Translational control of stem cell function

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Abstract | Stem cells are characterized by their ability to self-renew and differentiate into many different cell types. Research has focused primarily on how these processes are regulated at a transcriptional level. However, recent studies have indicated that stem cell behaviour is strongly coupled to the regulation of protein synthesis by the ribosome. In this Review, we discuss how different translation mechanisms control the function of adult and embryonic stem cells. Stem cells are characterized by low global translation rates despite high levels of ribosome biogenesis. The maintenance of pluripotency, the commitment to a specific cell fate and the switch to cell differentiation depend on the tight regulation of protein synthesis and ribosome biogenesis. Translation regulatory mechanisms that impact on stem cell function include mTOR signalling, ribosome levels, and mRNA and tRNA features and amounts. Understanding these mechanisms important for stem cell self-renewal and differentiation may also guide our understanding of cancer grade and metastasis.

Self-renewal
A process by which stem cells divide and maintain stemness.

Quiescent
A reversible state in which cells exit the cell cycle but can re-enter it in response to stimuli such as injury to the tissue in which they reside.

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Stem cells are collectively a group of diverse cell types that share two main properties: the ability to self-renew indefinitely and the ability to differentiate into one or more cell types. In adult tissues, stem cells give rise to the appropriate differentiated cell types of those tissues. By contrast, embryonic stem cells (ESCs), which are cell lines derived from pre-implantation embryos, are pluripotent and can give rise to all differentiated cell types in the adult body.

To date, most stem cell research has focused on how the processes of self-renewal and differentiation are controlled at a transcriptional level[1,2]. ESCs are characterized by a hypomethylated, hypertranscriptional state of euchromatin[3] and are known to express 'pioneer' transcription factors, which can bind to less-accessible DNA to promote transcription of distinct genetic programmes that dictate cell fate[4]. As such, changes in protein expression in ESCs are thought to be controlled primarily at the transcriptional level[2,5,6]. Over the past three decades, an extensive body of literature has described mechanisms that regulate protein expression at the translational level, and in the past decade, numerous studies have implicated these mechanisms in the control of ESC and adult stem cell function. It has become clear that tight regulation of the protein synthesis machinery — the ribosome and its associated factors — is essential for stem cell self-renewal and differentiation in ESCs and in many adult stem cell types.

Beyond the processes of self-renewal and differentiation, ESCs and adult stem cells can exist in quiescent or activated states characterized by distinct functional properties. Moreover, cells from differentiated tissues can be reprogrammed to become pluripotent, generating cells referred to as 'induced pluripotent stem cells' (iPSCs). Finally, when tissues are damaged or diseased, the normal properties of stem cells and differentiated cells can be disrupted, leading to dedifferentiation or uncontrolled proliferation in the absence of differentiation. Protein translation plays important roles in the regulation of each of these processes in ESCs and various adult stem cell types (depicted in FIG. 1). In this Review, we discuss how control over the ribosome and protein synthesis contributes to stem cell function by affecting the core properties of stem cell self-renewal, lineage commitment and differentiation, the transition between quiescent and activated states, the induction of pluripotency and the dysregulation of these processes in disease (FIG. 1). Recent reviews have detailed the role of translation in human disease and in tissue homeostasis[7–9]. Here, we focus on the translational regulation of stem cell function and provide some new interpretations of previous publications.

We first discuss general trends in ESC and adult stem cell systems with a focus on the unique stem cell signature of low global protein synthesis with high ribosome biogenesis (RiBi) under self-renewal conditions. We next consider stem cell differentiation as a translationally dynamic and sensitive phase in which protein synthesis and RiBi are tightly regulated to assemble the new proteome for the differentiated cell.

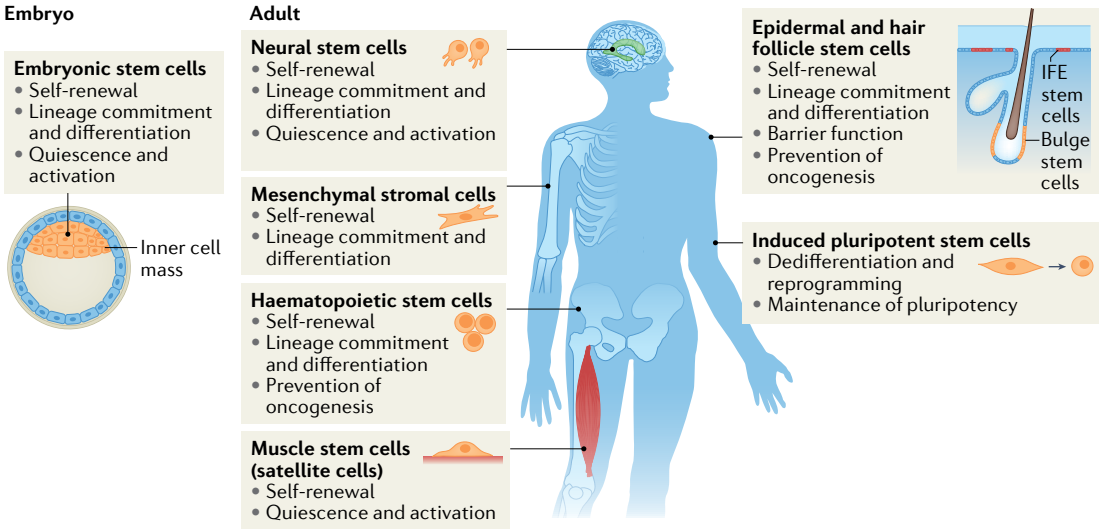


Fig. 1 | **Overview of stem cell types and functions.** Stem cells are derived from the inner cell mass of the pre-implantation embryo (embryonic stem cells) or from adult tissues (adult stem cells, also known as somatic stem cells). Embryonic stem cells are pluripotent and can differentiate into all differentiated tissues of the adult body, whereas adult stem cells are lineage restricted and retain the ability to differentiate into multiple discrete cell types derived from that tissue. Induced pluripotent stem cells are derived from adult tissue but have been reprogrammed into an embryonic-like pluripotent state. At least five stem cell properties are regulated through control of the translation machinery of the cell: self-renewal, lineage commitment and differentiation, quiescence and activation, induction of pluripotency, and dysregulation in disease. Stem cell types in which one or more of these functions have been studied in the context of regulation by the translation machinery of the cell are shown. IFE, interfollicular epidermis.

We discuss various signalling pathways and regulatory factors that impinge on protein synthesis, RiBi and the differentiation switch in stem cells. Finally, we end by discussing the extent to which the themes elucidated in stem cells may guide our understanding of cancer grade and metastasis. This Review highlights gaps in knowledge and provides motivation for future experiments to understand how regulation of protein synthesis contributes to stem cell biology.

Translation features in stem cells

Eukaryotic protein synthesis involves a series of steps in which an mRNA that was transcribed and processed in the nucleus and exported to the cytoplasm is translated into protein by the ribosome. Eukaryotic translation can be broadly broken down into three key steps: initiation (reviewed in[10]), elongation (reviewed in[11]), and termination and recycling (reviewed in[12]) (FIG. 2).

Initiation involves a number of protein factors, referred to collectively as 'eukaryotic initiation factors' (eIFs), and is broadly thought to be the rate-limiting step and a point of control for selective translation of different cellular mRNAs. During this step, the 43S pre-initiation complex (PIC) is assembled, consisting of eIF1, eIF1A, eIF2–$tRNA_i^{Met}$–GTP, eIF3, eIF5 and the 40S ribosomal subunit. The 43S PIC is recruited to the mRNA by the mRNA cap-binding complex, eIF4F, to form the 48S PIC. The 48S PIC scans the mRNA in the net forward direction until it encounters an AUG start codon, at which point GTP is hydrolysed on eIF2, the 60S ribosomal subunit is recruited by eIF5B to form the full 80S ribosome and translation of the open reading frame begins. The second step of eukaryotic translation is elongation, and is mediated by the eukaryotic elongation factors (eEFs). Elongation involves decoding of the mRNA codon in the ribosome aminoacyl site (A-site), eEF1A-mediated recruitment of a cognate aminoacylated tRNA, peptidyl transfer of the nascent peptide chain from the peptidyl site (P-site) tRNA to the A-site tRNA and eEF2-mediated translocation of the downstream mRNA codon into the A-site. Elongation repeats until one of three stop codons (UAA, UAG or UGA) is encountered in the A-site. The third and final step of eukaryotic translation is termination and recycling. In this step, eukaryotic release factor 1 (eRF1) and eRF3 recognize a stop codon in the ribosome decoding centre and release the nascent peptide chain, and then the recycling factor ABCE1 is recruited to dissociate the 40S and 60S ribosomal subunits for another round of translation[13]. Together, these steps form the basis for eukaryotic protein synthesis by the ribosome (FIG. 2). Points of regulation at each step and their involvement in stem cell functions are discussed throughout this Review.

Stem cells are characterized by low rates of global protein synthesis. An emerging theme in stem cell biology is that stem cells generally have low rates of global protein synthesis relative to the differentiated cell types they give rise to, as first documented in mouse ESCs[14] (BOX 1; FIG. 2). ESCs are pluripotent cells derived from the inner cell mass of the pre-implantation blastocyst and can be maintained indefinitely in culture or differentiated to three-dimensional spherical aggregates called 'embryoid bodies' (EBs) composed of all three primary germ layers (ectoderm, mesoderm and endoderm). To understand how protein synthesis rates change during differentiation, nascent protein synthesis was

Induced pluripotent stem cells
(iPSCs). Somatic cells that are reprogrammed by defined factors to acquire embryonic stem cell-like, pluripotent features.

Dedifferentiation
A process by which terminally or partially differentiated cells revert to less-differentiated cells within the same lineage.

Ribosome biogenesis
(RiBi). A concerted molecular process to build a ribosome involving more than 200 proteins and other factors.

tRNA
A small RNA molecule that links decoding of the mRNA to incorporation of the appropriate amino acid into the nascent peptide strand during translation elongation.

measured by [^{35}S]methionine labelling of nascent peptides and polyribosome profiling (polysome profiling) in mouse ESCs compared with 5-day differentiated EBs[14]. It was found that protein synthesis rates were increased approximately twofold in EBs. This was mirrored by a global increase in the relative number of ribosomes loaded per mRNA, which is indicative of translational efficiency[14]. Subsequent studies confirmed low rates of

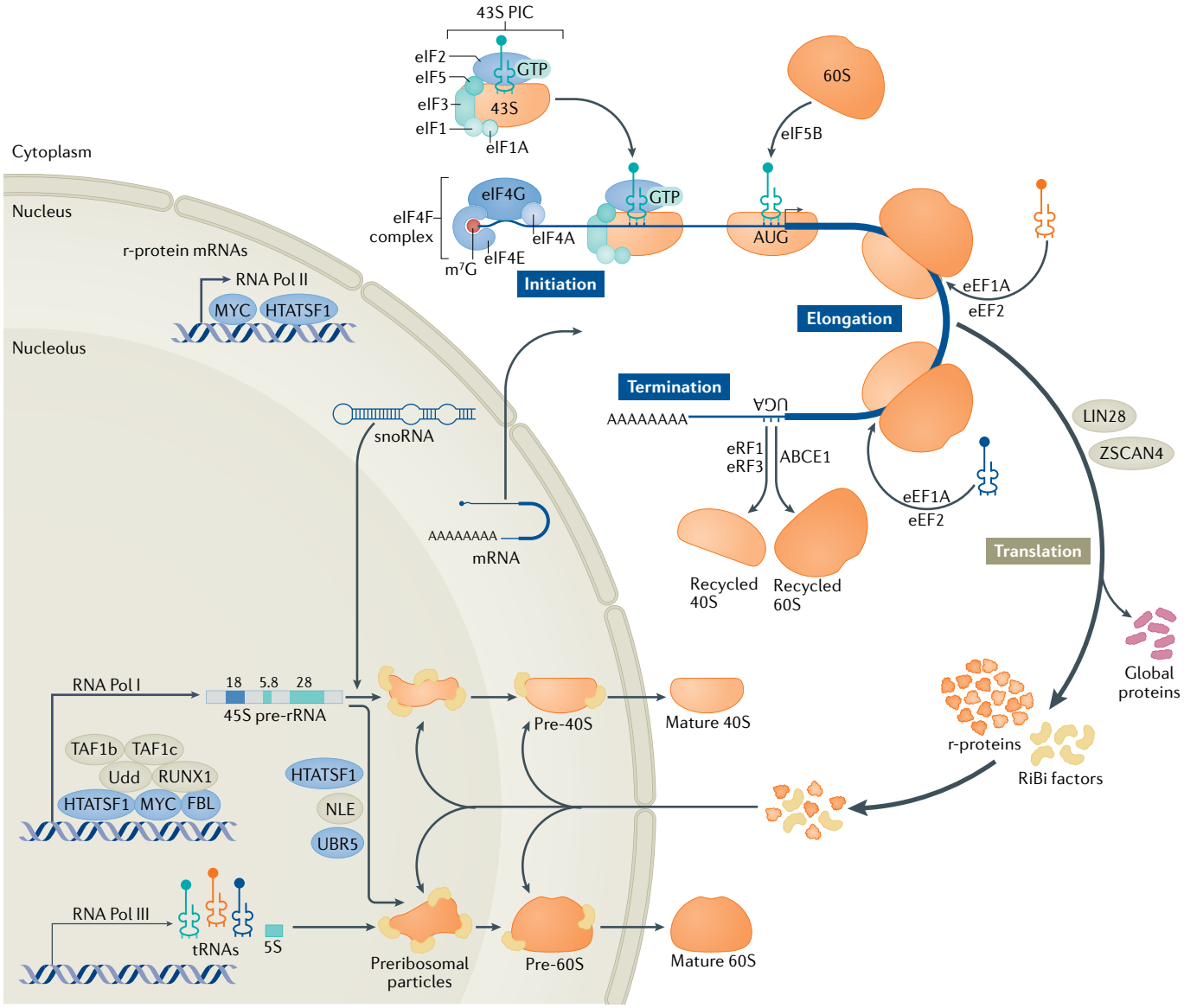


Fig. 2 | **Translation and ribosome biogenesis in stem cells.** Top: Eukaryotic translation is depicted as three main steps: initiation, elongation, and termination and recycling[29]. During initiation, eukaryotic initiation factors (eIFs) mediate recruitment of the 43S pre-initiation complex (PIC) to the mRNA and joining of the 60S ribosomal subunit. During elongation, eukaryotic elongation factors (eEFs) mediate recruitment of tRNAs and incorporation of an amino acid into the nascent peptide chain. During termination and recycling, eukaryotic release factors (eRFs) mediate recognition of a stop codon and release of the nascent peptide, while ABCE1 mediates ribosomal subunit dissociation and recycling. Embryonic and many adult stem cell lineages support ribosome biogenesis (RiBi) through the translation of ribosomal proteins (r-proteins) and other RiBi factors, while the synthesis of total proteins is relatively low compared with that in differentiated cell types. Bottom: RiBi begins in the nucleolus with the synthesis of preribosomal RNAs (pre-rRNAs) by RNA polymerase I (Pol I) and RNA Pol III. RiBi factors and r-proteins are imported into the nucleus and nucleolus, where the pre-rRNA is folded and processed and r-proteins are added to form the pre-40S and pre-60S particles, which are fully assembled in the cytoplasm. RNA Pol II transcribes mRNAs for r-proteins and RiBi factors, as well as small nucleolar RNAs (snoRNAs), which enter the nucleolus and function in rRNA processing. Multiple factors support RiBi in stem cells by promoting transcription of rRNA through RNA Pol I, including HIV Tat-specific factor 1 (HTATSF1 (REF.[33])), MYC[30], the rRNA 2′-*O*-methyltransferase fibrillarin (FBL)[32,35], TATA-box-binding protein-associated factors B and C (Taf1b and Taf1c)[41] and Under-developed (Udd)[41], which contribute to growth and proliferation in *Drosophila melanogaster* germline stem cells, and Runt-related transcription factor 1 (RUNX1), which contributes to growth of mouse haematopoietic stem and progenitor cells[44]. MYC and HTATSF1 also promote the transcription of r-protein mRNA by RNA Pol II through their upregulation in mouse embryonic stem cells. HTATSF1, Notchless protein homologue 1 (NLE)[43] and the E3 ubiquitin-protein ligase UBR5 (REF.[34]) are involved in pre-rRNA processing and maturation. Factors that are upregulated in stem cells are coloured in blue, whereas those that have been shown to be important for stem cell viability and/or growth are shown in grey. m^{7}G, 7-methylguanosine.

Box 1 | **Translational control in undifferentiated cell states**

While our Review focuses on the transition between stem cells and differentiated cells, there are also changes in the state of undifferentiated cells that involve translational control. Specifically, embryonic stem cells (ESCs) in vivo exist in multiple states, including (1) a totipotent state derived from the fertilized zygote, which is capable of giving rise to any embryonic or extraembryonic tissue[207,208], (2) a naive pluripotent state (also called the 'ground state') derived from the inner cell mass of pre-implantation epiblasts and able to differentiate into any embryonic tissue[209,210] and (3) a primed pluripotent state derived from the postimplantation epiblast and 'primed' to differentiate into specific tissues[209,210]. An intermediate naive-primed state has also been characterized, which is sometimes called 'rosette-stage pluripotency'[211].

Certain pluripotent states can be mimicked in vitro. Ground-state pluripotency is maintained by culture of ESCs in a combination of CHIR99021, a glycogen synthase kinase-β (GSK3β) inhibitor, PD0325901, a mitogen-activated protein kinase kinase (MEK) inhibitor and leukaemia inhibitory factor (LIF), an IL-6 class cytokine that inhibits differentiation (collectively referred to as '2i/LIF')[212]. Importantly, GSK3 and MEK are known to undergo crosstalk with the mTOR signalling pathway[213,214], and LIF has been suggested to promote eukaryotic initiation factor 2α (eIF2α) phosphorylation in ESCs[139]. A recent study demonstrated that Torin1-mediated mTOR inhibition converts primed human pluripotent stem cells to the ground state and that this state can be maintained indefinitely[215]. Thus, we can speculate that pharmacological inhibitors maintain ground-state pluripotency at least in part through regulation of the translation machinery. Additionally, a quiescent/paused pluripotent state can be induced by direct inhibition of either MYC[216] or mTOR[70], an effect that is also likely to be mediated through regulation of the translation machinery.

Undifferentiated state transitions have been studied most extensively at the transcriptional level[209,210,217], although a few studies have interrogated the role of translation regulation during these transitions[5,6,216]. One study found that ground-state ESCs have more polysome-associated mRNAs than do intermediate or postimplantation-state ESCs[6], which is surprising considering the general trend towards higher protein synthesis in more-differentiated cell types (FIG. 4). By contrast, protein synthesis rates are consistently reduced in quiescent/paused pluripotent states compared with activated stem cell states[26,70,73,216]. Translation inhibition of chromatin regulators — mediated by the RNA-induced silencing complex — has been documented during ground-state and primed-state transitions[218]. These results suggest that global protein synthesis may be similarly dynamic among undifferentiated stem cell states, although the functional consequences of this translation regulation is not fully understood. Despite these dynamics, multiple studies have found that transcriptional changes, rather than translation efficiency changes, are the major driver of gene expression changes in undifferentiated states[5,6]. This is consistent with reports that translational control of gene expression increases as differentiation progresses[17,26].

protein synthesis and/or low translational efficiency of global mRNAs in cultured mouse ESCs and human ESCs[15–20], haematopoietic stem and progenitor cells (HSCs)[21–23], quiescent epidermal and hair follicle stem cells (HFSCs)[24,25], quiescent neural stem cells (NSCs)[26], quiescent muscle stem cells (also known as satellite cells)[27] and *Drosophila melanogaster* germline stem cells (GSCs)[28]. Thus, low overall protein synthesis rates appear to be a general characteristic of stem cells.

Stem cells upregulate RiBi. In eukaryotes, RiBi consists of a sequence of carefully orchestrated steps that involve (1) synthesis of pre-ribosomal RNA (rRNA) molecules in the nucleolus, (2) nuclear import of ribosomal proteins (r-proteins) and a cohort of assembly factors that assist co-transcriptional folding, processing and assembly of the preribosomal particles (collectively referred to as the 'RiBi machinery'), (3) export of the pre-40S and pre-60S subunits to the cytoplasm and (4) further processing and addition of r-proteins in the cytoplasm[29] (FIG. 2). The 45S pre-rRNA is synthesized by RNA polymerase I as a polycistronic transcript from repetitive ribosomal DNA genes located on five chromosomes and processed into 18S rRNA (for the small ribosomal subunit) and 5.8S and 28S rRNA (for the large ribosomal subunit). RNA polymerase II transcribes mRNAs, which are translated into ~80 eukaryotic r-proteins, ~200 eukaryotic RiBi factors and small nucleolar RNAs, which are involved in rRNA processing. RNA polymerase III transcribes the 5S pre-rRNA (for the large ribosomal subunit) as well as tRNAs required for the elongation steps of translation.

Despite generally low rates of protein synthesis, ESCs selectively upregulate RiBi. This occurs through the upregulation of a number of protein factors that either associate with RNA polymerase I or polymerase II complexes to promote transcription of the 45S pre-rRNA and r-protein mRNAs, respectively, or function in pre-rRNA processing and maturation[30–34] (FIG. 2). Examples of these factors include fibrillarin (FBL), a nucleolar rRNA 2′-*O*-methyltransferase required for rRNA processing, which is highly expressed at the baseline in mouse ESCs and whose expression is required for the maintenance of pluripotency and sustains pluripotency in culture conditions that would normally promote differentiation[32,35], as well as MYC, a transcription factor that is protected from proteasomal degradation in mouse ESCs and is involved in the transcription of rRNA and various r-protein and RiBi genes[30,31,36]. MYC is also one of the four 'Yamanaka factors' (OCT4, SOX2, KLF4 and MYC; also commonly referred to as OSKM) that were discovered to reprogramme fibroblasts into iPSCs[37,38]. HTATSF1, involved in rRNA and r-protein mRNA transcription and splicing, is also upregulated in mouse ESCs and is required to maintain the pluripotent state. The E3 ubiquitin ligase UBR5, which is upregulated in human and mouse ESCs, was recently shown to be involved in rRNA maturation to promote pluripotency in human and mouse ESCs through interaction with the H/ACA complex, although the mechanisms involved have not been fully elucidated[34].

In addition to RiBi components, which have been directly demonstrated to be upregulated in ESCs, the loss of function of other factors involved in RiBi has been shown to be detrimental to stem cell growth and/or survival in many stem cell types. These factors include the RNA-binding protein LIN28 in human ESCs and mouse neural progenitor cells[39,40], the RNA polymerase I regulatory proteins Udd, Taf1b and Taf1c in *D. melanogaster* GSCs[41] and the 60S maturation protein Notchless in mouse HSCs and intestinal stem cells[42,43]. In an intriguing example, loss of function of the transcription factor RUNX1 induces mouse HSCs to adopt a quiescent state in which they become smaller and less proliferative but are also protected from genotoxic stress[44]. From the findings taken together, the sensitivity of many stem cell types to loss of function of RiBi components is in accordance with a reliance on upregulated RiBi for stem cell maintenance.

Beyond analysing ancillary components involved in RiBi, RiBi can be more directly monitored by measuring the synthesis rates of various constituents of the ribosome, such as rRNAs or r-proteins. With use of this approach, high rates of RiBi have been directly documented in stem cells compared with their differentiated

Polysome profiling
An experimental technique in which a cell lysate is separated by density gradient centrifugation and mRNAs associated with multiple ribosomes (polyribosomes or 'polysomes') can be fractionated; not to be confused with the sequencing method ribosome profiling.

H/ACA complex
A small nucleolar ribonucleoprotein complex involved in the pseudouridylation of ribosomal RNA.

Cap-dependent translation
Translation initiation by the binding of an mRNA cap protein, usually eukaryotic initiation factor 4E (eIF4E), to a modified guanine structure at the 5′ end of the mRNA. This interaction is required for canonical mRNA translation.

progeny. For example, mouse HSCs, despite having low protein synthesis rates[21], transcribe nascent rRNA at rates approximately twofold to fourfold greater than downstream lineage-restricted haematopoietic cells[22]. Similarly, human ESCs exhibit high levels of nascent rRNA synthesis compared with many other differentiated human cancerous and non-cancerous cell lines[30]. Upregulated rRNA synthesis in stem cells is not a mammal-specific phenomenon, as *D. melanogaster* GSCs also have high rates of rRNA and r-protein synthesis relative to their progeny cells[28,41]. If RiBi is higher in ESCs and adult stem cells than in their differentiating progeny, then it is expected that the rates of rRNA or r-protein synthesis will be downregulated upon the induction of differentiation. This prediction was confirmed in the differentiation of mouse ESCs to EBs[16], human ESCs to neural progenitor cells[17], human ESCs to cardiomyocytes[45], myoblasts to myocytes[46] and adipocyte stem cells to adipocytes[47]. Finally, stem cells exhibit phenotypic features consistent with increased RiBi, as they maintain a large nuclear-to-cytoplasmic ratio and have prominent nucleoli, which are known to reflect high rates of rRNA synthesis[14,28,41,48].

mTOR signalling coordinates protein synthesis and RiBi with stem cell function. The kinase mTOR belongs to the PI3K-related kinase family and integrates intrinsic and extrinsic signals to coordinate diverse cellular processes, including cell growth, proliferation and survival (reviewed in[49]). Relevant to this Review are the processes of protein synthesis and RiBi, which are critical outputs of mTOR signalling. In stem cells, mTOR acts as an important nexus that links these key outputs to self-renewal, differentiation and induction of pluripotency (BOX 2; FIG. 3).

mTOR functions in two distinct complexes, mTORC1 and mTORC2, of which mTORC1 is known to have a more direct role in controlling protein synthesis and RiBi through two key targets: eIF4E-binding proteins (4E-BPs) and r-protein S6 kinase (S6K)[49]. In the unphosphorylated state, 4E-BPs bind to eIF4E and displace its interaction with eIF4G, thus impairing initiation of 5′ cap-dependent translation. By contrast, when phosphorylated by mTOR, 4E-BPs cannot bind to eIF4E, and cap-dependent translation is stabilized. 4E-BP phosphorylation preferentially upregulates the translation of terminal oligopyrimidine (TOP)-motif containing mRNAs, which are highly represented by r-proteins, RiBi factors and translation machinery components[50,51]. In this way, mTOR-dependent translation through 4E-BPs promotes RiBi and consequent protein synthesis. Beyond the ribosomal machinery, 4E-BP phosphorylation also upregulates translation of mitochondrial proteins, which is probably important for coordinating metabolic demands with changes in cell fate and protein synthesis[52–55]. In a complementary manner, mTOR phosphorylation of S6K leads to activation of upstream binding factor (UBF) and transcription initiation factor 1A (TIF1A) to promote rRNA transcription through RNA polymerase I[56,57], MAF1 to upregulate tRNA transcription through RNA polymerase III[58], eIF4A (via PDCD4), eIF4B and eEF2 kinase (eEF2K) to promote translation initiation and elongation[59,60,61], and through mechanisms that are not fully elucidated, the transcription of as many as 75% of the RiBi processing machinery genes[62]. Surprisingly, a mechanistic understanding of the role of phosphorylation for S6K's namesake target RPS6 in global protein synthesis remains elusive[63]. Nonetheless, S6K signalling downstream of mTOR promotes protein synthesis and RiBi through multiple coordinated mechanisms. Finally, mTOR activation leads to phosphorylation of eIF4G to

Box 2 | Translational control in response to biophysical cues

Stem cells respond to signals from the local microenvironment (niche)[219], including physical cues[220]. Cell–cell adhesion and cell–extracellular matrix (ECM) adhesion transmit tensile forces. Several studies have focused on the mechanisms of mechanotransduction in transcriptional control[221]. However, recent studies suggest mechanical forces affect stem cell function through regulation of translation.

The cytoskeleton transduces mechanical signals from the extracellular space to the nucleus and cytoplasm, thereby influencing gene expression and protein synthesis[222]. Most core components of the translation machinery are closely associated with cytoskeletal proteins, and disruption of this link affects the initiation of protein synthesis[223–225]. Moreover, the cytoskeleton is important for mRNA transport, allowing spatially localized translation[226]. Thus, there is an intimate relationship between cytoskeletal remodelling — one of the key features of stem cell differentiation — and protein synthesis.

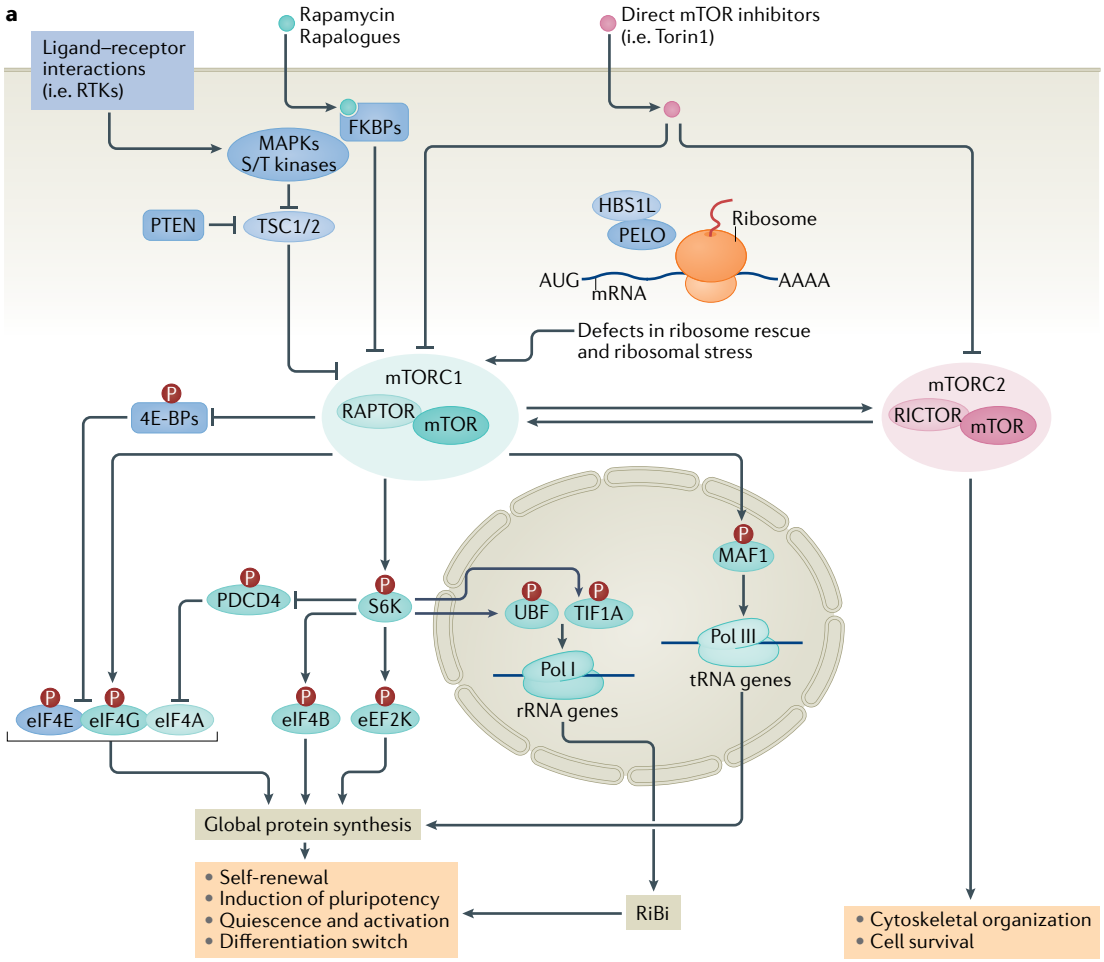
Keratin intermediate filament proteins are a major component of the cytoskeleton of epithelial cells. Keratin 17 (KRT17), which is expressed in hair follicles and upregulated on epidermal wounding, interacts with the adaptor protein 14-3-3σ to regulate protein synthesis. Deletion of *Krt17* reduces mTOR–AKT (also known as protein kinase B) activity, resulting in reduced global protein synthesis[227]. Indeed, many keratin proteins interact with non-structural proteins that are core components of translation pathways, including eukaryotic elongation factor 1γ (eEF1γ)[228,229].

Integrins, the transmembrane heterodimeric ECM receptors that link the cytoskeleton to the ECM via integrin-associated proteins, are known to associate with mRNA and ribosomes at focal adhesions, where they regulate protein synthesis[230]. Integrin-dependent translational regulation is well documented in cancer and a range of cell types[231,232]. It seems likely that similar regulation occurs in stem cells, since stem cells tend to express high levels of integrins and ECM adhesion regulates differentiation[219].

The Hippo pathway effectors Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ) are important players in mechanotransduction and regulating stem cell properties. YAP transcriptional activity and localization are regulated by cell spreading, ECM stiffness or rigidity of the substrate, exemplified in studies using various stem cell types[233–235]. A direct link between regulation of protein synthesis and YAP-mediated mechanotransduction has been documented. For example, skeletal muscle cells adjust their size in response to mechanical load, leading to alteration of protein metabolism through control of protein synthesis rate. YAP overexpression in skeletal muscle progenitors leads to upregulation of ribosome biogenesis genes, although independently of mTOR signalling[236–238]. Given the cytoplasm–nuclear shuttling of YAP, it is possible that mechanotransduction drives YAP to relocate to the nucleus to induce expression of ribosome biogenesis genes.

YAP/TAZ-regulated upregulation of key mTOR activators has been reported[239–241]. Moreover, several molecules implicated in the regulation of translation, such as S6K, AKT, stress-activated protein kinase and glycogen synthase, are mechanoresponsive[242–244]. Integration of YAP/TAZ with mTOR and translation-related proteins might explain how the cascade influences differentiation via global protein synthesis elicited by mechanical cues.

Another well-studied mechanoresponsive molecule is the mechanically gated ion channel PIEZO1. When activated upon mechanical stress, PIEZO1 can regulate the fate of embryonic, mesenchymal and neural stem cells[245–247]. PIEZO1 activation leads to activation of YAP and nuclear translocation in neural stem cells and osteoclasts[247,248]. There is no evidence suggesting direct involvement of PIEZO1 acting on components of the translation machinery. However, PIEZO1 levels regulate mTOR–AKT activation[249]. In addition, PIEZO1 channels facilitate Ca^{2+} influx, and intracellular Ca^{2+} signalling can directly activate mTOR, thereby promoting protein synthesis. It thus appears that the role of PIEZO1 in regulating mRNA translation is indirect, yet crucial for mechanically induced differentiation.


a
Ligand–receptor interactions (i.e. RTKs)
Rapamycin
Rapalogues
Direct mTOR inhibitors (i.e. Torin1)
FKBPs
MAPKs S/T kinases
PTEN
TSC1/2
HBS1L
PELO
Ribosome
AUG
mRNA
AAAA
Defects in ribosome rescue and ribosomal stress
mTORC1
RAPTOR
mTOR
mTORC2
RICTOR
mTOR
4E-BPs
MAF1
PDCD4
S6K
UBF
TIF1A
Pol III
tRNA genes
Pol I
rRNA genes
eIF4E
eIF4G
eIF4A
eIF4B
eEF2K
Global protein synthesis
• Self-renewal
• Induction of pluripotency
• Quiescence and activation
• Differentiation switch
RiBi
• Cytoskeletal organization
• Cell survival
b mTOR activity in different stem cell types
Quiescent stem cell (G0)
Active stem cell
Differentiated cell
ESC
Lowest mTOR
Low mTOR
Active ESC (or iPSC)
Transient drop at onset of differentiation
Low mTOR
↓ 4E-BP-P / 4E-BP
High mTOR
↑ 4E-BP-P / 4E-BP
Embryoid body (or somatic cell)
Drop at onset of differentiation
NSC
Low mTOR
High mTOR
Active 'primed' NSC
High mTOR
mTOR activity unclear
Neuron
Satellite cell
Low mTOR
High mTOR
Active (G_{alert}) satellite cell
High mTOR
mTOR activity unclear
Muscle cell
• Quiescence
• Stem cell maintenance
mTOR 4E-BP-P
• Differentiation
• Stem cell loss

◀ Fig. 3 | **mTOR signalling coordinates protein synthesis and ribosome biogensis to stem cell growth, survival and differentiation. a** | mTOR complex 1 (mTORC1) and mTORC2 have distinct roles in maintaining cell growth and proliferation, with mTORC1 having a more direct role in stem cell fate via downstream effectors that act in several steps of ribosome biogenesis (RiBi) and protein synthesis. Phosphorylation of eukaryotic initiation factor 4E (eIF4E)-binding proteins (4E-BPs) by mTORC1 potentiates the interaction between eIF4E and eIF4G, leading to global translation initiation as well as the preferential translation of mRNAs that encode ribosomal proteins, translation machinery and mitochondrial proteins. Phosphorylation of S6K activates multiple effectors that promote global translation initiation and elongation via phosphorylation of PDCD4, eIF4B and eukaryotic elongation factor 2K (eEF2K). Additionally, phosphorylated S6K (pS6K)-mediated phosphorylation of upstream binding factor (UBF) and transcription initiation factor 1A (TIF1A) upregulates ribosomal RNA (rRNA) gene transcription via RNA polymerase I (Pol I), and pS6K-mediated phosphorylation of MAF1 promotes tRNA gene transcription via RNA Pol III. Together, these events are essential to regulate stem cell self-renewal, induction of pluripotency, quiescence/activation and the differentiation switch through regulation of global protein synthesis and RiBi. By contrast, mTORC2 is known to regulate cytoskeletal organization and cell-survival pathways[49]. Extracellular signals regulate mTOR activity by binding to receptor tyrosine kinases (RTKs) and transducing signals that activate mitogen-activated protein kinases (MAPKs) or other serine/threonine kinase (S/T kinase) pathways[49]. Pathways and protein complexes are simplified for illustration purposes. Rapamycin and rapamycin analogues (rapalogues) can enter the cell and inhibit mTORC1 by binding to FK506-binding proteins (FKBPs)[49]. Direct mTOR inhibitors, such as Torin1, bind directly to mTOR and inhibit both mTORC1 and mTORC2 (reviewed in[49]). Ribosomal stress caused by knockout of the gene encoding the ribosome rescue factor Pelota or its interacting factor HBS1L can lead to compensatory activation of mTORC1 in epidermal stem cells and fibroblasts[25,206]. **b** | mTOR activity and its control over the activation and differentiation in embryonic stem cells (ESCs) and adult stem cell types. As a general rule, mTOR activation and 4E-BP phosphorylation (4E-BP-P) tend to promote differentiation and stem cell loss, while mTOR inhibition tends to promote quiescence and stem cell maintenance, although there are exceptions. In ESCs, inhibition of mTOR signalling induces a paused quiescent state, whereas activation of mTOR and/or increased 4E-BP phosphorylation promotes differentiation. The switch to differentiation is characterized by a transient drop in mTOR signalling before mTOR activity rises in the differentiated cell. In induced pluripotent stem cells (iPSCs), mTOR activation or increased 4E-BP phosphorylation prevents the induction of pluripotency. In neural stem cells (NSCs), mTOR activity is low in the quiescent state but rises in active NSCs, which are primed to differentiate. As for ESCs, a drop in mTOR signalling is required at the induction of NSC differentiation. mTOR activity in fully differentiated neural cells (that is, neurons and glia) relative to NSCs is not clear. In satellite cells (muscle stem cells), low mTOR signalling supports the quiescent state, whereas high mTOR signalling supports an active state. mTOR activity in fully differentiated muscle cells relative to satellite cells is not clear. PTEN, phosphatase and tensin homologue.

promote translation initiation by mechanisms that are not fully understood[64].

Many studies have demonstrated that mTOR is essential for survival and function of ESCs and tissues of the early embryo. Loss-of-function mutations in mTOR or its effectors lead to embryonic lethality and patterning defects in mice and prevent the establishment of ESCs[65–67]. In mouse oocytes, 4E-BP1 phosphorylation by mTOR is tightly regulated to promote specific translation programmes during meiosis[68], and conditional knockout of the gene encoding mTOR in oocytes leads to infertility[69]. While mTOR is essential for early embryonic development, its activity seems to be maintained at a low level in ESCs to coordinate low protein synthesis rates and stem cell function (FIG. 3). In mouse and human ESCs, low phosphorylation of 4E-BP1 maintains the undifferentiated state[14]. Likewise, human ESCs maintain high expression of the TSC1–TSC2 complex (an upstream inhibitor of mTORC1 activity) and concomitant low expression of mTOR activity compared with differentiated progeny; TSC2 depletion or constitutively active mutant S6K induces mTOR-dependent translation and ESC differentiation[19]. Partial inhibition of mTOR leads to a paused pluripotent state in ESCs, further supporting the notion that low protein synthesis coordinated by low mTOR activity promotes stem properties in ESCs and cells of the early embryo[70].

In somatic stem cells, direct connections between mTOR, protein synthesis, RiBi and stem cell function have been most extensively studied in the neural lineage. These studies provide key insights into how mTOR activity coordinates, at the translation level, the switch from a stem cell self-renewal programme to a differentiation programme. Like ESCs, mouse NSCs maintain low rates of protein synthesis in the quiescent state[26]. This is probably coordinated by low mTOR activity, as reductions in mTOR signalling or overexpression of constitutively active, non-phosphorylatable 4E-BP1 promotes mouse NSC self-renewal and disrupts differentiation, whereas knockdown of 4E-BP2, the major isoform expressed in the brain, promotes NSC differentiation[71]. Upon receipt of various upstream signals, such as cellular injury, NSCs are induced to enter a primed predifferentiation state characterized by increased protein synthesis[72,73]. In this activated state, mTOR signalling is high[26]. However, upon neural differentiation and exit from the cell cycle, mTOR signalling transiently drops, leading to translation repression of a subset of mTOR-dependent mRNAs, including those involved in maintenance of stem cell identity and the synthesis of ribosomes[17,26]. In this way, tight regulation of mTOR signalling controls the NSC differentiation switch through coordination of specific protein synthesis and RiBi (FIG. 3).

mTOR coordinates similar phenomena in stem cell types beyond NSCs. For instance, in response to cell injury, the transition of quiescent muscle satellite cells to an 'alert' state primed for differentiation is coordinated by mTOR signalling[74]. In the mouse epidermis, knockout of the genes encoding RAPTOR and RICTOR — the major proteins that constitute mTORC1 and mTORC2 — disrupts differentiation and leads to epidermal barrier dysfunction[75,76]. Tight regulation of mTOR activity is also necessary for induction and maintenance of pluripotency, as somatic cells with either overactive or inactive mTOR signalling fail to be reprogrammed into iPSCs[77], and repeated rounds of mTORC1 activation drive age-related stem cell loss in *Drosophila* intestinal epithelium and mouse tracheal epithelium[78]. Thus, dynamic regulation of mTOR signalling to coordinate the translation switch between self-renewal and differentiation programmes is probably prevalent and extends to stem cell lineages beyond NSCs.

Although the regulation of mTOR signalling is clearly integral to coordinating protein synthesis, RiBi and stem cell function, how stem cells maintain low rates of protein synthesis but high RiBi in the context of mTOR signalling is not immediately apparent, as these processes might be expected to be regulated concomitantly by mTOR. Low protein synthesis rates in mouse ESCs, HSCs and HFSCs do not reflect differences in cell proliferation rate, cell cycle stage, cell size and/or total mRNA or rRNA content[14,21–24], which are other phenotypes that might be expected to correlate with the level of mTOR signalling[49,79,80]. Instead, we speculate that the

Cell lineages
The developmental paths of a tissue or organ from single or multiple cell types.

coordination of mTOR signalling with other signalling regimes and regulatory mechanisms allows stem cells to maintain this unique signature.

MYC expression appears to be critical to maintain high transcription of rRNA and RiBi genes even under conditions when mTOR signalling is typically low in stem cells[30,36,38]. Further contributions to these distinct regulatory programmes are probably made by the developmentally controlled RNA-binding protein LIN28, which represses translation of endoplasmic reticulum-associated secretory proteins, but maintains translation of the RiBi machinery[39,40,81]. Interestingly, there appear to be differences in the translation signatures between fetal and adult stem cell populations[82] and these differences may in part be regulated by LIN28 (REFS[83–85]). Another RNA-binding protein, Musashi 2 (MSI2), directly modulates the translation of mTOR and MYC signalling components in blood and intestinal stem cell lineages[86–88]. Other RNA-binding proteins are known to be essential for maintenance of pluripotency and have been reviewed elsewhere

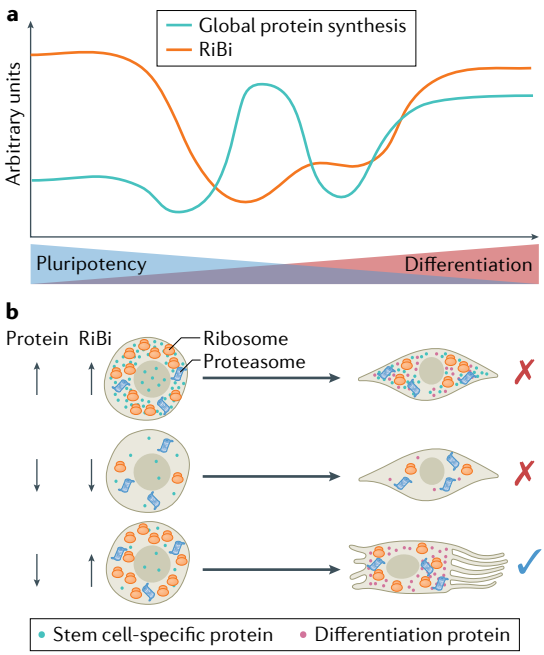


Fig. 4 | **The differentiation 'switch'. a** | Cartoon depiction of global protein synthesis and ribosome biogenesis (RiBi) during differentiation. Global protein synthesis rates tend to be lower in stem cells than in differentiated cells, while rates of RiBi are selectively high. During differentiation, rates of protein synthesis and RiBi are tightly and dynamically regulated in accordance with the needs of the differentiating cell. Upon exit from differentiation, rates of protein synthesis tend to be higher than in the stem cell to support the protein synthesis needs of the differentiated cell. **b** | 'Proteome reassembly' model. Global protein synthesis rates that are too high in stem cells impair differentiation by preventing clearance of the stem cell proteome. RiBi that is too low in stem cells impairs differentiation by maintaining too few latent ribosomes to rapidly synthesize the proteome of the differentiated cell. By maintaining globally low protein synthesis rates but a pool of latent ribosomes, stem cells are able to successfully differentiate by rapidly and efficiently reassembling their proteome.

(see[89] for one example), but parsing direct roles in translation versus alternative or complementary processes such as mRNA splicing, localization or stability has proved challenging. Finally, a minor contribution to translation repression in certain stem cell lineages may be made by ZSCAN4, which leads to global translation repression through expression of eIF1A-like genes[90]. Beyond these, major pathways that license selective protein translation in stem cells are discussed later.

Functional considerations of low protein synthesis and high RiBi in stem cells. The conservation of low rates of protein synthesis among many stem cell types suggests that it is important to stem cell function. One thought is that low protein synthesis rates decrease 'wear and tear' on the cell and are therefore essential for maintenance of stem cell self-renewal and longevity[21]. This idea is appealing but seems unlikely to be the full answer: ex vivo longevity of mouse blastocyst-derived ESCs is barely increased by treatment with the translation inhibitor cycloheximide, while it is greatly increased by inhibition of mTOR signalling[70]. Thus, rather than acting as a driver of longevity, low protein synthesis rates are more likely a by-product of signalling processes such as inhibition of mTOR signalling, which maintains longevity. Another idea is that low protein synthesis rates simply reflect the need for a less diverse proteome in stem cells as compared with differentiated cell types with directed functions[14]. Future proteomics experiments comparing the diversity of proteins expressed in stem cells relative to differentiated cells will probably shed light on this hypothesis.

Finally, maintaining a low abundance of total protein may be essential for stem cells to de novo create a proteome that is optimized to perform the specialized functions of the differentiated cell (FIG. 4). A low abundance of proteins would therefore allow stem cells to quickly 'erase' the old proteome when they receive signals to differentiate. Consistent with this hypothesis, many core pluripotency factors have short half-lives due to their targeted degradation by the ubiquitin–proteasome system[91,92]. Furthermore, high proteasome activity is a known contributor to ESC and iPSC function and viability[93–95]. This regulation may, however, be quite nuanced, as the pluripotency marker SOX2 appears to be stable in NSCs and only polyubiquitylated and targeted for degradation during neural differentiation[96]. An extreme example of proteome remodelling is observed during terminal erythroid differentiation, in which the E2/E3 ubiquitin ligase UBE2O targets a wide array of substrates to trigger clearance of progenitor proteins[97].

Like low protein synthesis, high levels of RiBi could be a by-product of other signalling processes in stem cells. For instance, in addition to the RiBi machinery, MYC has a large and diverse set of transcriptional targets, and so it is easy to imagine how activation of MYC (or other pluripotency transcription factors) could lead to an accumulation of ribosomes even if the RiBi machinery components are not the main transcriptional targets that maintain the pluripotent state[36,38]. However, we consider it unlikely that the cell would upregulate

such an energy-consuming process as a secondary effect. Additionally, ribosome abundance is thought to correlate with proliferation rates across diverse cell types[30,98,99]; therefore, high RiBi in the stem cell may be important for promoting self-renewal, potentially through signalling mechanisms that regulate cell cycle activity on the basis of ribosome numbers.

We and others[17,26,28,41] endorse the parsimonious idea that a pool of latent ribosomes is important to prepare stem cells to rapidly and effectively differentiate once the appropriate environmental cues are received (FIG. 4). Just as stem cells must remove their old proteome upon differentiation, they must also rapidly replace it with the correct proteins at the correct time to support the differentiation process. Keeping a large pool of latent ribosomes should allow a quick and efficient transition. Further studies are needed to test these hypotheses, but we propose that this 'proteome reassembly' model could functionally explain the unique stem cell signature of low global protein synthesis with concomitant high RiBi.

Translation dynamics in stem cells

Signalling pathways that impinge on the translation machinery (such as mTOR) allow cells to acutely modulate their translational output in accordance with intracellular and extracellular cues. In this section, we discuss translation dynamics in stem cells. We focus on differentiation as a stem cell function that features dynamic changes in the translation status of the cell and is exquisitely sensitive to perturbations that disrupt these dynamics.

Differentiation is a translationally dynamic period. To date, most studies in stem cells have visualized protein synthesis at one or a few discrete time points, leading to the consensus that the translation rate increases and RiBi decreases as stem cells differentiate[14–17,41]. Two recent studies have increased the number of time points examined and revealed that translation regulation is even more dynamic than previously appreciated[26,33] (FIG. 4).

NSCs and their progenitor cells were isolated at various stages of differentiation towards olfactory bulb interneurons and used to measure nascent protein synthesis rates[26]. Protein synthesis was found to rise and fall multiple times as neural differentiation progressed. These observations argue for multiple phases of dynamic regulation. A complementary study identified a RiBi factor, HTATSF1, which promotes the transcription and processing of rRNAs, r-proteins and translation factors in mouse ESCs[33]. The levels of HTATSF1 and RiBi markers transiently dropped over the first few days of differentiation of mouse ESCs to EBs, before rising again at later time points. These dynamics are recapitulated in vivo as the embryonic day 4.5 mouse epiblast differentiates into the embryonic day 7.5 neuroectoderm[33]. Together, these results suggest that precise and dynamic regulation of protein synthesis and RiBi are essential for differentiation of mouse ESCs. What unifies these two reports is how the changes during differentiation are not monotonic (FIG. 4). We anticipate that such dynamic regulation of protein synthesis and the translation machinery may be a more prevalent feature of differentiating cells than previously anticipated.

Differentiation is sensitive to ribosome levels. Insults that disrupt ribosome function or numbers are generally detrimental to cell function and viability[100]. The stem cell is no exception, as defects in the translation machinery can lead to stem cell death[28]. Furthermore, disruptions in RiBi and r-protein stoichiometry activate apoptosis through the well-established MDM2–p53 signalling axis in stem cells[34,42,43,101,102].

In addition to these general cell death responses, differentiation is particularly sensitive to variations in the levels of functional ribosomes, which reinforces the significance of the tight regulation of RiBi that is observed immediately before and during differentiation[17,26,33,41]. The importance of this regulation is made apparent by ribosomopathies, a class of human diseases often caused by mutations in r-proteins, which are thought to lead to differentiation defects towards specific cell lineages. One well-characterized example is Diamond–Blackfan anaemia, which is caused by heterozygous mutations in one of many possible r-proteins, which leads to disruptions in the differentiation from HSCs to the erythroid lineage[103–105]. In a cell model of Diamond–Blackfan anaemia, knockdown of TSR2, a RiBi factor involved in maturation of the r-protein RPS26, or knockdown of individual r-proteins each decreased total ribosome levels in HSCs and recapitulated the differentiation defects towards erythroid lineage cells[105]. This is an illustrative case, as it shows that alterations in the production of r-proteins in HSCs lead to a concomitant decrease — through translation repression — in the levels of all other r-proteins to maintain proper stoichiometry. Another well-studied ribosomopathy that disrupts myeloid lineage differentiation is Shwachman–Bodian–Diamond syndrome, which is caused by a homozygous loss-of-function mutation in the 60S maturation factor SBDS leading to defects in pre-rRNA processing[102,106].

Beyond these disease examples, studies in cell-culture systems highlight the particular sensitivity of differentiation to the levels of functional ribosomes within the cell. For example, hemizygous r-protein mutations lead to decreased polysome levels in ESCs and are enriched as a class among mutations that disrupt mouse ESC-to-EB differentiation[15]. These hemizygous r-protein mutations have no detrimental impact on stem cell self-renewal, highlighting the enhanced sensitivity of differentiation to the levels of functional ribosomes. In the case of the RiBi factor HTATSF1, it is observed that not only too few but also too many functional ribosomes may disrupt differentiation, as either deletion or overexpression of HTATSF1 prevents differentiation of ESCs to EBs[33]. Strikingly, the differentiation defect in HTATSF1-deficient mouse ESCs can be rescued by a loss-of-function mutation in the protein factor phosphatase and tensin homologue (PTEN), a negative regulator of mTOR (FIG. 3), presumably by rescuing the deficiency in RiBi through activation of the mTOR pathway[33]. Finally, in *D. melanogaster* GSCs RNAi knockdown of multiple RiBi factors leads specifically to a differentiation defect, while self-renewal

Ribosomopathies
Diseases caused by abnormalities in the structure and function of ribosomal proteins or genes encoding ribosomal RNA or genes involved in ribosome biogenesis.

Polysome
Multiple ribosomes loaded onto a single mRNA ('polyribosome').

remains intact[28], and overexpression of RNA polymerase I, which leads to increased rRNA synthesis, delays differentiation[41]. Together, these examples highlight the exquisite sensitivity of stem cell differentiation to inappropriate levels of functional ribosomes.

It is clear from these studies that an inappropriate number of ribosomes blocks stem cell differentiation, but it remains unclear whether this results from a disruption in ribosome levels per se or from the downstream effects on global protein synthesis rates. These processes are challenging to decouple: both are tightly controlled during differentiation and both tend to be regulated in a coordinated manner by signalling pathways such as mTOR. Furthermore, compensatory signalling processes are gaining traction as an important mechanistic link between ribosomal defects and downstream phenotypic consequences at a whole-cell or whole-organism level[25,107,108], and are probably implicated in stem cell fate decisions resulting from inappropriate ribosome levels. Creative experimental paradigms will be critical to experimentally decouple ribosome levels, protein synthesis and compensatory signalling to fully appreciate the individual mechanisms by which tight regulation of ribosome levels contributes to stem cell differentiation.

The differentiation 'switch'. We propose a differentiation 'switch', which integrates these themes into a cohesive framework describing how translation dynamics and RiBi fit into the larger picture of stem cell differentiation (FIG. 4). First, stem cells in the undifferentiated state maintain a low abundance of total protein through global translation repression. At the same time, stem cells maintain a high concentration of latent ribosomes through upregulated RiBi. Both of these properties prepare stem cells for differentiation by enabling the rapid and efficient reassembly of the proteome. At this stage, defects that lead to an overabundance of stem cell proteins or too few ribosomes may negatively impact the onset of differentiation. On receipt of signals to differentiate, RiBi is transiently downregulated in favour of allocating resources for the rapid synthesis of proteins and other factors that are essential for differentiation. Global translation enters a dynamic period in which protein synthesis output and RiBi are acutely tuned to the needs of the differentiating cell. At this stage, differentiating cells are especially sensitive to insults to the ribosome. Similarly to regulation of mTOR signalling[17,26,28,41,74,77], we predict that other signalling processes that control protein synthesis and RiBi are also tightly regulated during this period. Eventually, the cell enters a metastable translation state, corresponding to the completion of differentiation. In this state, the protein synthesis rate tends to be higher than in the stem cell, thereby supporting the protein synthesis needs and directed functions of the differentiated cell. While there may be exceptions to these general trends — for example, activated NSCs have relatively high protein synthesis rates relative to more-differentiated neural cell types[26,73] — the differentiation switch can serve as a useful framework for understanding how protein synthesis and RiBi dynamics contribute to stem cell differentiation success.

Translational control of non-differentiation stem cell functions. Differentiation is not the only aspect of stem cell function that requires tight translation control. The ability of HSCs to engraft (that is, to survive and proliferate in the bone marrow of a new host following transplantation) is compromised by a hypomorphic mutation in the gene encoding the r-protein RPL24 (*Rpl24*$^{Bst/+}$), which reduces ribosome levels and protein synthesis, and by knockout of the gene encoding PTEN, which increases mTOR signalling, RiBi and protein synthesis[21]. Therefore, HSC engraftment, like differentiation, is finely tuned to the overall levels of RiBi and protein synthesis. When knockout of the gene encoding PTEN is combined with the *Rpl24*$^{Bst/+}$ mutation, protein synthesis rates are normalized and engraftment success is restored. *Pten* knockout additionally increases the rate of leukaemogenesis when donor bone marrow is transplanted into an irradiated host, and this is suppressed when protein synthesis rates of the donor bone marrow cells are normalized by the *Rpl24*$^{Bst/+}$ mutation. This suggests that tight control over RiBi and protein synthesis rates is important to prevent cancer progression in HSCs or other cells of the bone marrow microenvironment[21,109].

In a study in mouse epidermal stem cells, the conditional knockout of the gene encoding the ribosome rescue factor Pelota (*Pelo*) leads to increased protein synthesis rates through mTOR signalling and, ultimately, lethal epidermal barrier defects caused by overproliferation and accumulation of aberrant cells in the epidermis and hair follicles[25,110] (FIG. 3). Although the mechanistic link between the *Pelo* knockout and increased protein synthesis is not fully understood, when the protein synthesis rate is normalized by treatment with rapamycin, an inhibitor of mTOR, the overproliferation and epidermal barrier defect phenotypes are largely rescued[25].

Taken together, these studies demonstrate that multiple aspects of stem cell activity beyond differentiation, including engraftment, tumour suppression and epidermal barrier function, are acutely tuned to the regulation of ribosome number and activity.

Selective mRNA translation in stem cells

Features encoded in the 5′ untranslated region (UTR), open reading frame or 3′ UTR of mRNAs can alter the rates of mRNA translation and therefore regulate cell function in different contexts[10,111]. Considering that stem cells are characterized by low global protein synthesis rates, how they selectively translate important mRNAs is not immediately apparent. Not surprisingly, mRNA features regulate protein expression in self-renewing stem cells and during cell differentiation[17,112]. Translation initiation is considered to be the major and consequently best-studied point of regulation for selective translation of mRNAs; therefore, many of the currently understood mechanisms exert control at this step, although we also discuss mechanisms that regulate translation elongation (FIG. 5).

eIF4F-regulated translation. eIF4F is a trimeric complex that recruits the 43S PIC to the 7-methylguanosine cap of mRNAs to promote 40S subunit scanning and translation initiation at the start codon, usually AUG[10].

Hypomorphic mutation
A mutation in a gene that confers less function than the wild-type copy of that gene but retains more function than a complete loss-of-function allele.

Ribosome rescue factor
A protein factor that is involved in dissociating the large and small ribosomal subunits of a ribosome stalled on an mRNA (for example, the Pelota–HBS1L complex).

The three protein subunits of the eIF4F complex are (1) eIF4E, which binds to the mRNA 7-methylguanosine cap, (2) eIF4G, which physically links eIF4E at the cap to poly(A)-binding proteins at the mRNA tail, and (3) eIF4A, a DEAD-box protein that promotes 5′ UTR unwinding. Although generally thought to work together as the eIF4F complex, eIF4E/G and eIF4A appear to regulate the translation of distinct subsets of transcripts that are important for stem cell function (FIG. 5).

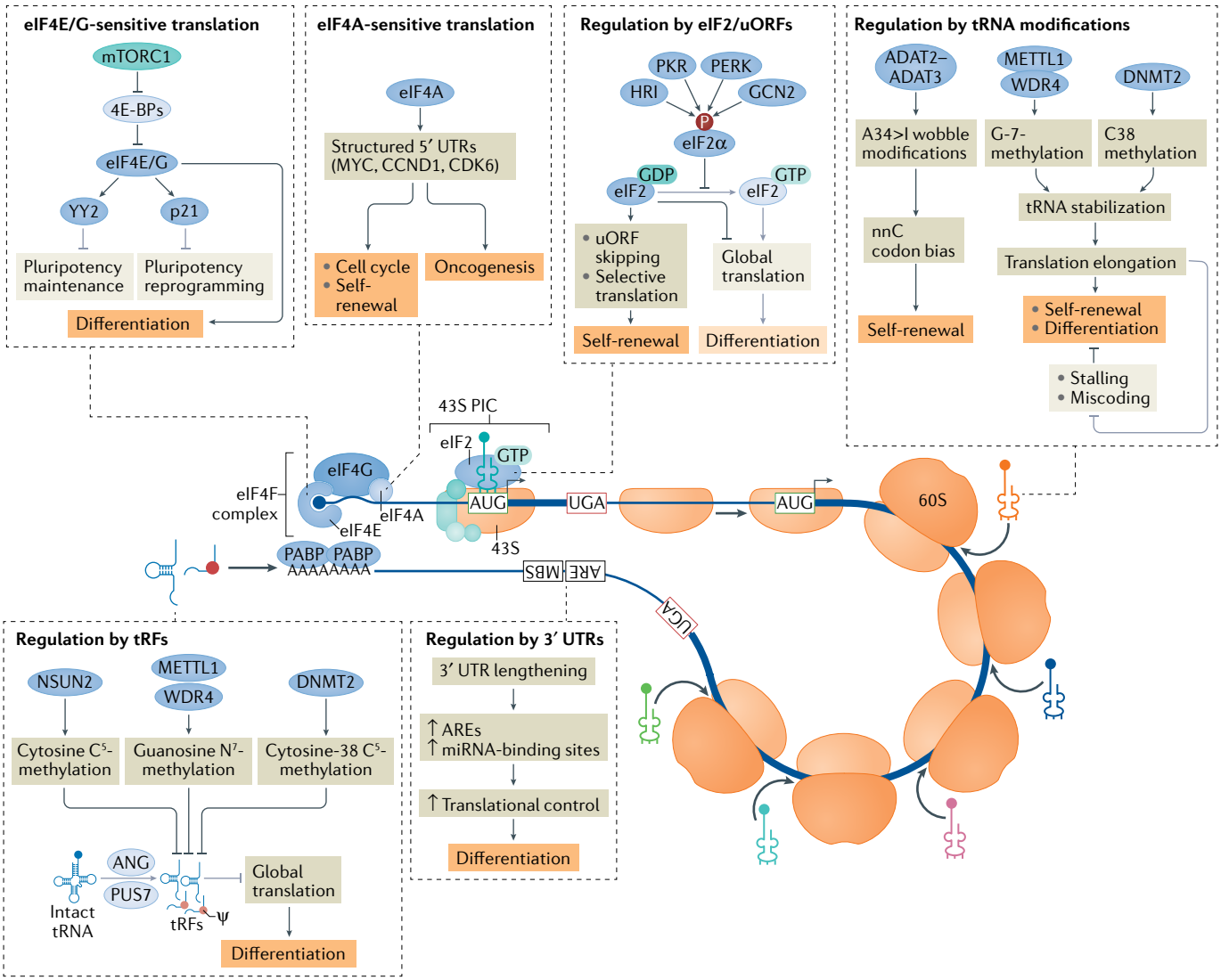


Fig. 5 | **mRNA and tRNA features regulate translation and stem cell function.** Top, from left to right: Components of the eukaryotic initiation factor 4F (eIF4F) complex mediate distinct translation programmes that regulate stem cell function. eIF4E is the mRNA cap-binding protein and eIF4G is the scaffold protein of eIF4F. The eIF4E/G translation programme is inhibited in stem cells, and its activation represses pluripotency maintenance and reprogramming, while promoting differentiation. eIF4A is the DEAD-box component of eIF4F and promotes cell cycle and oncogenesis through translation of mRNAs with structured 5′ untranslated regions (UTRs). The integrated stress response is coordinated through eIF2 phosphorylation and leads to global translation repression while promoting the selective translation of mRNAs, which promote self-renewal through upstream open reading frame (uORF) skipping. tRNA nucleotide modifications stabilize tRNAs and alter their mRNA cognate substrates, preventing tRNA depletion and ribosome stalling during translation elongation. Stabilization of tRNAs can support either a self-renewal or a differentiation regime by licensing increased translation of mRNAs with high codon density for the cognate tRNAs. Bottom, from left to right: Modifications of tRNAs prevent their degradation to tRNA-derived fragments (tRFs), which inhibit cap-dependent protein synthesis in stem cells. Stabilization of tRNAs decreases the levels of tRFs and increases global protein synthesis rates during differentiation. 3′ UTRs of mRNAs contain (A+U)-rich elements (AREs) and microRNA (miRNA)-binding sites (MBSs), which are bound by protein factors and miRNAs to repress and/or regulate translation from the mRNA. 3′ UTRs of mRNAs tend to be short in undifferentiated cell types. During differentiation, 3′ UTR lengthening and an increase in 3′ UTR elements license more-sophisticated translational control of mRNAs. 4E-BP, eukaryotic initiation factor 4E-binding protein; ADAT, tRNA-specific adenosine deaminase; ANG, angiogenin; CCND1, G1/S-specific cyclin D1; CDK6, cyclin-dependent kinase 6; DNMT2, tRNA (cytosine 38-C^5)-methyltransferase 2; GCN2, general control non-derepressible 2; HRI, eukaryotic initiation factor 2α kinase 1; METTL1, tRNA (guanine-N^7-)-methyltransferase; mTORC1, mTOR complex 1; NSUN2, RNA cytosine C^5-methyltransferase NSUN2; p21, cyclin-dependent protein kinase inhibitor 1; PABP, polyadenylate-binding protein 1; PERK, protein kinase R-like endoplasmic reticulum kinase; PIC, pre-initiation complex; PKR, protein kinase R; PUS7, pseudouridylate synthase 7 homologue; WDR4, WD repeat-containing protein 4; YY2, transcription factor YY2.

In mammals, three 4E-BPs bind to eIF4E in their unphosphorylated state and prevent its interaction with eIF4G[49]. The net result is a general inhibition of cap-dependent translation initiation as well as the translation inhibition of a subset of mRNAs that are particularly sensitive to eIF4E/G activity[50,51]. By contrast, when phosphorylated by mTOR, 4E-BPs cannot bind to eIF4E, and the translation of eIF4E/G-sensitive mRNAs is permitted[50,51]. As discussed earlier, the phosphorylation status of 4E-BPs is generally low in mouse ESCs and HSCs, consistent with low mTOR activity and suggesting that eIF4E/G-sensitive translation may be generally repressed in these stem cell types[14,21,23,113,114]. Ribosome profiling revealed that YY2, a transcriptional repressor of pluripotency genes, is translationally upregulated in 4E-BP1–4E-BP2 double-knockout mouse ESCs[113], while the cell cycle inhibitor p21 is translationally upregulated in 4E-BP1–4E-BP2 double-knockout mouse embryonic fibroblasts[114]. Thus, eIF4E/G-sensitive translation of YY2 and p21 downstream of 4E-BP phosphorylation is thought to promote differentiation and suppress pluripotency maintenance and reprogramming. Similarly, in NSCs, 4E-BP knockdown promotes differentiation by licensing translation of differentiation genes, whereas 4E-BP overexpression prevents expression of differentiation genes[71]. Together, these examples suggest that activation of eIF4E/G coordinates stem cell differentiation through selective mRNA translation. Nonetheless, how mRNA selectivity is achieved by eIF4E/G is not well understood.

Another component of the eIF4F complex, the DEAD-box protein eIF4A, is important for promoting translation of particular classes of mRNA with structured 5′ UTRs[115,116]. eIF4A has been studied primarily for its role in promoting oncogenesis[117–119], and it has become clear that the translation of mRNAs for cell cycle regulatory genes, notably those encoding MYC, cyclin D1 and CDK6, are sensitive to eIF4A inhibition. Because these cell cycle genes are important regulators of stem cell self-renewal, it is expected that eIF4A-dependent translational regulation is important for normal stem cell function. Furthermore, recent evidence suggests another DEAD-box protein, DDX3, to be important in human ESC maintenance[120]. DDX3 associates with the eIF4F complex and, like eIF4A, promotes the translation of cell cycle regulatory genes such as the gene encoding cyclin E1 (REFS[121,122]). Thus, maintenance of self-renewal in stem cells appears to be dependent on multiple DEAD-box proteins that unwind structured 5′ UTRs.

Although eIF4E/G and eIF4A work together as part of the eIF4F complex, they regulate the translation of distinct mRNAs[117,118], but how such distinction is achieved is unclear. One hypothesis that has gained traction is that differential sensitivity to these factors is conferred by specific 5′ UTR sequence motifs. While eIF4A is important for translation of mRNAs with structured 5′ UTRs and G-quadruplexes[115,117–119], eIF4E/G-sensitive translation appears to primarily involve 5′ UTRs containing TOP motifs[50,51], pyrimidine-rich motifs[26], C-rich motifs[123] and extremely short 5′ UTRs[53,124]. Beyond 5′ UTR motifs, further insight into this regulation is found in studies focused on the alternative eIF4G isoform, eIF4G2 (also known as DAP5 or NAT1), which complexes with eIF4A but not eIF4E to promote translation of specific mRNAs essential for differentiation of mouse ESCs[125]. eIF4G2 uses eIF3D as an alternative cap-binding protein to eIF4E, which explains how subsets of mRNAs may not rely on eIF4E for their cap-dependent translation[126,127]. More broadly, the eIF4A and eIF4G families each have three isoforms, which may contribute to explaining aspects of regulation in stem cells. eIF4E also forms alternative complexes with 4E-T to sequester mRNAs into P-bodies during neural differentiation[128]. From the findings taken together, it seems likely that eIF4E/G and eIF4A regulate translation of distinct mRNAs in part through involvement in protein complexes distinct from eIF4F.

uORF-regulated and eIF2-regulated translation. With the development of high-throughput sequencing and ribosome profiling methods, we have gained thorough understanding of the control of translation by upstream open reading frames (uORFs). For many years, there were only a few known examples of regulatory uORFs, while it is now argued that ~50% of human mRNAs contain uORFs, most of which are actively translated[129,130]. uORFs are encoded within the 5′ UTR of an mRNA and have been shown to act as *cis* regulators of translation of the downstream main open reading frame (mORF)[10,131]. Because translation reinitiation in eukaryotes is inefficient, translation of uORFs typically inhibits translation of the mORF. The regulatory capacity of uORFs is directly tied to the integrated stress response (ISR) and thus to the levels of eIF2α phosphorylation: while uORFs are generally inhibitory to mORF translation, eIF2α phosphorylation can lead to uORF skipping and therefore increased translation of the mORF for a number of uORF-containing transcripts[10,132]. In mammals, four kinases are known to phosphorylate eIF2α at Ser51: (1) haem-regulated eIF2α kinase 1 (HRI); (2) double-stranded RNA-sensing protein kinase R (PKR); (3) unfolded protein-sensing PKR-like endoplasmic reticulum kinase (PERK); and (4) amino acid-sensing kinase general control non-derepressible 2 (GCN2)[133]. While eIF2α phosphorylation at Ser51 leads to global translation repression by preventing GTP–GDP exchange of the eIF2–$tRNA_i^{Met}$–GDP ternary complex (which is essential for translation initiation), selective uptranslation of mORFs of uORF-containing mRNAs is known to be a critical output[10,134,135] (FIG. 5).

As in many other cell types, uORF regulation of translation is ubiquitous in stem cells[16,46,136–138]. In ESCs, the mRNA encoding the core pluripotency transcription factors Nanog and MYC both have actively translated uORFs[16,136]; as a result, Nanog and MYC expression is increased following eIF2α phosphorylation[139]. Furthermore, uORF regulation of the Sonic hedgehog (SHH) signalling pathway receptor PTCH1 is essential for proper neural differentiation, as shown by the inability of *Ptch1*$^{\Delta uORF/\Delta uORF}$ mouse ESCs to differentiate to neurons[137]. In muscle stem cells, high basal eIF2α phosphorylation promotes self-renewal through repression of global protein synthesis with selective mRNA translation downstream of uORFs[27]. By contrast, replacing

Ribosome profiling
A method to deep sequence ribosome-protected mRNA fragments, also known as ribo-seq.

G-quadruplexes
RNA secondary structures canonically formed by the stacking of planar guanine tetrads and stabilized by Hoogsteen base pairing and a central cation.

Upstream open reading frames
(uORFs). Translatable open reading frame sequences within the 5′ untranslated region of an mRNA.

Integrated stress response
(IRS). An extensive intracellular signalling network activated in response to various stresses to maintain cellular homeostasis.

endogenous eIF2α with a non-phosphorylatable form (S51A) leads to a failure of self-renewal and entry to a terminal differentiation programme[27].

The past decade has seen a large amount of work unravelling the mechanisms of uORF and ISR control of translation. uORF-encoded microproteins have been found to function in *trans* to regulate the function of the mORF protein[140]. We predict that future work will further highlight the control of stem cell function through uORF-mediated and eIF2-mediated translation programmes.

3′ UTR-regulated translation. 3′ UTRs encode multiple elements that determine the stability and translation status of an mRNA[141]. These include (A+U)-rich elements and microRNA-binding motifs, which recruit factors that cause mRNA destabilization and/or translation repression. Therefore, mRNAs with long 3′ UTRs tend to be extensively regulated and less permissive to translation on average[17,142] (FIG. 5). Importantly, 3′ UTR length can be modulated through multiple mechanisms, including alternative splicing and alternative cleavage and polyadenylation.

Intriguingly, widespread shortening of 3′ UTRs and a reduction in 3′ UTR regulatory capacity through alternative polyadenylation were observed in highly proliferative cancerous and non-cancerous cells[143,144]. Human ESCs and human neural progenitor cells also appear to express mRNA isoforms that have shortened 3′ UTRs compared with those of differentiated neurons[17]. By contrast, widespread lengthening of 3′ UTRs is observed during differentiation of mouse ESCs to neurons[17]. Differentiating neurons on average select not only for mRNA isoforms containing longer 3′ UTRs but also for mRNA isoforms containing increased density of microRNA-binding sites and (A+U)-rich elements[17]. Together, these findings suggest that cells that divide rapidly and/or self-renew may preferentially select for expression of mRNA isoforms with decreased regulatory capacity by 3′ UTR elements. In support of this conclusion, widespread lengthening of 3′ UTRs is also observed during mouse embryonic development and differentiation of mouse myoblasts to muscle cells[17,145]. Recent evidence obtain by single-cell RNA sequencing demonstrates that alternative cleavage and polyadenylation mechanisms contribute to 3′ UTR selection during neural differentiation[146]. In the context of tight control of protein synthesis rates and RiBi (FIG. 4), it seems likely that 3′ UTR lengthening with increased density of regulatory elements is an important mechanism that enables fine-tuning of the translational regulation of specific mRNAs during differentiation. Selective translation through 3′ UTR elements is also known to regulate cell fate in epidermal stem cells, muscle stem cells and adipocyte stem cells[147–149], together suggesting 3′ UTRs to be a prevalent feature that regulates cell fate decisions in many stem cell types.

Ribosome concentration-regulated translation. Models and experimental data going back to the 1970s suggest that mRNA translation is differentially sensitive to ribosome concentrations[105,150,151]. These models proposed that when the concentration of assembled cellular ribosomes drops, the translation of mRNAs with low initiation rates might be more sensitive to ribosome depletion, while the translation of mRNAs with high initiation rates may remain more stable[150,151]. More recently, these models were assessed in the context of ribosomopathies and potential connections to ribosome quality control pathways[104,105]. What was lacking in these models and later discussions was a clear understanding of what the rate-limiting step in translation initiation might be and how ribosome concentration might impact this step. For example, it is easier to understand how the second-order reaction of the small ribosomal subunit being recruited to an mRNA would be dependent on ribosome concentrations, while it is less clear how the first-order scanning reaction of the small ribosomal subunit on an mRNA might be impacted by ribosome concentration. Models are currently limited by our lack of understanding of which of these steps is rate limiting in different cellular contexts and on mRNAs of differing composition.

The contribution of ribosome concentration to stem cell function has been extensively studied in the haematopoietic lineage. These studies have revealed a key gene that is sensitive to r-protein depletion, the gene that encodes GATA1, a core transcription factor essential for the proper fate specification of HSCs to erythroid lineage cells[104,105,152]. While it was originally proposed that GATA1 is expressed with a long and highly structured 5′ UTR that impedes efficient scanning by the small ribosomal subunit and causes *GATA1* mRNA to be poorly translated[104], a more recent study by the same group proposed that the *GATA1* mRNA actually contains a short and unstructured 5′ UTR[105]. More broadly, the study authors suggest that depletion of multiple distinct r-proteins results in a translational signature in which a common group of mRNAs is sensitive to this depletion. These results are consistent with similar work performed in yeast[99]. Collectively, these studies provide support for the idea that dynamic fluctuations in ribosome concentration during stem cell differentiation could regulate the translation of specific mRNAs in time and space to support differentiation[26,33]. The molecular explanation for how certain mRNAs are sensitive to these concentration differences will require further exploration.

tRNA modifications in stem cell function

tRNAs can carry nucleotide modifications, which affect translation elongation, and thus mRNA stability, by regulating tRNA stability (usually increased)[153] or tRNA affinity for cognate mRNA codons (when the modification is in the anticodon loop)[154]. Indeed poorly translated mRNAs — owing to stalling on mRNA codons recognized by depleted tRNAs or reduced tRNA affinity — are prone to degradation[130,155,156]. Beyond having this role of regulating translation elongation and mRNA stability, tRNA nucleotide modifications protect against the accumulation of tRNA-derived fragments (tRFs), which may function as important signalling molecules in the cell[24,153,157,158]. Through these mechanisms, tRNA/tRF modifications and abundance are emerging as important regulators of stem cell function[153] (FIG. 5).

The upregulation of specific tRNAs can contribute to cell fate decisions by leading to the preferential translation and stabilization of mRNAs that are enriched in their cognate codons. For example, genes that are involved in cell-autonomous functions preferentially use different codons, on average, to encode the same amino acid compared with genes involved in multicellularity processes[159]. To exploit this, proliferating cells upregulate tRNAs that correspond to the codon usage in pro-proliferation genes, whereas differentiating cells upregulate tRNAs that correspond to the codon usage in prodifferentiation genes[159]. Thus, the abundance of specific tRNAs is coordinated with codon usage for the mRNAs present for that stage of cell growth. In mouse ESCs, the tRNA N^7-methylguanosine methyltransferase complex METTL1–WDR4 is highly expressed and important for both self-renewal and differentiation, and the same is true of the tRNA cytosine C^5-methyltransferase DNMT2 in HSCs and mesenchymal stem cells[160,161]. These factors stabilize their tRNA substrates to protect against degradation and subsequent ribosome stalling and/or miscoding during translation elongation. In the case of METTL1–WDR4, deficiencies in these factors impair mouse ESC self-renewal and differentiation towards neural lineages, while a deficiency in DNMT2 impairs HSC self-renewal and differentiation towards lymphoid lineages. Other tRNA modifiers are differentially expressed to promote either a pluripotent state or a differentiated state. For example, adenosine-to-inosine editing catalysed by the tRNA-specific adenosine deaminase 2 (ADAT2)–ADAT3 heterodimer allows a base-pairing interaction of inosine with cytosine in the wobble position of multiple tRNAs[154]. This wobble base pair is important for proper codon recognition in human ESCs and mouse ESCs, which do not highly express other cognate tRNAs to recognize nnC codons[154]. When ESCs are induced to differentiate, ADAT2 and ADAT3 are downregulated, leading to decreased recognition of nnC codons in mRNAs that promote self-renewal[154].

tRNA modifications can modulate the abundance and function of tRFs in stem cells. Angiogenin (ANG) and other RNases cleave tRNAs to produce tRFs, which can inhibit global protein synthesis through mechanisms that are still being worked out[153,157,162–164]. For example, ANG-mediated tRF generation is known to contribute to maintenance of stemness through global translation repression in mouse HSCs[157]. By contrast, tRNA methylation protects against tRNA cleavage to produce tRFs, and in doing so appears to promote differentiation. Loss-of-function mutations in the tRNA cytosine C^5-methyltransferase NSUN2 cause a build-up of 5′ tRFs, altered translation and differentiation defects, leading to impaired spermatogenesis in GSCs and improper lineage commitment of HFSCs in mice[24,165,166].

Recent evidence in human ESCs has revealed mechanistic insight into how tRFs inhibit translation initiation[158]. PUS7 is a pseudouridine synthase that is highly expressed in ESCs and the knockout of which increases global protein synthesis. PUS7 was found to pseudouridylate short ~18-base oligo-tRFs at the U8 position (Ψ8). Importantly, tRF co-immunoprecipitation experiments revealed that only Ψ8 (but not U8) oligo-tRFs interact with the poly(A)-binding protein and in this way displace it from interacting with the eIF4F cap-binding complex, an interaction thought to be important for mRNA looping and cap-dependent translation initiation[167]. In accordance with this model, PUS7 expression decreases as mouse ESCs differentiate to EBs to relieve translation repression as differentiation progresses[158].

From the findings taken together, tRNAs and tRFs coordinate distinct translation programmes of pluripotent and differentiated cell states. Recently developed methods that enable careful quantification of tRNA abundance and modifications[168] will probably be instrumental in further defining tRNA regulatory roles during stem cell differentiation.

Dysregulation of translation in cancer

Cancer is a disease of malignant overproliferation. In general, cancers are thought to be characterized by increased protein synthesis rates, a result of metabolic reprogramming which permits biomass accumulation and high metabolic output necessary for sustained growth and proliferation. Many reviews have described the role of translation regulation in cancer (see, for example, REFS[169,170]). In this section we discuss emerging themes for how the same translation control mechanisms regulating stem cell self-renewal and differentiation may also contribute to the differentiation status of a tumour and its propensity to metastasize (FIG. 6).

Contributions of translation control to tumour 'grade'. Tumours contain heterogeneous cell populations of differentiated and stem-like cells[171–174]. In the clinic, the average differentiation status of a tumour specimen serves as a diagnostic, therapeutic and prognostic indicator for the aggressiveness of that tumour. Clinical pathologists grade tumours under the microscope from well differentiated (low grade) to poorly differentiated (high grade). In this sense, well-differentiated tumours resemble the tissue of origin and form a defined mass consisting of cells with uniform nuclei and low mitotic index. By contrast, poorly differentiated tumours feature poor structural organization and contain cells with a high nuclear-to-cytoplasmic ratio, nuclear atypia, enlarged or 'prominent' nucleoli and a high mitotic index. Poorly differentiated tumours also tend to be more aggressive and resistant to treatment, carry increased risk of invasion and metastasis and have an overall worse prognosis. While these clinical pathology designations do not strictly represent the molecular status of a tumour (that is, expression of markers of stem cells versus differentiated cells), there may be considerable overlap between these clinical definitions and our molecular and biochemical understanding of pluripotency and differentiation[175–177].

Recent evidence suggests that the repression of global translation through the same mechanisms elaborated in stem cells may support less-differentiated, higher-grade cancer phenotypes. For example, the cytosine C^5-methyltransferase NSUN2 is maintained

Codon usage
The use of one codon instead of another one to encode the same amino acid; two codons encoding the same amino acid may be recognized by different tRNAs.

Wobble position
The third nucleotide of a codon in which recognition by the cognate tRNA may occur by certain non-Watson–Crick base pairing.

Mitotic index
A measure of proliferating cells defined as the percentage of cells in mitosis. Used for tumour grading.

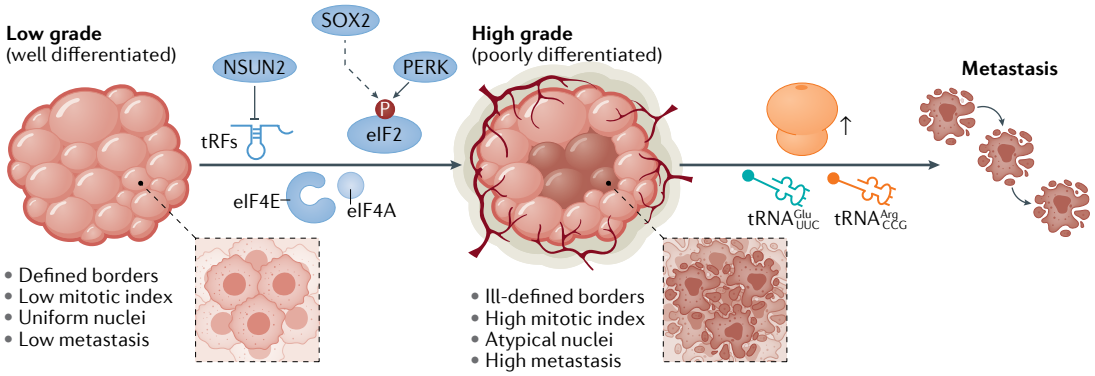


Fig. 6 | **Translational control of tumour grade and metastasis.** Mechanisms that confer global translation inhibition and selective translation programmes in stem cells also support poorly differentiated, high-grade tumours. These tumours have a higher likelihood of metastasis. Cytosine C^5 methylation of tRNAs by NSUN2 in mouse epidermal and hair follicle stem cells prevents the accumulation of tRNA-derived fragments (tRFs) and supports a low-grade tumour phenotype while promoting global protein synthesis[24]. SOX2 induction in the mouse embryonic epidermis leads to phosphorylation of eukaryotic initiation factor 2α (eIF2α), reduced global protein synthesis (through indirect mechanisms) and a squamous cell carcinoma-like phenotype[178]. SOX2 is upregulated in many human cancers and is associated with poor survival[179]. Protein kinase R-like endoplasmic reticulum kinase (PERK) phosphorylation of eIF2α reduces global protein synthesis and is involved in the malignant progression of prostate and mammary cancers[180,190]. eIF4A and eIF4E support translation of mRNAs involved in cancer progression and metastasis in multiple cancer models[51,117–119,123,181–183]. An increased pool of ribosomes promotes metastatic potential of circulating tumour cells derived from hormone receptor-positive breast cancer[192]. The expression of $tRNA^{Glu}_{UUC}$ and $tRNA^{Arg}_{CCG}$ is upregulated in human breast cancer cells as they gain metastatic potential and promotes the translation of mRNAs involved in metastasis[191].

at low levels in HFSCs, and its knockout leads to increased levels of tRFs and global translation repression[24]. In an epidermal cancer model in mice (*K5-Sos*), knockout of *Nsun2* decreases protein synthesis rates and leads to less-differentiated, more-aggressive tumours and shortened mouse lifespan. In human patients, NSUN2 levels inversely correlate with malignancy by the tumour–node–metastasis staging system[24]. These results suggest that translation repression induced by increased levels of tRFs may contribute to a less-differentiated cancer that is more aggressive in nature. Similarly, in mouse embryos, epidermis-specific induction of the pluripotent marker SOX2 — which is essential for stem cell self-renewal and reprogramming — leads to a squamous cell carcinoma phenotype that features globally repressed translation through phosphorylation of eIF2α[178]. SOX2 is similarly upregulated in many cancers and is widely associated with poor survival[179]. Finally, in a prostate cancer model, advanced-stage tumours feature increased ISR activation through the PERK–eIF2α pathway, which is required for malignant progression by reducing global protein synthesis rates, compared with less-malignant prostate cancer cells[180]. In human patients, increased eIF2α phosphorylation is associated with poorer prognosis of prostate cancers[180]. Together, these examples suggest that tumour grade and 'dedifferentiation' status may be mechanistically linked to common nodes by which stem cells repress global translation.

Selective translation mechanisms implicated in stem cells also contribute to a high-grade cancer phenotype. eIF4A promotes translation of specific oncogenic transcripts containing 5′ UTR secondary structure in T cell acute lymphoblastic leukaemia[118], breast cancer[117,119] and multiple myeloma models[181], which may promote an aggressive stem-like cancer phenotype in part through increased translation of *MYC* and consequent increased RiBi. Similarly, increased eIF4E levels promote malignant transformation through the translation of specific oncogenes such as the gene encoding cyclin D1 (REFS[123,182,183]). In SOX2-induced epidermis, ISR activation leads to selective translation of oncogenic transcripts through uORF-related mechanisms[178]. In lung and liver cancers, ISR activation promotes the selective translation of the mRNA encoding programmed death ligand 1 (PDL1) through decreased uORF translation in the PDL1 5′ UTR[184,185]. PDL1 is directly involved in immune evasion and is widely associated with more-aggressive tumours across many cancer types. Finally, just as stem cells feature shortened 3′ UTRs compared with differentiating cells[17,145], 3′ UTR shortening is commonly observed in cancer and leads to the loss of translational regulation of oncogenic mRNAs[144]. Interestingly, recent evidence suggests 3′ UTR shortening in both stem cells and cancer is coordinated by low availability of the splicing and polyadenylation factor U1 small nuclear ribonucleoprotein relative to other factors in the cell[186,187]. Together, these examples suggest that the regulatory mechanisms that maintain low protein synthesis rates and selective translation in stem cells may be hijacked by cancer to promote a less-differentiated tumour associated with an aggressive nature and a poor prognosis.

Contributions of translation control to tumour metastasis. There are at least five steps in cancer metastasis: (1) local invasion of the basement membrane, (2) intravasation into the bloodstream, (3) survival in the bloodstream, (4) extravasation into a new tissue and (5) survival and colonization in the new tissue microenvironment (reviewed in[188]). Epithelial-to-mesenchymal transition (EMT), in which carcinoma cells acquire an intermediate

Epithelial-to-mesenchymal transition
A process by which epithelial cells lose cell polarity and cell–cell adhesion properties and become mesenchymal-like cells with increased migratory and invasive potential.

Transdifferentiation
The transformation of cells other than stem cells into a different cell type.

Synthetic ribosomes
Engineered artificial small molecules that can mimic ribosome function by synthesizing peptides in a sequence-specific manner.

epithelial–mesenchymal phenotype, is thought to confer increased invasive potential and molecular and phenotypic changes that permit survival and colonization within new tissue microenvironments, collectively referred to as 'cellular plasticity'[189].

It seems plausible that many of the same translation mechanisms governing stem cell differentiation may also govern the plastic dedifferentiation and transdifferentiation processes observed in cancer metastasis and EMT. For example, eIF4E/G-sensitive translation downstream of mTOR signalling promotes metastasis in prostate cancer[51], and induction of EMT in mammary tumour cells induces eIF2α phosphorylation through the PERK pathway, which is required for invasion and metastasis[190]. Furthermore, just as expression of distinct tRNA pools regulates stem cell differentiation[159], metastatic breast cancer cells increase expression of a common set of tRNAs that promote cancer metastasis through increased codon usage of metastasis-related mRNAs[191]. In terms of direct regulation of metastasis by ribosome levels, a recent study showed that CRISPR activation of distinct large-subunit r-proteins increases the metastatic potential of circulating tumour cells derived from hormone receptor-positive breast cancer[192]. In this study, activation of single large-subunit r-proteins led to a concomitant upregulation of all other r-proteins, suggesting that ribosome levels are stoichiometrically co-regulated. Interestingly, MCF10A breast cancer epithelial cells featured a decrease in RiBi upon EMT induction by TGFβ (measured as decreased r-protein and rRNA expression) as well as a decrease in global protein synthesis. It is therefore intriguing to speculate that the same tight regulation of RiBi and protein synthesis observed during stem cell differentiation is also essential during EMT and metastasis. Nonetheless, how increasing the number of ribosomes promotes metastasis remains unclear. It may be that, just as a large pool of latent ribosomes enables efficient stem cell differentiation, maintaining this unique signature allows circulating tumour cells to rapidly reassemble their proteome during colonization of the new tissue microenvironment.

Conclusions and perspectives

RiBi and the synthesis of proteins are essential for the function of all cells from archaea to humans. The past three decades have seen a great expansion in our understanding of the vast and complex regulatory processes that control translation, from signalling processes such as mTOR, to mRNA-encoded sequence features, to RNA-binding and ribosome-binding factors. For example, our understanding of uORF regulation of protein synthesis has burgeoned since the development of ribosome footprint profiling in 2009[129]. Likewise, in stem cells, these regulatory mechanisms are now known to exert exquisite control over the translation of mRNAs in time and in place, and this control is essential for stem cell function and differentiation. Technical advances will continue to reveal new paradigms.

We expect that the next decade will see a similar increase in our understanding of these processes in stem cells and perhaps new mechanisms that are at present unimagined. Long non-coding RNAs, long thought to be translationally inactive, are now understood to translate functional peptides in some cases[16,136,140,193]. Specific long non-coding RNA populations are also known to regulate self-renewal and differentiation[194,195], but whether this occurs through translation of functional peptides, or through other mechanisms, is not currently known. Translation of chromatin modifiers maintains an open chromatin state in mouse ESCs[3], but the functional and mechanistic links between transcription and translation in stem cells remain to be fully explored. Beyond tRNAs, other RNA modifications, such as 6-methyladenosine in mRNA, are known to regulate cell fate through mechanisms that increasingly appear to impinge on the translation machinery of the cell[196–200]. The full spectrum of RNA modifications and their implications for cell fate and in disease states will undoubtedly continue to emerge. In cancer, differentiation therapy — the idea of terminally differentiating a heterogeneous population of cancer cells — has been successful in the treatment of only a few select cancers[176,177]. However, we wonder whether differentiation therapy or other similarly creative paradigms may see a resurgence as our understanding of the translational control of cancer heterogeneity, plasticity and stem-like features continues to mature. Finally, diverse signalling pathways downstream of insults to the ribosome have now been defined, and we expect that future years will reveal detailed mechanisms through which these signalling processes regulate cell fate[25,107,108,201]. In particular, recent advances such as single-cell proteogenomics[202] and the visualization of pulsatile signalling in single cells[203,204] will probably provide important insights into the contribution of signalling pathways to regulating stem cell fate.

Use of simple and accessible stem cell systems will enable us to address these and new questions. For example, lineage tracing methods within the epidermis can be feasibly combined with labelling of newly synthesized proteins or ribosomes to allow visualization along multiple differentiated lineages. Similarly, the use of small-molecule synthetic ribosomes may provide a tractable system through which novel insights can be obtained[205]. What is clear is that we are only at the beginning of unravelling the intricacies and functional implications of the translational control of stem cell function.

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