Translational control during developmental transitions

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Abstract

The many steps of gene expression, from the transcription of a gene to the production of its protein product, are well understood. Yet, transcriptional regulation has been the focal point for the study of gene expression during development. However, quantitative studies reveal that mRNA levels are not necessarily good predictors of the respective proteins' levels in a cell. This discrepancy is, at least in part, due to developmentally regulated, translational mechanisms that control the spatiotemporal regulation of gene expression. In this review, we focus on translational regulatory mechanisms mediating global transitions in gene expression: the shift from the maternal to the embryonic developmental program in the early embryo and the switch from the self-renewal of stem cells to differentiation in the adult.

Introduction

The control of fate transitions is essential during development, the process by which an entire organism is built from a single totipotent cell, and for homeostasis in adults, where stem cells sustain tissue integrity by contributing newly differentiated progeny cells. In both cases, changes in cellular fate rely on changes in gene expression, which instruct transitioning cells with new directives and provide the means to remodel cellular structure and to achieve a new identity. Not surprising, gene expression is regulated at practically every level during development and stem cell differentiation, from transcription and splicing of pre-messenger RNAs (mRNAs), through mRNA localization and decay, to translation and protein maturation, modification and degradation.

From plants to mammals, accumulating evidence indicates that translational control plays critical roles during organismal development, tissue homeostasis and tumorigenesis (Buszczak et al. 2014; Simsek and Barna 2017). While global quantification of gene expression in different cell systems shows a good correlation between mRNA and protein levels, mRNA levels seem poor predictors for protein abundance during cell state transitions, particularly during early embryonic development (Peschkin et al. 2015; Liu et al. 2016). Yet, the study of spatiotemporal regulation of gene expression and its function in controlling fate transitions has been mostly limited to chromatin-based and transcriptional mechanisms. Among other reasons, this biased attention was facilitated by the advent of new technologies to systematically study changes in mRNA accumulation and chromatin modifications at the genome level. Indeed, until recently, the study of translational changes occurring in a given developmental transition was limited to small

sets of individually tested candidate genes. With the advance of techniques able to tease apart translational changes from fluctuations in mRNA accumulation at the genome level and at nucleotide-resolution – such as ribosome profiling (Ingolia et al. 2009; Brar and Weissman 2015; Ingolia et al. 2018) – it is now possible to comprehensively visualize changes in translation during development. This has led to the realization of a much broader scale at which translation affects fate decisions and has considerably expanded our mechanistic understanding.

Historically, the most studied examples of translational control during development have been described at developmental periods during which transcriptional input is minimal, such as during oogenesis and early embryogenesis. In the absence of transcriptional activity, gene expression control is restricted to later regulatory steps, most notably translation, mRNA localization, and mRNA and protein decay. Indeed, a large amount of genetic, molecular and biochemical evidence, mostly gathered from flies and frogs, has provided an impressive detailed picture of the multitude of post-transcriptional mechanisms driving development. More recently, genome-wide approaches revealed the widespread importance of certain regulatory mechanisms, and allowed researchers to uncover new layers of regulation and to interrogate other important developmental and cell fate transitions, including stem cell differentiation and cancer.

Here, we review the knowledge gathered from studies conducted in animal models on how translational control plays essential roles during two critical developmental

processes relying on cell fate transitions: embryonic development and adult stem cell differentiation. We argue that, as the processes of post-transcriptional regulation are closer to the final protein product than the process of mRNA transcription, this type of regulation may be preferred and advantageous during rapid cell fate transitions. Finally, we discuss how recent studies revealed new mechanisms controlling translation during development.

Translational control during embryo development

With little or no transcriptional input, the late stages of egg maturation and the first steps of embryo development rely exclusively on maternally provided gene product pools (mRNAs and proteins) to lay down the basic structures upon which a new organism will be built. Far from being static, these pools rapidly evolve during the course of early development to both drive and adapt to transitioning states, creating a complex and synchronized ballet of dynamically changing mRNA and protein levels. At these developmental stages, sophisticated gene expression modules are in place to spatially and temporally regulate the expression of proteins required to control growth, establish the embryonic axes and define new cellular fates (Figure 1). The dependency on maternally provided pools ceases once the zygotic genome is activated and transcription is re-established, in a process known as the maternal-to-zygotic transition (MZT, Figure 1A) (Lee et al. 2014). While the length of the transcriptional quiescence phase varies depending on the species, reflecting evolutionary and developmental strategies, its pervasiveness in animals and plants suggests a widespread requirement for post-transcriptional regulatory

mechanisms during early embryo development (Baroux et al. 2008; Tadros and Lipshitz 2009).

mRNA localization and the spatial control of translation

The most compelling and well-characterized examples of spatially regulated expression of proteins at the subcellular level were delineated by work focusing on embryonic axis specification in *Drosophila*. In this system, the anteroposterior and dorsoventral embryonic axes are pre-determined during oogenesis by the targeted intracellular localization of mRNAs encoding key factors that are at the top of cascades establishing embryonic protein gradients. At first, an active and polarized microtubule-dependent mechanism (minus-end directed), driven by dynein (Bullock and Ish-Horowicz 2001; Dienstbier et al. 2009) and dependent on Bicaudal-D and on the RNA-binding protein Egalitarian (Egl), delivers master-regulator mRNAs - namely *oskar* (*osk*), *bicoid* (*bcd*), and *gurken* (*grk*) - from transcribing nurse cells to the transcriptionally quiescent oocyte. Once inside the oocyte, *osk* mRNA-containing particles are brought to the posterior side of the *Drosophila* oocyte through a kinesin-dependent mechanism (Brendza et al. 2000; Zimyanin et al. 2008; Lehmann 2016; Gaspar et al. 2017). Concurrently, *bcd* and *grk* mRNAs are actively delivered to the anterior and anterodorsal cortexes, respectively, through the action of dynein motors (plus-end directed) (Davidson et al. 2016; Trovisco et al. 2016). Localization specificity is mostly provided by sequences and secondary structures present in the 3' untranslated region (UTR) of target mRNAs, which are in turn recognized by double-stranded RNA-binding proteins like Egl and Staufen (Stau) (Figure 1B) (St Johnston et al. 1991; St Johnston et al. 1992; Micklem et al. 2000; Jambor et al. 2011; Le

Bouteiller et al. 2013; Jambor et al. 2014). mRNA-protein interactions required for transport are normally established in the cytoplasm, and form ribonucleoprotein complexes (RNPs) that may contain multiple mRNA copies per particle and are charged into the microtubule network (Jambor et al. 2011; Little et al. 2015; Trcek et al. 2015; Gaspar et al. 2017). Nevertheless, meaningful protein-mRNA interactions can equally well be established inside the transcribing nuclei (Chin and Lecuyer 2017). In the best-studied example, *osk* mRNA splicing and the interaction of *osk* mRNA with the exon junction complex (EJC) are critical for its final posterior oocyte localization (Hachet and Ephrussi 2004; Ghosh et al. 2012; Marchand et al. 2012). This indicates that at least in some cases, target mRNA recognition is initiated before nuclear export (Chin and Lecuyer 2017).

Regardless of the mechanism required for RNP assembly and recruitment to molecular motors, the general role for localized mRNA in determining embryo axial polarity seems to be widespread in animals. In fact, the importance of localized mRNA in defining cellular polarity goes far beyond the above described example, as it has been implicated in the polarization of migrating cells, in the establishment of apicobasal structure, and in asymmetric cell divisions (reviewed in Medioni et al. 2012; Chin and Lecuyer 2017; Moor et al. 2017).

Very important for the successful establishment of a protein gradient, localized mRNAs have to be translationally silenced during transport. Frequently, translational silencing is actively established upon targets by RNA-binding proteins that recognize sequences within

the transcript UTRs. In contrast to mechanisms involved in recruitment of mRNAs to molecular motors, which seem to rely on secondary-structure RNA motifs, translational repression is regulated by sequence-specific RNA-binding proteins that directly interact with motifs in the 3'UTR of the target RNA (Figure 1C). For instance, specific motifs in the *osk* 3'UTR (known as Bruno response elements, BRE) are recognized by the RNA-binding protein Bruno (Bru) (Kim-Ha et al. 1995; Webster et al. 1997; Snee et al. 2008), which in turn is thought to recruit Cup. The Cup protein binds to the cap-binding protein eIF4E and antagonizes eIF4E binding to the translation initiation factor eIF4G, thereby blocking *osk* translation (Wilhelm et al. 2003; Nakamura et al. 2004; Nelson et al. 2004; Zappavigna et al. 2004; Chekulaeva et al. 2006). The fact that Cup can interact with eIF4E in an RNA-independent manner further demonstrates the key role of sequence-specific RNA-binding proteins such as Bru in specifying the set of translationally regulated mRNAs (Nakamura et al. 2004). Similarly, Bru binds to the *grk* 3'UTR and, together with another RNA-binding protein (Squid), is thought to repress *grk* translation during transport (Norvell et al. 1999; Saunders and Cohen 1999; Filardo and Ephrussi 2003; Caceres and Nilson 2009; Weil et al. 2012). In addition to repressive mechanisms relying on protein-protein interactions, it is nonetheless likely that translational repression during transport may also be accomplished by physical exclusion of the translation initiation apparatus, either due to the fact that target mRNAs are compacted into large RNP complexes (Chekulaeva et al. 2006) or due to the preferential enrichment of translational activators only at the final destination (Davidson et al. 2016).

Much less understood are the mechanisms by which localized mRNAs become translationally active. Generally, poly(A) polymerases are recruited to target mRNA transcripts during translational activation or derepression (Figure 1D) (Castagnetti and Ephrussi 2003; Norvell et al. 2015). For instance, *osk* and *grk* translation is activated during oogenesis and requires the Oo18 RNA-binding protein (Orb, the homolog of the *Xenopus* cytoplasmic polyadenylation element binding protein, CPEB), a protein that is enriched inside the oocyte and that can directly associate with the poly(A) polymerases (Chang et al. 1999; Chang et al. 2001; Castagnetti and Ephrussi 2003; Weil et al. 2012; Norvell et al. 2015). Although *grk* and *osk* activation differ in many other mechanistic aspects, such as the fact that Orb directly recognizes sequences in the *osk* 3'UTR while the physical link between Orb and *grk* has not been reported, the poly(A) tails of both *grk* and *osk* mRNAs increase in length during activation. Indeed, translational activation and poly(A) polymerase activity are often correlated, although translation of maternally deposited mRNA *per se* does not necessarily require an increase in poly(A) tail length during early embryogenesis (Eichhorn et al. 2016). Such is the case of *bcd* mRNA, which is silenced during oogenesis and activated during embryogenesis. Global analysis of mRNA poly(A) traits in *Drosophila* oocytes and early embryos revealed that *bcd* mRNA translational activation is not associated with an increase in poly(A) tail length, suggesting that its temporal regulation is defined by the release of a repressive state rather than the promotion of translational activation and poly(A) polymerase activity (Eichhorn et al. 2016).

During oogenesis, regulated translation of localized *osk* mRNA at the posterior cortex of the forming egg nucleates the formation of a specialized cytoplasm – known as germ plasm

– and is required for the entrapment of a set of mRNAs passively moving in the cytoplasmic stream created during oogenesis (Lehmann 2016). Among these is *nos*, a master determinant of posterior fate, as well as many other mRNAs required for the formation of germ cells at the embryonic posterior cortex, including *pgc* and *gcl* (Wang and Lehmann 1991; Gavis and Lehmann 1992; Gavis and Lehmann 1994; Little et al. 2015; Trcek et al. 2015). Posterior localization is determined by sequences and secondary structures present in their 3'UTRs, even though mechanistically the movement of mRNAs does not include their recruitment to molecular motors or the assembly of large RNPs (Crucs et al. 2000; Forrest et al. 2004; Rangan et al. 2009; Little et al. 2015; Trcek et al. 2015). Regardless of such differences, translation of germ plasm-enriched mRNAs is also tightly regulated, with most of them being repressed during oogenesis and individually activated during embryogenesis (Rangan et al. 2009). For instance, *nos* mRNA translation is repressed during oogenesis by Glorund and Smaug (Smg), two RNA-binding proteins that interact with the *nos* 3'UTR (Smibert et al. 1999; Kalifa et al. 2006; Tamayo et al. 2017). Similarly, to Bru, Smg interacts with Cup, which then binds to eIF4E and inhibits *nos* translation initiation by precluding the eIF4E-eIF4G interaction and formation of the pre-initiation complex at the 5'end of *nos* mRNA (Nelson et al. 2004; Andrews et al. 2011). Transcript-specific translational regulation may be achieved via association of mRNAs within large, membraneless RNP granules. For example, at the *Drosophila* oocyte anterior cortex, translationally active *grk* is preferentially distributed at the edges of RNP granules, while translationally repressed *bcd* is found both in the center and at the edge of the granule (Weil et al. 2012). This correlation is not observed for mRNAs localizing to the germ granules at the posterior pole. Here multiple copies of a single RNA organize into homotypic RNA

clusters, which occupy fixed locations with respect to the protein germ granule. This spatial organization, however, does not reflect translational activity (Trcek et al. 2015). In line with the importance of such post-transcriptional mechanisms for embryonic development, 71% of the 2314 maternally deposited mRNAs tested in *Drosophila* embryos were shown to be subcellularly localized (Lecuyer et al. 2007). Beyond embryos, accumulating evidence indicates that mRNA localization is more prevalent than expected and may regulate other aspects of cellular biology, including cell signaling, neuronal axon growth, and dendritic plasticity (reviewed in (Chin and Lecuyer 2017)).

Translational efficiency and mRNA decay

Thousands of maternally transcribed mRNAs are deposited into the transcriptionally quiescent oocyte during oogenesis and the vast majority of these mRNAs are found evenly distributed in the egg cytoplasm. To drive the first steps of embryonic development, their translation is temporally – and in some cases even spatially – regulated. For instance, prominent developmental transitions such as oocyte maturation and egg activation, as well as the activation of the zygotic genome during the MZT, are characterized by large-scale changes in gene expression (Figure 1A) (reviewed in Laver et al. 2015; Yartseva and Giraldez 2015). Mechanistically, these changes are mostly achieved by the bulk regulation of translation efficiency and mRNA decay, although global changes co-exist with the more targeted regulatory mechanisms described in the previous section.

During the stages involving oocyte maturation and egg activation in which meiotic divisions proceed towards completion, regulation of gene expression is mostly achieved at the translational level, with minimal fluctuation at the mRNA accumulation level (Su et al. 2007; Tadros et al. 2007; Thomsen et al. 2010; Chen et al. 2011; Kronja et al. 2014; Eichhorn et al. 2016). In *Drosophila*, ~1350 out of ~5800 mRNAs assessed by ribosome profiling were shown to be under translational control during egg activation, with 70% of those being translationally activated and 30% repressed (Kronja et al. 2014). Interestingly, most of the observed changes in translational efficiency depend on the activity of the serine/threonine kinase PAN GU (Tadros et al. 2007; Kronja et al. 2014), although the details of how this is achieved and which PAN GU substrates are involved in translational regulation are not yet fully understood. Given that translational changes during egg activation are mainly determined by changes in mRNA poly(A) tail length, which are dictated by the opposing activities of deadenylases and the poly(A) polymerase Wispy (Benoit et al. 2008; Cui et al. 2013; Subtelny et al. 2014), it is possible that PAN GU acts directly upstream of such modifying enzymes (Eichhorn et al. 2016).

In contrast to what happens during oocyte maturation and egg activation, MZT is largely accompanied by widespread changes in mRNA levels (affecting 30-40% of maternal mRNAs) that are mainly driven by mRNA decay mechanisms (reviewed in Laver et al. 2015; Yartseva and Giraldez 2015). Maternal mRNA clearance starts prior to zygotic transcriptional activation, but is intensified upon its onset. Accumulating evidence indicates that mRNA destabilization is often preceded by translational inhibition, and that both types of regulation are mediated by the same machineries involving the deadenylation

of target mRNAs (Figure 1E). For instance, the RNA-binding protein Smaug mediates most of the maternal mRNA translational repression and decay prior to zygotic activation in *Drosophila* (Nelson et al. 2004; Tadros et al. 2007; Benoit et al. 2009; Chen et al. 2014). Indeed, PAN GU activates *Smaug* mRNA translation during egg activation (Tadros et al. 2007), with Smaug protein accumulating during early embryogenesis but decreasing soon after the MZT (Benoit et al. 2009). Smaug binds to secondary structure RNA motifs (Smaug recognition elements, SRE) (Dahanukar et al. 1999; Smibert et al. 1999) within target mRNAs, and mediates either decay by recruiting the CCR4/NOT deadenylase complex or translational repression by interacting with Cup (Nelson et al. 2004; Laver et al. 2015; Eichhorn et al. 2016). However, it is still unclear what determines the recruitment of either downstream effector.

Upon genome activation, zygotically transcribed microRNAs play a crucial role in translational control and maternal mRNA clearance in many animal species (Figure 1F). This is notably the case for *miR-430* in zebrafish*, miR-309* in *Drosophila*, and *miR-427* in *Xenopus*, which are responsible for destabilization of hundreds of transcripts during early embryogenesis (Giraldez et al. 2006; Bushati et al. 2008; Lund et al. 2009). Mechanistically, microRNA specificity is determined by base complementarity with target mRNAs (Duchaine and Fabian 2018), but the mode of action can vary between translational repression and target mRNA cleavage and decay. Interestingly, careful analysis of *miR-403*-mediated regulation in zebrafish using genome-wide approaches revealed that targeting initially leads to translation initiation, which is then followed by mRNA deadenylation and decay (Bazzini et al. 2012).

In parallel to the regulation mediated by RNA-binding proteins and microRNAs, recent work shed light on a new mechanism by which the process of translation elongation influences mRNA stability (see Dever et al. 2018) and dictates mRNA decay during the MZT in *Drosophila*, zebrafish, *Xenopus*, and mouse (Presnyak et al. 2015; Bazzini et al. 2016; Mishima and Tomari 2016). Genome-wide approaches indicate that mRNAs encoding ORFs enriched with uncommon, suboptimal codons decay faster than those enriched with optimal codons. 'Codon optimality' is mostly defined by tRNA availability, and slower ribosome translocation rates over transcripts containing suboptimal codons trigger mRNA deadenylation by the CCR4/NOT complex, promoting decay (Figure 1G). Interestingly, deadenylation activity is increased when suboptimal codons are placed closer to the stop codon and in mRNAs containing shorter 3'UTRs (Mishima and Tomari 2016), suggesting that physical proximity between the ribosome and the poly(A) tail may be required for the decay. The molecular mechanism by which ribosome elongation speed is interpreted and how this connects to the decay machinery remain to be determined. Nevertheless, analysis of mRNA decay kinetics during zebrafish embryo development indicates that the magnitude of codon composition-mediated mRNA decay regulation is comparable to that observed for microRNA regulation (Bazzini et al. 2016). In addition, a third mechanism, which can promote the degradation of transcripts enriched for the RNA modification N^6-methyladenosine (m^{6}A) (Wang et al. 2014; Peer et al. 2018), complements microRNA- and codon composition-mediated decay during the MZT in zebrafish embryos (Zhao et al. 2017).

Finally, translational regulation of evenly distributed mRNAs is not solely regulated in time, but can also be differentially regulated in space. A notable example is provided by the uniformly distributed *hunchback* mRNA (*hb*) in *Drosophila* (Lehmann and Nusslein-Volhard 1987; Tautz 1988; Irish et al. 1989). Like thousands of other mRNAs, *hb* is maternally provided and only translated during early embryogenesis. *hb* translation is inhibited at the posterior and forms a protein gradient that is required during the MZT for regulating the expression of genes defining the anterior-posterior embryonic axis. *hb* translation repression is mediated by the sequence-specific binding of Pumilio (Pum) and its partners Nanos and Brain tumor (Brat) to the 3'UTR of *hb* mRNA (Irish et al. 1989; Wharton and Struhl 1991; Murata and Wharton 1995; Chagnovich and Lehmann 2001; Sonoda and Wharton 2001). Mechanistically, Nanos modulates Pum target-specificity and binding to the Nanos response element (NRE) found at the 3'UTRs (Murata and Wharton 1995; Weidmann et al. 2016). In the case of *hb* mRNA, Nanos also recruits Brat, forming the NRE complex (Sonoda and Wharton 2001). Brat then interacts with the eIF4E-Homologous protein (4EHP), which interacts with the mRNA 5'cap structure and negatively regulates translation by blocking eIF4E binding and formation of the pre-initiation complex at the cap (Cho et al. 2006; Arvola et al. 2017). Spatially, this regulation is defined by Nanos protein distribution, due to the posterior localization and local translation of *nanos* mRNA (Gavis and Lehmann 1992). Therefore, the spatial regulation of evenly distributed mRNAs may ultimately depend on an initial subcellular enrichment of translational repressors.

Translational control during stem cell maintenance and differentiation

Throughout development and adult life, precursor cells transit between quiescent, self-renewing, and differentiating states in a spatiotemporally coordinated fashion to promote embryonic development, tissue formation, and later to sustain organismal homeostasis. While particularities exist in different stem cell lineages and developmental stages, transitions are fundamentally driven and accompanied by changes in gene expression, including translational control (Buszczak et al. 2014). For instance, many stem cell lineages present lower global protein synthesis rates in comparison to their immediate differentiating daughters. This was observed both *in vitro* (Sampath et al. 2008; Ingolia et al. 2011) and in stem cell systems *in vivo*, the latter including *Drosophila* adult germline stem cells (Sanchez et al. 2016), mouse adult hematopoietic stem cells (Signer et al. 2014; Signer et al. 2016), mouse adult neural stem cells (NSCs) (Llorens-Bobadilla et al. 2015), mouse adult skeletal muscle stem cells (or satellite cells) (Zismanov et al. 2016), and mouse adult hair follicle stem cells (Blanco et al. 2016) (Figure 2). In the studied cases, changes in protein synthesis rate during self-renewal and differentiation were mediated by regulatory mechanisms rather than being a consequence of changes in cell size, proliferation rate, or cell-cycle status (Sampath et al. 2008; Buszczak et al., 2014; Signer et al. 2014; Blanco et al. 2016; Sanchez et al. 2016).

Genetic experiments using mutations affecting translational control indicate that changes in protein synthesis rates are required and sufficient to modulate self-renewal and differentiation capacities in many stem cell systems. For instance, mutations inducing a global reduction in protein synthesis favor the accumulation of undifferentiated cells at the expense of differentiation. This is the case for *Nsun2*-deficient mouse hair follicle stem

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cells and male germline stem cells (Blanco et al. 2011; Hussain et al. 2013), for knockdowns of mechanistic target of rapamycin (mTOR) or transformation/transcription domain-associated protein (TRRAP) – both belonging to the phosphatidylinositol 3-kinase-related kinase (PIKK) family – in *Drosophila* female germline stem cells (Sanchez et al. 2016), and for mTOR-inhibited cultured mouse embryonic stem cells (Bulut-Karslioglu et al. 2016). Similarly, phosphorylation of the eukaryotic initiation factor 2α (eIF2α) decreases protein synthesis rates and promotes mouse skeletal muscle stem cell self-renewal rather than differentiation (Zismanov et al. 2016). Muscle stem cells unable to phosphorylate eIF2α exit the quiescent state and differentiate, while pharmacological treatment inhibiting eIF2α dephosphorylation promotes the regenerative capacity of muscle stem cells (Zismanov et al. 2016). Mirroring this, a genetically- or chemically-induced increase in protein synthesis, for instance by ectopically activating the mTOR pathway in stem cells, induces premature differentiation and stem cell depletion in many of the above-mentioned systems (Yilmaz et al. 2006; Zhang et al. 2006; Castilho et al. 2009; Sun et al. 2010; Bonaguidi et al. 2011; Deng et al. 2015). The central role for protein synthesis in this process is exemplified by the fact that a hypomorphic mutation affecting the ribosomal protein Rpl24 is sufficient to counterbalance the activation of the mTOR pathway in mouse hematopoietic stem cells, restoring normal self-renewal and differentiation functions (Signer et al. 2014). Yet, strong translation abrogation by means of affecting ribosome biogenesis can lead to stem cell loss as well as anomalous differentiation (Fichelson et al. 2009; LaFever et al. 2010; Le Bouteiller et al. 2013; Zhang et al. 2014; Sanchez et al. 2016). This illustrates how fine-tuning mechanisms, rather than

drastic changes in protein synthesis, balance self-renewal and differentiation functions, possibly in a lineage-specific manner (Buszczak et al. 2014).

The mechanisms by which the protein synthesis rate is limited in wild-type stem cells are still poorly understood, but current evidence suggests that it may be achieved by different means depending on the specific stem cell lineage. For instance, translation inhibition in mouse adult hematopoietic stem cells and in mouse embryonic stem cells is at least partially mediated by the regulation of the eukaryotic initiation factor 4E-binding proteins 4E-BP1 and 4E-BP2 (Signer et al. 2016; Tahmasebi et al, 2016). In these systems, 4E-BP proteins are preferentially hypophosphorylated; this favors 4E-BP binding to the eukaryotic initiation factor 4E (eIF4E), leading to inhibition of translation initiation. As a consequence, the translation of certain subsets of target mRNAs becomes more strongly inhibited (Roux and Topisirovic 2012; Morita et al. 2013; Proud 2018). In mouse skeletal muscle stem cells, on the other hand, high levels of eIF2α phosphorylation, another fulcrum of translation initiation regulation (Hinnebusch and Lorsch 2012; Merrick and Pavitt 2018; Wek 2018), are responsible for limiting protein synthesis (Zismanov et al. 2016). In contrast to these systems, mouse neural stem cells seem to employ ribosome biogenesis regulation rather than translation initiation to modulate protein synthesis; here, stem cells express lower levels of ribosomal subunits in comparison to their immediate daughters (Llorens-Bobadilla et al. 2015). This type of regulation may not be generally used in other stem cell systems, however. For instance, rRNA synthesis and ribosome biogenesis are up-regulated in *Drosophila* adult germline stem cells compared to their differentiated progeny; yet, stem cells show lower protein synthesis rates (Neumuller et al. 2008; Zhang et al. 2014;

Sanchez et al. 2016) (reviewed in Brombin et al. 2015) (Figure 2). It remains to be determined how ribosome biogenesis and protein synthesis rates are uncoupled in some stem cell systems. Recurrent observations in several independent systems suggest that active, yet unknown processes limit protein synthesis specifically in stem cells (such as the above-mentioned regulation of translation initiation) and/or conversely boost it in differentiating progeny. Given that differentiation relies on higher protein synthesis rates, it has been proposed that a surfeit of ribosome biogenesis in stem cells may prime daughter cells with enough ribosomes required for efficient differentiation (Ingolia et al. 2011; Sanchez et al. 2016).

In parallel to the global regulation of the translation machinery, specific translational regulators drive fate transitions during adult stem cell self-renewal and differentiation, with many examples being described in the *Drosophila* female germline stem cell system. In this system, a series of RNA binding proteins initially identified through genetic screens delineates a regulatory cascade that is both required and sufficient for self-renewal and differentiation (reviewed in Slaidina and Lehmann 2014). These include conserved translational repressors, such as Nanos and Pum (Forbes and Lehmann 1998; Tsuda et al. 2003; Miller and Olivas 2011), as well as the DExH-box RNA helicase Benign gonial cell neoplasm (Bgcn) (Bailey et al. 2017), the TRIM-NHL tumor suppressor proteins Mei-P26 and Brat (Neumuller et al. 2008; Harris et al. 2011; Insco et al. 2012), and the cytoplasmic RNA-binding Fox 1 (Rbfox1) (Carreira-Rosario et al. 2016). It is important to notice that, in addition to its function as translational repressor, Mei-P26 also regulates translation by

directly interacting with Ago1 and inhibiting the microRNA pathway (Neumuller et al. 2008).

By regulating each other's expression through mutual translational repression, the translational repressor factors establish a self-limiting regulatory network that promotes precise fate transitions during female germline stem cell differentiation. Similar to the mechanisms described in embryos, translational silencing during germline stem cell self-renewal and differentiation is generally mediated by repressor complexes that bind to sequences within the target mRNA 3'UTRs. In germline stem cells, for instance, translational repressors Nanos and Pum cooperate to promote self-renewal by inhibiting the translation of differentiation factors including Brat and Mei-P26, likely through the recruitment of the CCR4/NOT decay machinery to target mRNAs (Harris et al. 2011; Joly et al. 2013). In differentiating daughter cells, the expression of the differentiation factor Bag-of-marbles (Bam) leads to the assembly of a complex containing the sex determination factor Sex-lethal (Sxl) and translational repressors Bgcn and Mei-P26 (Li et al. 2009; Chau et al. 2012; Li et al. 2013). Sxl and Mei-P26 are able to interact with the *Nanos* mRNA 3'UTR and repress its translation in differentiating daughter cells (Chau et al. 2012; Li et al. 2012; Li et al. 2013), although the mechanism by which this repression is mediated remains to be determined. In the absence of Nanos, Pum is free to interact with Brat, and the Pum-Brat complex inhibits the translation of other self-renewal factors in differentiating cells, including transcription factors Myc and Mad (Harris et al. 2011). Interestingly, in more advanced differentiation stages, cytoplasmic Rbfox1 is expressed and binds to a sequence-specific motif in *Pum* mRNA 3'UTR, thereby repressing Pum

translation and ensuring the progression to terminally differentiated states (Tastan et al. 2010; Carreira-Rosario et al. 2016).

Finally, it has recently been proposed that changes in tRNA concentrations may account for some of the gene expression differences observed in proliferating and differentiating cells (Gingold et al. 2014). Given that codon optimality is likely to be determined by tRNA availability (Presnyak et al. 2015; Bazzini et al. 2016), it is to be expected that in addition to a direct effect on translation (Gingold et al. 2014), the observed variation in tRNA abundance in these two states may also induce changes in mRNA stability like those observed during embryogenesis (Bazzini et al. 2016). While it remains to be determined whether tRNA repertoires vary during stem cell fate transitions, it has recently been shown that changes in the expression of specific tRNAs modulate protein expression and metastatic activity during cancer progression through both translational control and mRNA decay (Goodarzi et al. 2016).

Concluding remarks

Regulation at the post-transcriptional level is increasingly recognized as an important enabler of cell fate transitions in a variety of developmental and stem cell systems. At a global level, regulation of translational efficiency and RNA or protein turnover may be used to facilitate transitions between cell states by relinquishing entire gene programs in order to make way for synthesis of a new program. It may not be surprising that these post-transcriptional mechanisms are found so prominently at transitions that require gross turn-

over, like the transition from the maternally provided gene products of the egg to new gene synthesis by the embryo or during the transition from long-term, self-renewing stem cells to differentiating progeny. While broad, these transitions do not affect all mRNAs and our understanding of the mechanisms of regulation and target selection is still limited. Although this review has focused on 3'UTR-mediated regulation during the early stages of embryogenesis and global regulation at the stem cell–differentiation transition, additional mechanisms mediate translational efficiency via 5'upstream sequences. These include control via RNA secondary structures, internal ribosome entry sites, upstream ORFs and specialized ribosomes (Hinnebusch 2005; Chew et al. 2016; Johnstone et al. 2016; Shi et al. 2017; Leppek et al. 2018).

While the intricacies of transcriptional regulation have long been recognized to modulate gene expression, post-transcriptional mechanisms were thought of as 'house-keeping' and permissive. Recent discoveries and technologies challenge this perception and promise opportunities for continued discoveries of fundamental significance. As developmental biology moves towards single-cell level analysis, one of main challenges is the development of technologies to globally assess changes in translational control in individual cells, thereby complementing the recently establish techniques (such as single-cell RNA-sequencing) that measure mRNA accumulation levels at single-cell resolution.

Acknowledgements

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Figure Legends

Figure 1. Mechanisms of translational control during embryo development. Examples of modules regulating protein synthesis during the first steps of embryo development, a period during which the zygotic genome is mostly transcriptionally quiescent. **A**: Maternal to embryonic transition (MZT). *Left,* fertilized *Drosophila* (top), *Xenopus* (bottom) and zebrafish (right) embryos are transcriptionally silent and contain maternally synthesized gene products that sustain development and lay down the basic structures and embryonic axes. *Right,* during the MZT, maternal products are largely degraded and zygotic transcription ensues. This allows asynchrony in cell divisions and morphogenetic cell movements of gastrulation needed for body plan building. **B**: mRNA localization is mediated by RNA-binding proteins that recognize secondary structures present in the 3'UTR of target mRNAs. Assembled adaptor RNPs are recruited to molecular motors and delivered to specific intracellular domains. **C**: Translational repression can be actively
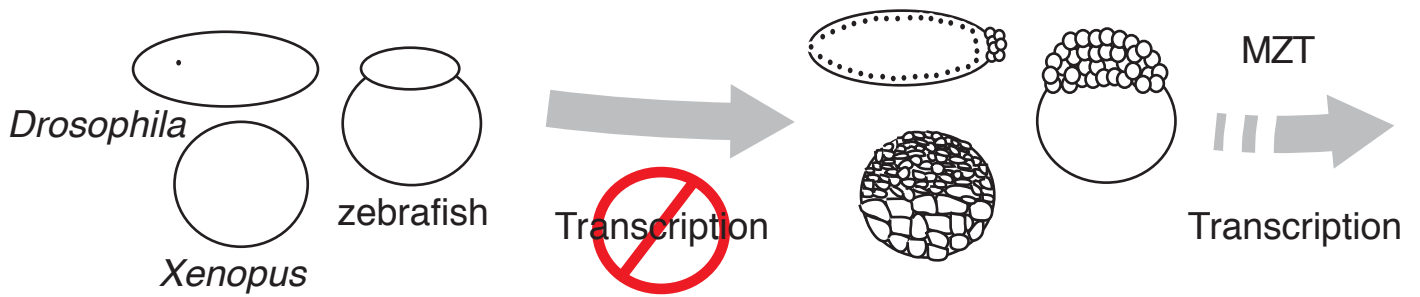
established upon targets by RNA-binding proteins – such as Bruno (Bru) and Smaug (Smg) – that recognize sequences within the transcript 3'UTRs and recruit other factors to block translation initiation. **D**: Translational activation is associated with the recruitment and activity of poly(A) polymerases and interactions between the poly(A)-binding protein and translation initiation factor eIF4G and eIF4E on target mRNA transcripts. **E**: Base complementarity-targeted microRNA regulation is mediated by the RNA-induced silencing complex (RISC) and induces large scale translational repression and CCR4/NOT-dependent target mRNA decay during the MZT. **F**: Local protection of evenly distributed mRNAs against bulk degradation, which is mediated by localized RNA-binding proteins recognizing specific motifs in the 3'UTR of target mRNAs leads to spatially regulated expression at the subcellular level. **G**: Slower ribosome translocation rates over transcripts containing suboptimal codons trigger mRNA deadenylation and decay during embryo development.

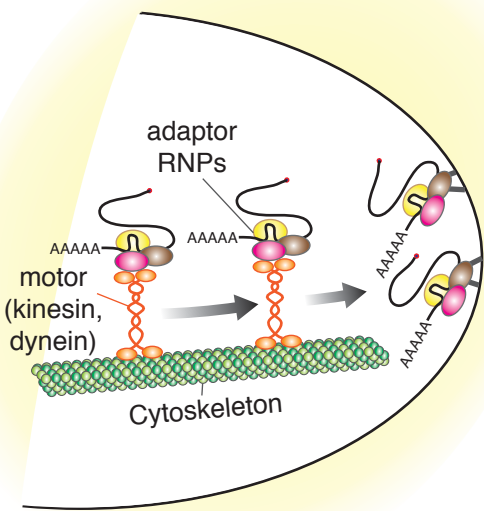**Figure 2. Translational control during stem cell maintenance and differentiation.** Actively dividing stem cells (*left*) present lower global protein synthesis rates (fewer ribosomes attached to mRNAs) in comparison to their immediate differentiating daughters (*right*). Yet, rRNA synthesis and ribosome biogenesis (shown by a black factory) are up-regulated compared to their differentiated progeny in many stem cells, such as *Drosophila* adult female germline and mouse embryonic stem cells, indicating that ribosome biogenesis and protein synthesis rates may be uncoupled in such stem cell systems.
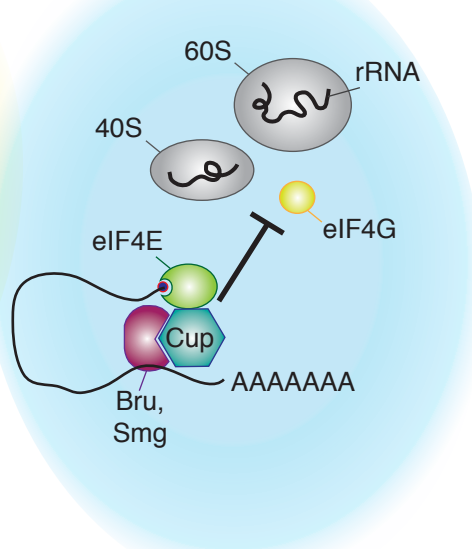
Figure 1. Teixeira and Lehmann
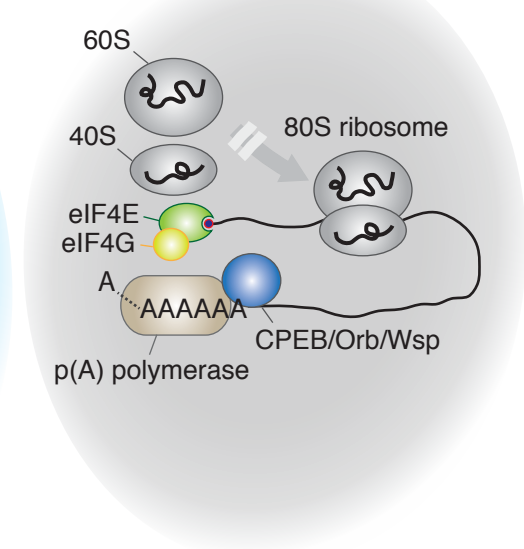
A. Maternal to Zygotic Transition

Drosophila

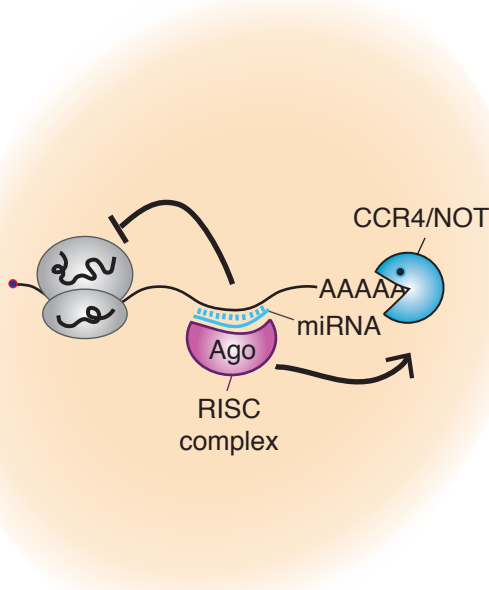*Xenopus*

zebrafish

Transcription

MZT

Transcription

B. mRNA localization

adaptor RNPs

AAAAA

motor (kinesin, dynein)

Cytoskeleton

C. Translational repression via RNA-binding proteins

60S

rRNA

40S

eIF4E

eIF4G

Cup

AAAAAAA

Bru, Smg

D. Translational activation

60S

40S

80S ribosome

eIF4E

eIF4G

A

AAAAAA

p(A) polymerase

CPEB/Orb/Wsp

E. microRNA-mediated repression and decay

CCR4/NOT

AAAAA

miRNA

Ago

RISC complex

F. Local protection from degradation

RNA-binding protein

degradation machinery

AAAAA

AAAAA

AAAAA

AAAAA

AAAAA
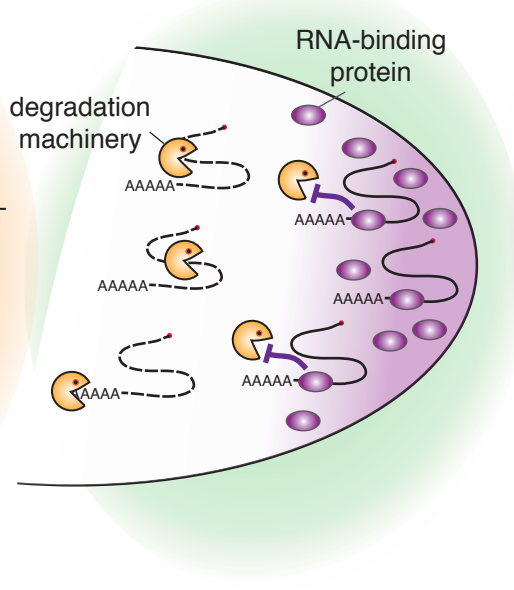
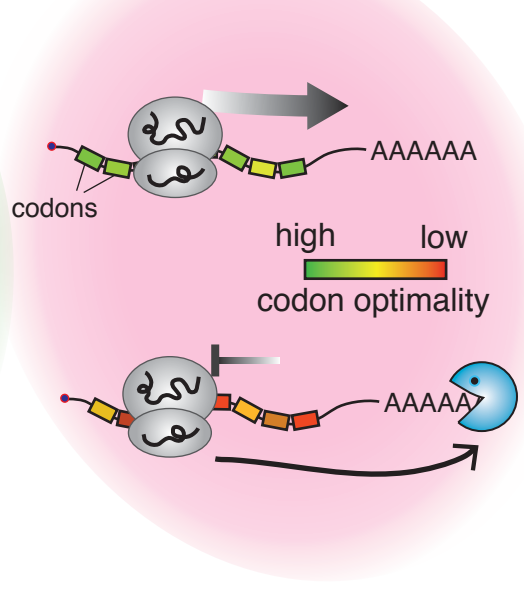G. Codon optimality-mediated decay

AAAAAA

codons

high   low

codon optimality

AAAAA

Figure 2. Teixeira and Lehmann

Active adult stem cell

Nucleolus

Nucleus

High ribosome
biogenesis rates

Differentiation

Ribosomes

mRNAs

Differentiating cell

High global translation rates