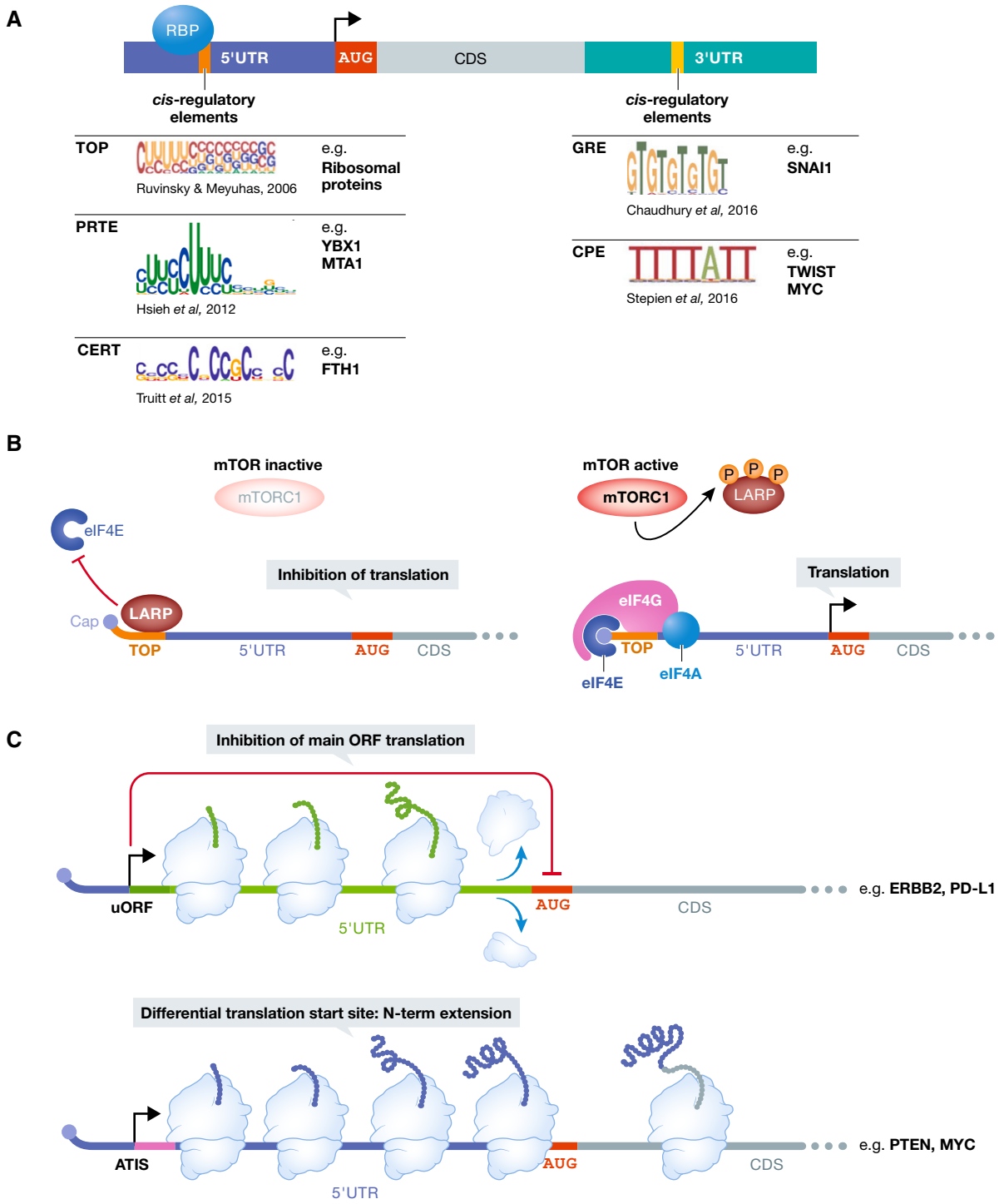

Figure 4.

efficient, and eIF4A-independent translation of genes with short 5′UTRs without scanning (Elfakess & Dikstein, 2008; Elfakess *et al*, 2011). TISU element-dependent translation mediates the synthesis of proteins involved in core biological processes such as protein biogenesis and degradation, RNA metabolism, and mitochondrial health (Sinvani *et al*, 2015; Haimov *et al*, 2017). Translation of TISU-containing transcripts requires the cap-binding complex eIF4F; however, the 48S ribosomal subunit recognizes and directly contacts the motif to promote the assembly of the complete 80S ribosomal complex and initiate translation without scanning to sustain translation of genes required for a cellular response to energy stress (Haimov *et al*, 2017). In line with its role in regulating core biosynthetic pathways, TISU-dependent translation is sensitive to mTOR inhibition, implicating the TISU motif as another mediator of

selective translation that promotes cancer cell growth and survival (Gandin *et al*, 2016). The TISU regulon demonstrates the capacity for 5′UTR sequence to selectively impinge on the translation of transcripts necessary to respond rapidly to maintain a pro-growth cellular state.

Interactions between RNA regulatory elements and RNA-binding proteins (RBPs) in the 3′UTR also contribute to the selective translational control of pro-oncogenic programs (Fig 4A). One notable motif is the guanine/uridine-rich element (GRE) found in the 3′UTR of genes important for epithelial-to-mesenchymal transition (EMT), including the EMT master regulator *SNAI1* (Chaudhury *et al*, 2016). The GRE motif resembles the binding motif for the RBP CELF1 and indeed CELF1 directly binds the motif in the 3′UTR of these genes to regulate their translation downstream of TGFβ activation through an unknown mechanism. Another example of a translational control feature in the 3′UTR is the cytoplasmic polyadenylation element (CPE). The CPE was originally described as a regulator of protein synthesis in embryonic development, but evidence also suggests a role in modulating the translation of several pro-tumoral mRNAs such as *PLAT* (encoding tPA), *TWIST1,* and *MYC* (Burns & Richter, 2008; Nairismägi *et al*, 2012; Ortiz-Zapater *et al*, 2012). The CPE is bound by a family of RBPs known as CPE-binding proteins (CPEBs) that differ in their affinities for the CPE consensus motifs and can act as either repressors or activators (Fernández-Miranda & Méndez, 2012). Of note, CPEB4 is overexpressed in pancreatic cancer and glioblastoma and is a key regulator of oncogenic driver expression in early melanoma formation (Ortiz-Zapater *et al*, 2012; Pérez-Guijarro *et al*, 2016).

The tumor-specific overexpression of certain CPEBs can be exploited to program cancer-specific translation. For example, researchers have employed an oncolytic adenovirus with CPE regulatory sequences in the 3′UTR of the E1A gene to selectively kill tumor cells in an *in vivo* pancreatic cancer model with high CPEB4 protein levels (Villanueva *et al*, 2017). Although the precise mechanisms of 3′UTR-driven translational control and potential collaborations with 5′UTR elements are only beginning to be revealed, they present great therapeutic potential for cancer-specific treatments.

The mechanistic details of how RNA sequence elements interface with *trans*-factors to control gene expression in cancer remains poorly understood. A working hypothesis is that upstream oncogenic pathways coordinate the recruitment of distinct RBPs and translation factors to these motifs (Fig 4B). A prime example is the proposed mechanism of how mTOR signaling modulates the interaction between the RBP LARP1 and the 5′ TOP motif to regulate translation (Tcherkezian *et al*, 2014; Fonseca *et al*, 2015). Detailed mechanistic experimentation has revealed that when mTOR is inactive, LARP1 competes with the eIF4F cap-binding complex to block translation initiation. However, when activated, mTORC1 phosphorylates LARP1 to promote mRNA release and mTORC1 directly sequesters LARP1 via interaction with mTORC1 complex member raptor, thus promoting efficient translation initiation (Lahr *et al*, 2017; Philippe *et al*, 2017; Jia *et al*, 2021). Importantly, the expression of LARP1 itself is also altered in cancer and its dysregulation predicts poor survival in a broad array of cancer types, including lung, colorectal, ovarian, and hepatocellular carcinoma (Xie *et al*, 2013; Mura *et al*, 2015; Hopkins *et al*, 2016; Ye *et al*, 2016). The mechanism of LARP1 and 5′TOP-dependent translation demonstrates the ability of oncogenic signaling to coordinate translation of core cell growth genes through the dynamic binding of a distinct RBP. More broadly, RBPs are emerging as key interpreters of upstream oncogenic signals and microenvironmental stressors conveying the message into rapid and specific translation of functionally related transcripts, as has been recently demonstrated downstream of Myc signaling and under hypoxic conditions (Ho *et al*, 2020). Current research has yet to uncover how the repertoire of RNA motifs in the 5′ and 3′UTR interface with these RBPs to coordinate translation of mRNA networks underlying diverse cellular processes associated with tumor development.

Alternative translation initiation sites

Another important 5′UTR feature that regulates selective translation initiation is the alternative translation initiation site (ATIS), which is located upstream of the start AUG initiation codon of the main open reading frame (Fig 4C). Many ATIS are part of small ORFs, called upstream ORFs (uORFs), which are found in nearly 50% of all human genes and typically act to repress translation of the downstream main ORF (Calvo *et al*, 2009). Genome-wide ribosome profiling in mammalian cells has shown that many uORFs are associated with translating ribosomes (Ingolia *et al*, 2011). Mechanistically, uORFs modulate protein abundance through either ribosomal dissociation from the mRNA after termination of the uORF or through stalled ribosomes on the coding sequence of the uORF (Zhang *et al*, 2019). Therefore, efficient translation of the main ORF requires bypass of the uORF start codon, referred to as "leaky" scanning, where ribosomes can reinitiate downstream of the uORF through a mechanism that is poorly understood. Researchers have developed new selective mRNA footprinting techniques that allow comparison of the location of 40S versus 80S ribosomes and their association with initiation factors (eIF3B, eIFS21, eIF4G, and eIF4E) on mRNA transcripts in human cells (Bohlen *et al*, 2020; Wagner *et al*, 2020). The data show that 80S ribosomes located within uORFs can maintain contact with eIF4E and the translation initiation machinery to promote efficient downstream reinitiation at the AUG of the main ORF (Bohlen *et al*, 2020). Future studies using this technique to compare the footprints of different ribosomal complexes in non-transformed and oncogenically transformed cells will help illuminate the mechanisms by which cancer cells rewire translational control through uORF regulation.

How do cancer cells exploit uORF regulation to selectively synthesize a pro-tumorigenic proteome? The 5′UTRs of oncogenes are enriched for uORFs, enabling the fine tuning of their expression levels under homeostatic conditions (Kozak, 1987). Specifically, in non-transformed cells, uORFs can repress the translation of mRNAs encoding oncogenes. However, cell stress conditions can favor uORF bypass and translation of the main ORF, enabling an appropriate, but temporally limited response until the cell regains homeostatic balance. A prominent set of genes under tight uORF translational control are the majority of human tyrosine kinase genes. Experiments have demonstrated that removal of their respective uORFs results in enhanced translation of the main ORF (Wethmar *et al*, 2016). One specific example is the translation of *ERBB2,* which encodes the HER2 receptor and is a common breast cancer driver. The 5′UTR of *ERBB2* contains a uORF close to the main start codon that impairs main ORF translation under physiological conditions (Child *et al*, 1999). However, oncogenic stress can promote *trans*-factor binding to an element in the 3′UTR to repress

this inhibition and promote efficient translation of the main ORF (Mehta *et al*, 2006; Spevak *et al*, 2006). Additionally, cancer cells can selectively drive oncogene or tumor suppressor translation through the use of an alternative promoter/transcription start site such that the 5′UTR of the resulting mRNA isoform either includes uORFs upstream of tumor suppressors, such as in *BRCA1*, or excludes uORFs upstream of oncogenes such as in the negative p53 regulator *MDM2 (*Brown *et al*, 1999; Sobczak & Krzyzosiak, 2002*)*. Additionally, changes in the cancer cell microenvironment can also suppress expression of uORF-containing transcripts. In particular, a recent study showed that in response to hypoxia and anti-cancer therapies, breast cancer cells preferentially express *NANOG*, *SNAIL*, and *NODAL* transcripts that lack repressive uORFs in their 5′UTRs, which facilitates a stem cell-like state and cancer persistence in unfavorable conditions (Jewer *et al*, 2020). Cancer cells have found varied means to exploit and circumvent uORF-mediated translational regulation to promote oncogenic growth. Cancer cells' unique reliance on uORF bypass to tailor their proteomes also provides a selective vulnerability, which can be targeted therapeutically.

Previous research has provided some insights into the mechanisms underlying uORF-dependent selective translation. Intensive dissection of the translation of *ATF4*, a transcription factor that is the main downstream regulator of the integrated stress response (ISR), has shed light on factors that suppress uORF translation to promote main ORF translation. Under a variety of cellular stress conditions, global translation is repressed while the ATF4 protein is newly synthesized to orchestrate the cell's transcriptional response to stress (Wortel *et al*, 2017). Work has shown that cancer cells depend on the translation of *ATF4* and the integrated stress response to mitigate the persistent stress of both uncontrolled oncogenic signaling leading to an increased metabolic load as well as external stressors, such as hypoxia, to promote continued tumor growth and therapy resistance (Bi *et al*, 2005; Falletta *et al*, 2017; Nguyen *et al*, 2018). The *ATF4* 5′UTR contains two uORFs that under physiological conditions repress downstream translation. However, stress conditions cause a reduction in the eIF2-GTP-Met-tRNA ternary complex resulting in "leaky" scanning such that ribosomes reinitiating after uORF1 bypass the uORF2 start codon to translate the main ORF encoding ATF4 (Vattem & Wek, 2004). Additionally, recent work demonstrated that the DENR-MCTS1 ribosome recycling complex is necessary for this reinitiation downstream of uORF1 to translate *ATF4* and may be a more general mechanism for selectively translating key oncogenes such as *ARAF*, *RAF1*, and *CDK4* (Bohlen *et al*, 2020).

An important recent example of selective uORF-dependent translation initiation during *in vivo* tumorigenesis is suppression of uORF translation to increase expression of the immune checkpoint protein PD-L1 in aggressive cancers (Xu *et al*, 2019). This study suggests that similar mechanisms promoting *ATF4* translation also act on the *CD274* mRNA (encoding PD-L1), thereby diminishing the anti-tumor T-cell response and fostering tumor immune evasion. Another study implicates eEF2K, an atypical protein kinase that negatively modulates the translation elongation stage, in fostering uORF bypass for increased PD-L1 protein synthesis (Wu *et al*, 2020b). Other interesting research demonstrated that during the ISR, eIF5B plays a role in promoting efficient *CD274* translation (Suresh *et al*, 2020). Intriguingly, a genome-wide study of translation in a mouse model of skin squamous cell carcinoma formation demonstrated a counterintuitive increase in the occupancy of uORFs during early tumorigenesis, particularly in cancer-related genes *NRAS*, *CD44*, *Ki67*, and *RAC1* (Sendoel *et al*, 2017). The authors show that under stress conditions, alternative translation initiation factor, eIF2A, is required for the increase in the translation of the main downstream ORF in these uORF-containing transcripts, although the mechanism of action is unclear. In support of the human disease relevance of this finding, eIF2A is upregulated in many squamous cancer types and correlates with poor patient outcomes (Sendoel *et al*, 2017). Further research into the functional impact of uORF number, length, and location will be key to understanding how oncogenic signaling regulates the translational control of cancer-related genes to allow precise adaptation to rapidly changing internal and external cellular environments.

Many studies have documented genetic events that result in uORF gain or loss in specific pro-tumorigenic genes, promoting oncogenic transformation and progression. A classic example is a hereditary mutation in the 5′UTR of *CDKN2A* that generates a *de novo* uORF, which decreases the expression of the encoded tumor suppressor p16(INK4A) and predisposes carriers to melanoma (Liu *et al*, 1999). A similar mechanism of action is observed with a deletion in the 5′UTR of *CDKN1B*, decreasing expression of the tumor suppressor p27(Kip1) to cause inherited multiple endocrine neoplasia syndrome type 4 (Occhi *et al*, 2013). Given the strong impact of inherited, germline mutations altering uORFs, researchers undertook a systematic search for cancer-associated changes in uORFs that identified ~400 mutations that could impact uORF loss or gain of function (Schulz *et al*, 2018). They highlight loss-of-function uORF mutations in *EPHB1* in breast and colon cancer, and in *MAP2K6* in a colon adenocarcinoma sample, which functionally enhanced translation. With the increasing availability of whole-genome sequences, new germline mutations that predispose individuals to cancer are being described, such as a study of 15,708 individuals that identified mutations in the 5′UTR of *NF2* that cause loss of a uORF, causing neurofibromatosis (Whiffin *et al*, 2020). Interestingly, germline polymorphisms can generate *de novo* uORFs that influence response to therapy, such as the novel uORF in the 5′UTR of the DNA damage repair gene, *ERCC5*, that promotes its selective translation after treatment with platinum-based chemotherapy to foster therapy resistance (Somers *et al*, 2015). Excitingly, the ever-expanding whole-genome sequencing of both normal and cancerous tissues will likely pinpoint more mutations that alter uORFs to modulate downstream translation, which will provide new means to identify oncogenic drivers as well as guide the selection of appropriate targeted therapeutics.

Finally, cancer can modulate the efficiency of translation from an upstream ATIS that is in frame with the main ORF to generate longer, alternative proteoforms that exhibit unique functions (Fig 4C). A paradigm of a gene which gives rise to N-terminally extended isoforms is *PTEN*, a key negative regulator of PI3K/Akt signaling. At least four extended translational variants of PTEN have been described, although the nomenclature differs among publications (Malaney *et al*, 2017). The best studied is PTEN long (equivalent to PTENα), which is produced from an upstream CUG to generate a 173 amino acid N-terminal extension (Hopkins *et al*, 2013; Liang *et al*, 2014a). The N-terminal extension permits PTEN long secretion from the cell to non-cell autonomously inhibit PI3K signaling and repress tumorigenesis (Hopkins *et al*, 2013). Additional work demonstrated a role for PTEN long/PTENα in promoting mitochondrial respiratory chain function and promoting ATP production; while PTENβ specifically

localizes to the nucleolus where it suppresses ribosomal RNA processing and decreases cell proliferation (Liang *et al*, 2014a, 2017). Finally, recent research functionally described another ATIS-encoded N-terminal extended form, PTENε, which includes an additional 72 amino acids and acts to suppress filopodia formation and diminishes the invasion and migration of cancer cells (Zhang *et al*, 2021). The example of the intricate regulated expression of diverse *PTEN* proteoforms highlights the need to look beyond the mRNA transcript level towards the role of selective translation in shaping a diverse proteome suited to cancer cell survival and adaptation to stressors. Another critical cancer-related gene that encodes an ATIS-produced N-terminal extension is the oncogene *MYC*. The long form of c-Myc is produced from an alternative CUG upstream of the main AUG and its expression depends in part on the activity of the ribosomal pause relief factor eIF5A (Hann *et al*, 1988; Manjunath *et al*, 2019). This longer form of c-Myc appears to have a tumor suppressive function via its occupancy of unique DNA-binding sites and pro-apoptotic properties (Hann *et al*, 1994; Benassayag *et al*, 2005). However, the role of the long form of c-Myc in cancer initiation and progression *in vivo* remains unknown.

Structural regulatory elements

RNA structures play a powerful role in regulating the translation of key cancer-related genes. Early on, it was noted that the majority of transcripts encoding oncogenic drivers had longer than average 5′UTRs, which promotes the formation of secondary structure and hinders efficient 40S ribosomal scanning (Kozak, 1987). Under homeostatic conditions, these structures precisely regulate translation initiation to selectively limit protein levels and maintain appropriate composition of the expressed proteome. Cancer cells can hyperactivate the eIF4F complex, in particular the eIF4A helicase, to promote unwinding of highly structured 5′UTRs and selectively increase the translation of pro-oncogenic genes, such as cell cycle regulators and growth factor receptors (Wolfe *et al*, 2014). For example, the mRNA encoding the master transcription factor and proto-oncogene c-Myc has a highly structured 5′UTR that enables exquisite regulation of its translation (Stoneley *et al*, 2000; Cobbold *et al*, 2008). Although many RNA structures that drive the translation of cancer essential genes have been identified, research is only beginning to understand the mechanisms behind this very diverse class of RNA elements. However, it is clear that RNA structures in both the 5′ and 3′UTRs enable critical switching between modes and efficiency of translation to permit the sustained expression of vital cancer genes even under the many inhospitable conditions of the tumor microenvironment.

Many genes encoding the key effectors of the "hallmarks of cancer" contain structured elements in their 5′UTRs known as internal ribosome entry sites (IRES), which permit cap-independent translation (Fig 5A). IRES-dependent translation enables efficient translation of certain genes when cap-dependent translation is downregulated, particularly under the stress conditions of hypoxia, low nutrient states (e.g., amino acid starvation), ER stress, and DNA damage, which are all common during cancer progression and/or after chemotherapeutic treatment (Kawai *et al*, 2004; Qin & Sarnow, 2004; Blais *et al*, 2006; Bushell *et al*, 2006; Thomas & Johannes, 2007). Important and diverse mediators of cancer progression contain cellular IRESs in their 5′UTRs, such as genes that promote cellular survival (e.g., *BCL2*, *XIAP*, and *APAF-1*), metabolic rewiring (e.g., ferritin), angiogenesis (e.g., *VEGF* and *FGF2*), the response to hypoxia (e.g., *HIF1A*), and EMT (e.g., *SNAIL* and *ZEB2*) (Stein *et al*, 1998; Holcik *et al*, 1999; Coldwell *et al*, 2000; Lang *et al*, 2002; Sherrill *et al*, 2004; Braunstein *et al*, 2007; Beltran *et al*, 2008; Evdokimova *et al*, 2009; Daba *et al*, 2012; Morfoisse *et al*, 2014; Philippe *et al*, 2016). Genome-wide polysome profiling analyses of different stress states as well as functional, high-throughput discovery of cap-independent translation elements in the human genome indicate that ~10–15% of all human 5′UTRs harbor the potential for cap-independent translation through a "cellular IRES" (Spriggs *et al*, 2008; Weingarten-Gabbay *et al*, 2016). Interestingly, some of the genes preferentially translated in a cap-independent manner under different stress conditions are non-overlapping, suggesting coordinated translation of subsets of IRES-containing genes in response to unique pathophysiological states.

The precise mechanisms that mediate selective IRES-dependent translation in cancer are only beginning to be elucidated. IRES structures are bound by RNA-binding proteins referred to as IRES transacting factors (ITAFs) as well as select translation factors, which, together, enable the recruitment of the 40S ribosome to promote cap-independent translation initiation. For example, selective translation through the cellular IRES in the *MYC* 5′UTR is dependent upon a subset of canonical cap-dependent factors, such as eIF4G and eIF3, as well as the RBPs PTBP1 and YBX1 (Fig 5A) (Spriggs *et al*, 2009; Cobbold *et al*, 2010). In multiple myloma, mutations within the *MYC* IRES strengthen the binding of PTBP1 and YBX1, upregulating c-Myc protein expression, and promoting a feed-forward loop wherein c-Myc increases the transcription of *YBX1* to drive tumorigenesis (Cobbold *et al*, 2010; Bommert *et al*, 2013). YBX1 has emerged as a key ITAF involved in the cap-independent translation of genes with diverse, pro-oncogenic functions. For example, YBX1 binds the 5′UTR of the gene encoding p16/INK4A to mediate response to hypoxia, to the IRES of *SNAIL* to coordinate an EMT translational program as well as mediating the cap-independent translation of *TGFB1* (Hu *et al*, 1999; Chappell *et al*, 2000, 2004). Additionally, the initiation complex eIF3 can bind directly to the mRNA of the anti-apoptotic factor, *XIAP*, where it appears to function as a scaffold to recruit additional RBPs and the 40S ribosome to promote translation initiation (Thakor *et al*, 2016). Excitingly, inhibition of ITAF interaction with its corresponding IRES may be a potent anti-cancer treatment. Research has demonstrated that inhibiting the binding of hnRNP A1 to both the *MYC* and *CCND1* (Cyclin D1) transcripts may be a potential therapy for glioblastoma (Holmes *et al*, 2016). Finally, ITAFs can also act to repress the translation of their target transcripts, such as the tumor suppressor PDCD4. PDCD4, whose protein level is regulated by activated ribosomal protein S6 kinase 2 (S6K2), can bind *XIAP* and *BCLXL* mRNAs and directly repress their translation to block tumorigenesis (Liwak *et al*, 2012). Only a small subset of ITAFs and other factors that regulate IRES-dependent translation have been identified. Hopefully, new technologies that enable high-throughput, functional identification of proteins that bind to and regulate translation through these structures will shed light on the complex mechanisms that modulate the cancer translatome.

While many studies have focused on structural elements upstream of the start codon, RNA structures in the 3′UTR can also mediate the translational efficiency of critical oncogenic programs (Fig 5B). One notable structural RNA regulon in the 3′UTR is the

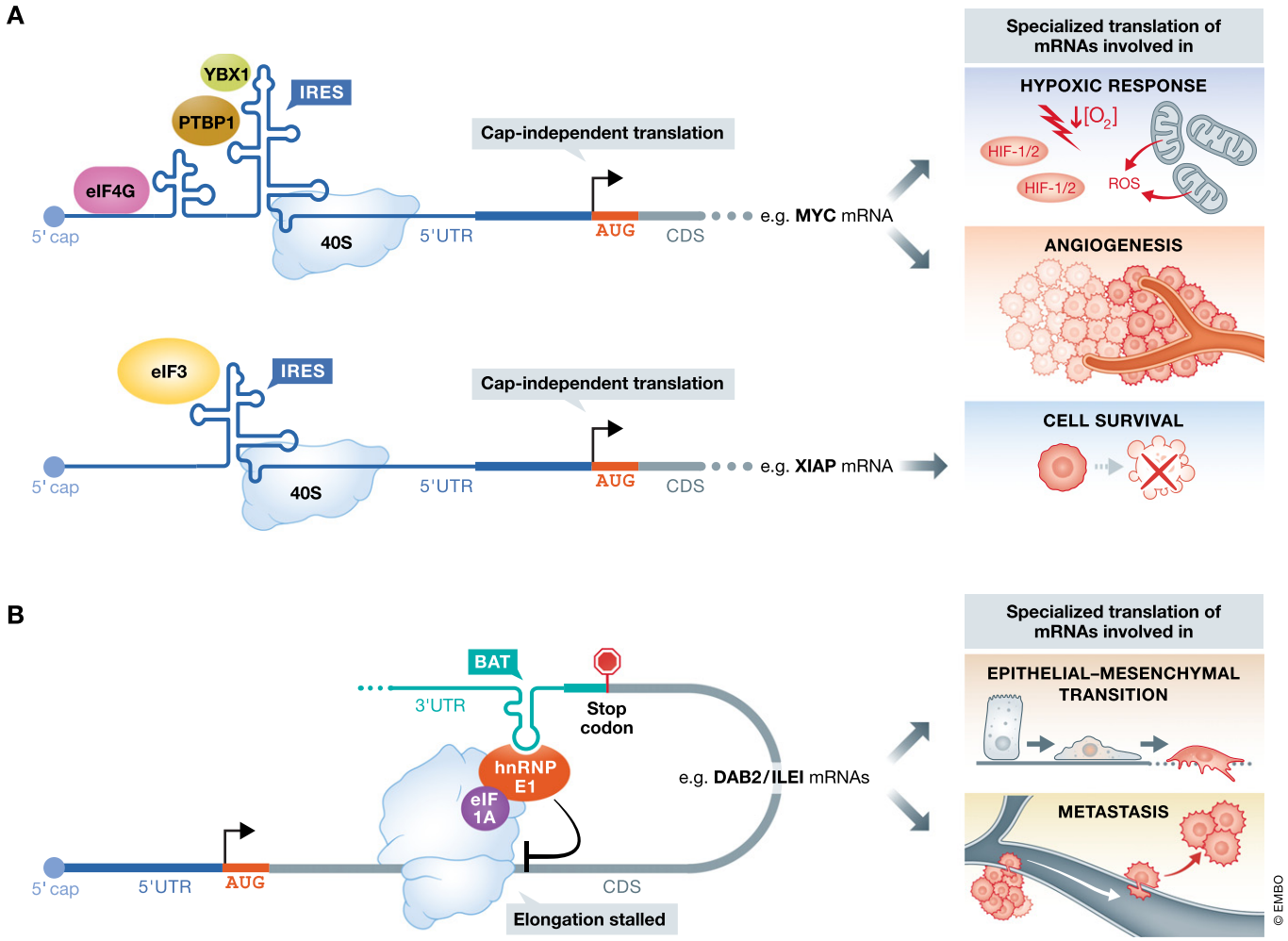

Figure 5. RNA structures mediate translational control.

(A) The 5′UTRs of key pro-tumorigenic transcripts contain RNA structures that promote selective translation initiation. The internal ribosome entry site (IRES) allows for cap-independent translation of mRNAs critical to cancer cell growth and survival in the setting of decreased cap-dependent translation, such as under low nutrient conditions or hypoxia. A key example of IRES-dependent translational control is the oncogene *MYC*. The coordinated binding of the RBPs YBX1 and PTBP1 and the translation initiation machinery to the IRES-like structure in the *MYC* 5′UTR can initiate cap-independent translation in a cancer setting. Another example is the anti-apoptotic factor *XIAP*. Binding of eIF3 to the cellular IRES located in the *XIAP* 5′UTR drives cap-independent translation. (B) Structures in the 3′UTR of mRNAs can regulate translational elongation to coordinate the selective expression of key hallmarks of cancer. One important example is the function of the TGFβ-activated translational (BAT) element in mediating the synthesis of proteins involved in EMT processes.

TGFβ-activated translational (BAT) element that consists of a stem loop with an asymmetrical bulge, which coordinates a translational program that promotes EMT. Active TGFβ signaling promotes phosphorylation and subsequent release of hnRNP E1 from the BAT element in the 3′UTRs of both *DAB2* and *ILEI*, which are both necessary for the induction of EMT (Chaudhury *et al*, 2010). Under physiological conditions, hnRNP E1 blocks translational elongation by inhibiting the release of eEF1A from the ribosomal A site (Hussey *et al*, 2011). Consequently, depletion of hnRNP E1 is sufficient to promote EMT and metastasis of breast epithelial cells (Hussey *et al*, 2011). TGFβ is a fascinating nexus of post-transcriptional regulation in that its own translation is also modulated in a cap-independent fashion through an IRES (Kim *et al*, 1992; Jenkins *et al*, 2010). In the future, it would be interesting to assess whether and how the structure of 5′ and 3′UTR regulatory elements change in real time during different steps in cancer development. The results of these studies would be particularly important for the development of new small molecules that recognize specific RNA structures that can be exploited as cancer therapeutic interventions.

Current and emerging technologies to study translational control in cancer

Methods to study how RNA and proteins interact to modulate translation in non-transformed cells, cancer cells, and within the tumor microenvironment have been pivotal in deciphering the oncogenic translational program. To directly assay the translation of specific mRNAs, the gold standard methodology remains polysome profiling. Polysome profiling entails the isolation of the cell type of

interest and centrifugation of the cytoplasmic fraction on a sucrose gradient to enable separation of the free ribonuclear proteins (RNPs), mRNA occupied with few polysomes, and the high polysome fraction. Specific mRNA abundance within each fraction can be analyzed via qRT–PCR or high-throughput sequencing. Alternatively, genome-wide ribosome profiling, in which mature ribosomes are bulk isolated, treated with nuclease, and the resulting "footprints" are analyzed with high-throughput sequencing can be employed (Ingolia *et al*, 2009; McGlincy & Ingolia, 2017). Ribosome profiling has contributed to both our fundamental understanding of selective translation initiation, for example, the use of alternative initiation codons and the role of uORFs in suppressing translation, as well as how cancer cells, anti-cancer therapies, and the tumor microenvironment reshape the translation landscape (Ingolia *et al*, 2011, 2018; Hsieh *et al*, 2012; Xu *et al*, 2019). While ribosome profiling is an important tool for the study of translation control, the technique has certain limitations. Ribosome profiling data present a single snapshot in time and, for example, cannot differentiate a stalled from a translating ribosome or a *bone fide* start codon from an internal ATG. However, experimental modifications, such as harringtonine treatment, which immobilizes the ribosome immediately after initiation and results in footprint enrichment at initiation sites can provide more detailed insights (Ingolia *et al*, 2011; McGlincy & Ingolia, 2017). Additional considerations for ribosome profiling experiments versus other techniques have been reviewed elsewhere (Brar & Weissman, 2015; Ingolia *et al*, 2018). Overall, ribosome profiling remains a valuable technique to assess translation control in a relatively straightforward and unbiased manner.

While ribosome profiling remains an incredibly powerful tool for transcriptome-wide analysis of translation efficiency, many groups have evolved the technique to provide more detailed insights into translational control (Table 2). One key new set of techniques enable the profiling specifically of the small ribosomal subunit during the course of translation initiation, extending beyond analysis of the complete 80S ribosomes assayed by the traditional method. Referred to as translation complex profile sequencing (TCP-seq), this family of techniques enable the dissection of the steps of translation initiation at nucleotide resolution across the whole genome, which will be useful in understanding how oncogenic signaling programs direct the translation of specific pro-tumorigenic RNA transcripts (Archer *et al*, 2016; Bohlen *et al*, 2020). Other groups have further modified ribosome profiling to assess both tissue-specific translation from a mixed cell population as well as to measure translational efficiency in subcellular compartments (Sanz *et al*, 2009; Jan *et al*, 2014). In the future, both techniques can provide critical insights into the regulation of translation both within a cancer cell as well as in other cell types that constitute the tumor microenvironment. All of these existing technologies require material from many cells. However, ribosome profiling at the single cell level will shed light on how a cancer cell responds to the complex mixture of internal oncogenic stressors as well as the many external insults. Toward this goal, a technique called Ribo-STAMP was recently published that harnesses an RNA editing enzyme coupled to a ribosomal protein to tag bound mRNAs, which can be analyzed with single-cell RNA sequencing (scRNA-seq) (Brannan *et al*, 2021). Excitingly, a new single-cell ribosome profiling technique employs enzymatic reactions at the single-cell scale, followed by pooled sequencing and machine learning-based data analyses, which was successfully applied to specific, rare populations of primary colon cells (VanInsberghe *et al*, 2021). These constantly evolving ribosome profiling technologies will be powerful tools to precisely, yet in an unbiased manner, measure the translation of cancer cell-specific genetic programs.

Many new techniques have been developed to capture RNA–protein interactions which will be very important in the study of different steps of tumor development and therapeutic response (Table 2). This family of approaches generally referred to as cross-linking and immunoprecipitation followed by sequencing (CLIP-seq) typically employ UV to cross-link bound RNA to an RBP of interest in a specific cell or tissue type and have proven invaluable in assessing the compendium of RNAs bound by an RBP (Ramanathan *et al*, 2019; Hafner *et al*, 2021). This technique has also been extended to translation factors, such as eIF4E, and could be implemented to identify RNAs bound by the translation initiation machinery *in situ* within a cancer (Jensen *et al*, 2021). Enormous effort has been put into generating a baseline catalog of the RNAs bound by more than 100 different RBPs, which provides a starting point in understanding how RBPs orchestrate the translation of specific functional groupings of transcripts (Nostrand *et al*, 2020). Ultimately, the power of these techniques will be to illuminate how certain oncogenic signals direct RBPs to cooperate with each other and the translational machinery to promote cancer cell survival and growth.

Computational prediction of RNA structure based on phylogenetic comparison or free energy minimization has been the most readily available method to predict RNA secondary structures; however, the accuracy of these prediction algorithms is limited (Mailler *et al*, 2019). Biophysical approaches, such as X-ray crystallography, nuclear magnetic resonance (NMR), and cryogenic electron microscopy (Cryo-EM), can produce very powerful structural data but face many limitations with respect to RNA length, RNA flexibility, and lack of native cellular environment (Mailler *et al*, 2019). In the last decade, new RNA probing approaches to map nucleotide accessibility have dramatically improved the analysis of RNA structure in living cells (Table 2). Most techniques to probe RNA structure are based on chemical compounds that modify specific RNA bases in a manner that causes the reverse transcriptase (RT) enzyme to stop, which can be read out with high-throughput sequencing as a shortened cDNA (RT stop), or cause a mutation at the modified base. For studies of single transcript or genome-wide RNA structure in living cells, which requires cell permeable reagents, the most commonly used methods are dimethyl sulfate (DMS)-Seq and selective 2-hydroxyl acylation analyzed by primer extension (SHAPE) (Rouskin *et al*, 2014; Spitale *et al*, 2015). The nucleotide accessibility data can be incorporated into *in silico* predictions to generate high-confidence RNA structure models. These structural predictions can then be verified through mutate and map strategies, in which mutations are introduced one at a time along the RNA sequence and the structural changes are analyzed to see if they match the prediction of the model (Kladwang *et al*, 2011). This technique has traditionally been applied *in vitro* but has recently been developed into an *in vivo* technique called in-cell mutate and map (icM^2) (Kladwang *et al*, 2011; Byeon *et al*, 2021). In the future, the combination of in cell RNA structural measurements with the rates of translational

Table 2. Methods to study translational control.

	Methods	Description	Reference
Methods to study translational efficiency	Ribosome Profiling	Genome-wide analysis of ribosome footprints.	Ingolia *et al* (2009)
	Translation complex profile sequencing (TCP-seq)	Specific profiling of the footprints of small ribosomal subunit during translation initiation. Adaptations of the technique isolate-specific translation initiation complexes interacting with 40S ribosomes.	Sanz *et al* (2009), Jan *et al* (2014), Archer *et al* (2016), Bohlen *et al* (2020), Wagner *et al* (2020)
	Ribo-STAMP	Exogenous expression of RNA editing enzyme coupled to a ribosomal protein to tag ribosome-bound mRNAs, which are analyzed with single-cell RNA sequencing (scRNA-seq).	Brannan *et al* (2021)
	Single-cell ribosome sequencing (scRibo-seq)	Single-cell-level ribosomal footprinting and sequencing. Requires machine learning algorithms to provide codon resolution. Can be applied to rare populations from *in vivo* tissue samples.	VanInsberghe *et al* (2021)
Protein Centric Methods to Study RNA–Protein Interactions	CLIP-seq	UV Cross-linking and immunoprecipitation followed by sequencing. Commonly used protocols include HITS-CLIP, iCLIP, eCLIP, and irCLIP. Another variation, PAR-CLIP, uses 4-thiouridine and/or 5-thioguanine as a nucleotide analog when UV penetration is insufficient.	Ramanathan *et al* (2019), Hafner *et al* (2021), Porter *et al* (2021), Licatalosi *et al* (2008), Konig *et al* (2011), Nostrand *et al* (2016), Hafner *et al* (2010), Zarnegar *et al* (2016)
	Chemical cross-linking Seq	Formaldehyde is used to cross-link protein and RNA. Main protocols are fCLIP-seq and xRIPiT-Seq which are useful for RBPs that do not UV cross-link to RNA, such as double stranded binding RBPs. Formaldehyde cross-links protein–protein interactions producing indirect associations.	Singh *et al* (2014), Kim and Kim (2019)
RNA Centric Methods to Study RNA–protein Interactions	Affinity capture of target mRNA	RNA is *in vitro* transcribed (IVT) and labeled with a molecular handle, such as biotin, for pull down after incubation with protein lysate. Alternatively, an aptamer (e.g., MS2, PP7, and S1m) can be appended to the RNA of interest, the RNA is expressed in the cell or IVT, incubated with protein lysate, and pulled down with cognate ligand.	Leppek and Stoecklin (2014), Zheng *et al* (2016), Gemmill *et al* (2020), Hogg and Collins (2007), Slobodin and Gerst (2010)
	Endogenous RNA Pull down	ChIRP-MS, CHART, TRIP, and RAP-MS: these techniques use antisense oligos to capture the RNA of interest, followed by mass spectrometry for unbiased identification of proteins associated with the RNA.	Chu *et al* (2015), West *et al* (2014), Matia-González *et al* (2017), McHugh and Guttman (2018)
	RNA-based Proximity Labeling	Recruitment of promiscuous biotin ligase to RNA sequence of interest, which then tags proteins proximal to the RNA. The biotin tag enables purification of the proximal proteome with streptavidin beads. Most commonly BioID- (RaPID and CARPID) and APEX2-based approaches.	Ramanathan *et al* (2018), Han *et al* (2020), Yi *et al* (2020)
Methods to Study RNA Structure	DMS-Seq/DMS-MaP-seq	DMS (dimethyl sulfate) modifies unpaired adenine and cytosine, which stop the reverse transcriptase (RT-stops) during cDNA generation. High-throughput sequencing can be used to identify the RT-stops across the transcriptome.	Rouskin *et al* (2014), Zubradt *et al* (2017)
	Selective 2-hydroxyl acylation analyzed by primer extension (SHAPE)	SHAPE reagents react with and modify all four nucleotides, which are read out as RT-stops; most common is the icSHAPE method.	Spitale *et al* (2015), Sun *et al* (2019)
	Mutate and Map	M^2 and icM^2 (in cell M^2) entail systematic mutagenesis of nucleotides in an RNA sequence followed by chemical mapping to provide "two dimensional" base pairing information.	Kladwang *et al* (2011), Byeon *et al* (2021)

Table 2 (continued)

	Methods	Description	Reference
Proteomic Methods to Measure Protein Synthesis	Stable isotope labeling with amino acids in cell culture (SILAC)-based techniques	Pulsed treatment of cells with isotopically labeled amino acids followed by mass spectrometry analyses identifies and quantifies newly synthesized peptides during the labeling period (e.g., dynamic SILAC, p-SILAC, & mePROD). Certain variations can be used in living organisms and for subcellular resolution.	Selbach *et al* (2008), Doherty *et al* (2009), Rhoads *et al* (2015), Mardakheh *et al* (2015), Baughman *et al* (2016), Klann *et al* (2020)
	Non-canonical amino acid labeling	Treatment with non-canonical amino acids that can be incorporated into the nascent peptide chain, but are suitable for click chemistry addition of a fluorescent moiety for visualization (e.g., FUNCAT) or biotin "handle" for stringent purification and mass spectrometry analysis (e.g., BONCAT & HILAQ).	Dieterich *et al* (2006), Elliott *et al* (2014), Dieterich *et al* (2010), Calve *et al* (2016), Ma *et al* (2017), Evans *et al* (2019)
	Puromycin and puromycin- derivative labeling of nascent chains	Cells are treated with puromycin, biotinylated puromycin, or the cell-permeable and azide-reactive derivative OPP (O-propargyl-puromycin), which are incorporated into the nascent peptide chain to cause chain termination and can be visualized or purified and subjected to mass spectrometry analyses. Common variants include Puromycin-associated nascent chain proteomics (PUNCH-P) and OPP-mediated protein identification (OPP-ID).	Liu *et al* (2012), Aviner *et al* (2013), Forester *et al* (2018)
Methods to Study Protein Abundance at Single-Cell Level	Cytometry by Time of Flight (CyTOF)	Single-cell-level protein abundance detected through antibodies conjugated to rare heavy metal isotopes, which can be distinguished by mass cytometry. Can be used to detect 30–100 unique surface and intracellular proteins.	Spitzer and Nolan (2016)
	Single-cell mass spectrometry (scMS)	Single cells are isolated, each proteome is barcoded, and the samples are pooled for mass spectrometry analysis. Currently, not widely employed due to technical limitations of low input protein.	Budnik *et al* (2018), Schoof *et al* (2021)
Methods to Measure Temporal and Spatial Dynamics of Translation	Single-molecule FRET (smFRET)	Specific components of the translation machinery are labeled with fluorophores—ribosome, tRNA, other translation factors, or the mRNA. FRET is used to track the proximity of the two fluorophores and thus the relative positions of the labeled components.	Uemura *et al* (2010), Choi *et al* (2018), Lawson *et al* (2021)
	Spatiotemporal translation of a single mRNA in a living cell	These approaches combine simultaneous labeling of nascent peptide formation and the RNA of interest in live cells to track subcellular location and rate of translation (e.g., SunTag system).	Wu *et al* (2016), Yan *et al* (2016), Wang *et al* (2016), Morisaki *et al* (2016)

efficiency of those same transcripts will shed light on how RNA structures guide translational control under different oncogenic signaling programs.

Another important set of methodologies to assess the cancer-specific translational landscape focus on proteomic techniques. It has been appreciated that mRNA transcript abundance does not strictly correlate with protein level across the entire genome (Buccitelli & Selbach, 2020). Therefore, quantitative proteomics is an important tool to assay the expression level of each protein in the proteome of different cell types and cell states (Table 2). On a single-cell scale, proteomic technologies have lagged behind scRNA sequencing approaches, which has resulted in many scRNA-seq experiments being used to infer protein expression on the single-cell scale. Therefore, a great deal of effort is being invested in assaying protein composition at the single-cell scale (Marx, 2019). Although not capable of measuring the whole proteome, cytometry by time of flight (CyTOF) has proved to be a very valuable tool to multiplex the quantification of proteins (30 to 100) at a single-cell level (Spitzer & Nolan, 2016). Excitingly, very recently several groups have begun to push the boundaries to create relatively accessible techniques for single-cell mass spectrometry (scMS). The most promising methods rely on scaled-down sample prep in conjunction with a strategy of barcoding and multiplexing single cells for greater throughput and sensitivity (Budnik *et al*, 2018; Schoof *et al*, 2021). As scMS becomes a more widely available technique, it will be fascinating to examine how RNA and protein expression correlate at the single-cell level within various cells of complex biological systems, such as the tumor and its surrounding microenvironment.

To further focus on the evaluation of rapid translational changes, proteomic technologies that capture nascent protein production can provide important insights into how cells respond to different environmental cues, such as response to cancer therapies (Aviner *et al*, 2013; Forester *et al*, 2018). Unbiased mass spectrometry analysis of the cellular proteome is a fundamental technique to understand how oncogenic signaling acts upon the transcriptome through translation to tailor protein expression. To assay nascent protein synthesis and protein turnover quantitatively, there are an array of proteomic techniques, employing alternatives to native amino acids to enable differentiation of newly synthesized peptides from the rest of the proteome. Briefly, cells or in some cases organisms can be pulsed with isotopically labeled amino acids (e.g., stable isotope labeling of amino acids in culture (SILAC)), non-canonical amino acids, or peptide chain terminating chemicals like puromycin and O-propargyl-puromycin (OPP), which label newly synthesized peptides (Dieterich *et al*, 2006; Selbach *et al*, 2008; Doherty *et al*, 2009; Aviner *et al*, 2013; Ma *et al*, 2017; Forester *et al*, 2018). Subsequently, mass spectrometry can be used to distinguish and quantify the labeled, new synthesized peptides compared to the total proteome. Continued development and refinement of these techniques to a single-cell scale will open a window into the heterogeneity of the proteome within the tumor, its microenvironment, and metastases that aids in tumorigenesis and progression.

Unbiased proteomics can also be applied to protein–RNA interactions to identify proteins bound to a specific transcript or RNA sequence (Table 2). A first set of methods use *in vitro* transcribed (IVT) RNA that is connected to a "molecular handle," such as biotin or an RNA aptamer structure, which can be coupled to a matrix, mixed with protein lysate, and the bound proteins identified through mass spectrometry (Leppek & Stoecklin, 2014; Zheng *et al*, 2016). Additionally, to assay proteins bound to a specific RNA within cells, techniques have been developed that utilize chemical cross-linking of the proteins to the RNA followed by antisense oligo pull down to isolate the RNA-bound proteins, as exemplified by the ChIRP-MS and CHART-MS methods (West *et al*, 2014; Chu *et al*, 2015). As these methods require either abundant RNAs or high cell numbers, additional methods have been developed, such as RaPID, which exploits proximity biotinylation technology to covalently tag with biotin proteins that are in proximity to the RNA sequence of interest (Ramanathan *et al*, 2018). The biotin-tagged proteins can then be easily extracted from the lysate with a streptavidin-coated matrix and identified via mass spectrometry. All of these approaches can illuminate the identity of the proteins bound to and potentially mediating the selective translation of an mRNA of interest. Moreover, these proteomic methods can be applied to unique RNA sequence or structural elements determined through RNA-sequencing–based technologies (e.g., CLIP or ribosome profiling) to reveal what proteins may be acting mechanistically to specify and coordinate translational programs.

Finally, there are a broad array of methods to assay the spatial and temporal dynamics of translation. These techniques are critical to understanding where within a cell and on what time scale translation is occurring (Table 2). A number of groups have developed techniques to assay the translation dynamics of single mRNAs. Single-molecule Förster resonance energy transfer (smFRET) can be used to precisely monitor all stages of translation at the single codon level, which allows insights into the contributions various components of the translational machinery to the translation efficiency, for example, mRNA epigenetic modifications (Uemura *et al*, 2010; Choi *et al*, 2018; Lawson *et al*, 2021). Additionally, several techniques have been published that examine the spatiotemporal translation of a single mRNA in a living cell (Morisaki *et al*, 2016; Wang *et al*, 2016; Wu *et al*, 2016; Yan *et al*, 2016). All of these methods share a common approach where the target mRNA transcript is fused to RNA structures that can recruit a fluorescent protein while the translated nascent peptide is bound by a different fluorescent molecule such that the mRNA and the nascent peptide can be independently tracked. Importantly, these techniques can provide dual insight into the location of translation and the rate of translation and can even incorporate forced localization to the membrane or potentially another subcellular compartment (Yan *et al*, 2016). Further development of these technologies into methods that can be applied to more complex systems that contain multiple cell types will uncover how cancer cells and the microenvironmental cells precisely respond to signaling cues to synthesize just the right set of proteins at the right time and the right subcellular location.

Conclusion

Translational control has emerged as a critical nexus integrating upstream oncogenic signals into protein synthesis—the ultimate expression of the cellular proteome. For this reason, the selective control of protein synthesis is increasingly recognized as an important focus of cancer research. The rapidity of translational control provides a first line of action for cancer cells to respond to a myriad of cues including intra- and extracellular stressors, whereas transcriptional changes require a longer time scale. Importantly, through the coordination of the translational machinery, RNA-binding proteins, and 5′ and 3′UTR RNA regulatory elements, translation can precisely, yet pervasively, mediate expression of an array of mRNA networks that are required for sustained tumorigenesis.

Building evidence suggests there is a "translation control code" composed of RNA sequence and structure regulatory elements contained in every mRNA in the cell. Each transcript's unique set of RNA regulons—the "regulatory syntax" or "grammar"—allow the cell to selectively translate the appropriate genetic program in response to a variety of external and internal signals. Cancer cells can exploit this code to selectively modulate the expression of distinct transcripts that contribute to the hallmarks of cancer. It is already apparent that different RNA motifs often co-occur within the same 5′UTR, such as the 5′TOP and PRTE motifs (Hsieh *et al*, 2012). We are only beginning to understand how cancer cells tailor networks of expressed RBPs and levels of translation factors to coordinate the translation of specific and functionally linked mRNAs to engage the genetic programs to respond to stress states, such as nutrient level changes or hypoxia (Ho *et al*, 2020). The integration of experimental methods to dissect the mechanisms of translational control will shed light on the rules of RNA regulatory element "grammar" that dictate the translational efficiency of a specific transcript.

Translational control also contributes to risk factors and predispositions for cancer formation. The translation machinery has emerged as a critical mediator of the cellular response in a variety of pathological states, for example, the metabolic dysregulation associated with obesity, and inflammation. Obesity is a significant risk

factor for cancer development, particularly of the liver, as a result of non-alcoholic fatty liver disease (NAFLD) (Font-Burgada *et al*, 2016). Intriguingly, research has demonstrated that inhibition of eIF4E activity and other translation factors such as eIF6 through genetic or pharmacological means can diminish weight gain on a high nutrient diet, which may result in a reduced risk of cancer development (Moore *et al*, 2016; Conn *et al*, 2021; Scagliola *et al*, 2021). Translational control is also important in the inflammatory response, which is a key consequence of obesity as well as a major risk factor for developing other cancers, such as pancreatic adenocarcinoma (Mazumder *et al*, 2010; Font-Burgada *et al*, 2016; Gameiro & Struhl, 2018; Klein, 2021). Translational control is at the heart of many disease states that can contribute to cancer formation and, therefore, is a potent therapeutic target to not only diminish tumorigenesis but potentially blunt cellular transformation.

Cancer cells hijack specific components of the translation machinery to regulate the synthesis of select proteins that are central to cancer-specific processes, distinguishing cancer cells from non-transformed cells. Therefore, compounds that target these translational components or the function of *cis*-regulatory elements of key transcripts are attractive therapeutic opportunities. Already, several molecules that target translation factors are in clinical trials. Most prominently are several clinical trials for cancer patients that are testing compounds which inhibit the activity of eIF4E and eIF4A (NCT01675128; NCT03616834; NCT04622007; NCT04092673). Studies have identified small molecules that bind RNA structures in the 5′UTR of oncogenes, such as the Ras proteins, to block translation and promote cancer cell death; however, there has been limited success in translating this approach to the clinic (Katsuda *et al*, 2016; Miglietta *et al*, 2017). Additionally, the elongation and termination steps of translation are highly regulated. However, there is scant evidence regarding the mechanisms of selectivity of these processes, although the BAT structural regulon in the 3′UTR provides a window into the possibilities. Compounds targeting the translation elongation factor eEF1A, such as the synthetic ternatin variant dA3, have shown promise in preclinical experiments, and another eEF1A inhibitor, Plitidepsin, has been clinically approved for the treatment of multiple myeloma (Carelli *et al*, 2015; Keysar *et al*, 2020). Research supports the robust efficacy of combinational therapies of translation inhibitors with known therapeutic compounds, such as inhibitors of anti-apoptotic proteins and targeted therapeutics (Anderson *et al*, 2016; Kuzuoglu-Ozturk *et al*, 2021; Thompson *et al*, 2021). Targeting translation from initiation through termination is an exciting new therapeutic avenue that may help overcome resistance to other anti-cancer agents, providing more effective treatments.

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The authors declare that they have no conflict of interest.

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