

Cellular Differences in Protein Synthesis Regulate Tissue Homeostasis

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Although sometimes considered a “house-keeping” function, multiple aspects of protein synthesis are regulated differently among somatic cells, including stem cells, and can be modulated in a cell-type-specific manner. These differences are required to establish and maintain differences in cell identity, cell function, tissue homeostasis, and tumor suppression.

Introduction

Much of what is known about protein synthesis has been learned from studies in yeast and mammalian cell lines (Hinnebusch and Lorsch, 2012; Lorsch and Dever, 2010). Moreover, analysis of uncultured tissues or tumors provides an aggregate picture of protein synthesis across the tissue without revealing differences among individual cells. Nonetheless, it is clear that protein synthesis differs among mammalian somatic cells. For instance, cells of the exocrine pancreas display some of the highest rates of protein synthesis of any adult cell type (Case, 1978; Logsdon and Ji, 2013). Protein production rates vary greatly among liver, kidney, and muscle cells (Alfrey et al., 1953; Garlick, 1972; Garlick et al., 1980; Garlick et al., 1991). Related cells within the same lineage can also exhibit different rates of mRNA translation. For example, in the immune system, primed T cells experience a global attenuation of mRNA translation, which is subsequently reversed upon restimulation (Scheu et al., 2006). However, we are only beginning to appreciate the ways in which these differences in protein synthesis are necessary for tissue development and homeostasis. This is because new approaches are making it possible to more generally compare protein synthesis and its regulation among different kinds of cells, revealing an unexpected richness in the biology.

Studies of stem cell function and tissue homeostasis offer the opportunity to better understand differences in protein synthesis among somatic cells and their physiological significance. The balance between stem cell self-renewal and differentiation must be tightly regulated: self-renewal without differentiation leads to tumorigenesis while differentiation without self-renewal depletes stem cells and tissue regenerative capacity. Great effort has been invested in determining the transcriptional and epigenetic networks that govern stem cell identity and function, but gene expression programs are ultimately governed at the level of mRNA translation (Schwanhäusser et al., 2011). Cell-type-specific differences in translation regulate development, differentiation, and responses to stresses such as nutrient deprivation. Differences in the regulation of translation among cells may actually help to establish and maintain differences in cell identity and function.

In this review we focus on differences in the regulation of translation among cells and the physiological consequences. Some well-characterized mechanisms that regulate differences in protein synthesis among cells, such as differences in microRNA expression, are beyond the scope of this review. We focus instead on differences in protein synthesis, protein stability, and ribosome assembly among cells that are critical for tissue development and homeostasis. The mechanisms that underlie these differences are only beginning to be elucidated, but this is providing fundamental new insights into development, tissue regeneration, and how these processes go wrong in degenerative diseases and cancer.

Dynamic Protein Synthesis among Embryonic Cells

Cellular function depends upon proteostasis—appropriate regulation of protein synthesis, protein folding, and protein degradation. Each of these are likely to exhibit cell-type-specific differences in regulation that influence stem cell function, tissue development, and homeostasis (Vilchez et al., 2014). However, differences among somatic cells in protein folding and protein degradation are not as well characterized as differences in protein synthesis. We will thus focus mainly on protein synthesis even though this is only one component of the proteostasis network.

Developmental studies have begun to reveal the extent to which related cells exhibit functionally significant changes in protein synthesis as they differentiate. In mammals, these changes can first be seen immediately after fertilization. Most proteins exhibit 2-fold or greater changes in abundance as mouse embryos transition from the one-cell to two-cell stage (Latham et al., 1991). Studies of *Drosophila* have shown that the changes in protein levels during the oocyte-to-embryo transition largely occur at the level of translation, not transcription (Kronja et al., 2014). Translational control mechanisms also help to promote germ cell differentiation in *Drosophila* males (Insko et al., 2012). Building upon these studies, the functional importance of differences in global protein production levels between undifferentiated cells and their progeny has recently begun to come into focus.

mRNA translation changes on a global scale as mammalian embryonic stem cells (ESCs) differentiate to form embryoid bodies (Ingolia et al., 2011; Sampath et al., 2008). Continuously

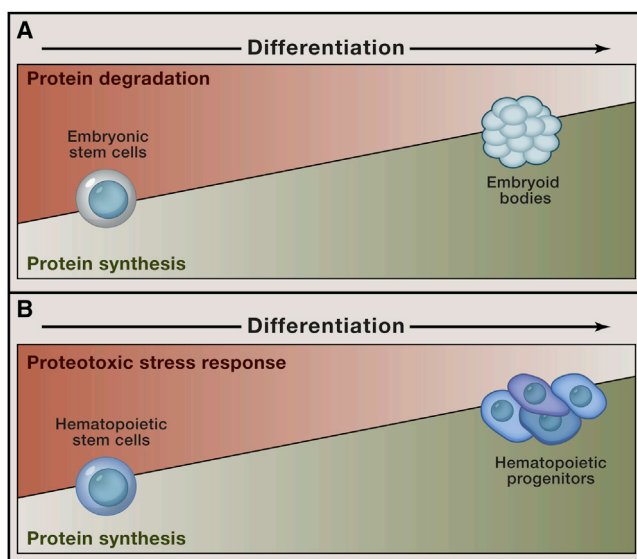


Figure 1. Proteostasis Differs between Stem Cells and Their Daughters

(A) Global protein translation levels remain low in embryonic stem cells (ESCs) but increase as these cells differentiate to form embryoid bodies (EBs) (Ingolia et al., 2011; Sampath et al., 2008). By contrast, the activity of protein degradation mechanisms appears high in ESCs relative to EBs (Vilchez et al., 2012). (B) Adult HSCs display low levels of global protein synthesis relative to differentiating hematopoietic progenitor cells (Signer et al., 2014), and proteotoxic stress response appears enhanced in umbilical cord blood (fetal) HSCs (van Galen et al., 2014). Whether these trends in proteostasis regulatory mechanisms will be seen in other stem cell populations remains unclear, but keeping overall protein levels low in long-lived stem cell populations may promote their fitness and longevity.

dividing cells tend to synthesize more protein than nondividing cells, but ESCs are distinct in that they maintain lower levels of bulk mRNA translation and protein accumulation than their differentiated progeny despite continuous cell division. ESCs display a marked reduction in overall translational efficiency (a lower fraction of mRNAs associate with actively translating polysomes) relative to other cells. Induction of differentiation increases global transcript levels and leads to more efficient loading of mRNAs into polysomes, increasing protein synthesis (Ingolia et al., 2011; Sampath et al., 2008).

Differences in protein synthesis among ESCs and their differentiating progeny correlate with global translational changes. For example, ESCs exhibit increased translation of upstream open reading frames (uORFs) (Ingolia et al., 2011). uORFs form when translation begins at initiation sites within the 5' UTRs of mRNAs that are upstream of the initiation sites of recognized coding sequences. While uORFs can promote reinitiation at downstream ORFs in specific cases, many act to decrease the translation of the actual coding sequence by impeding scanning by the pre-initiation complex (Barbosa et al., 2013). uORFs can be found in 49% of human transcripts and influence protein expression in a variety of different contexts, including during stress responses (Barbosa et al., 2013). Additional studies will be required to determine whether the global change in uORF translation during ESC differentiation commonly occurs during the differentiation of other stem cells and whether it influences cell fate.

Global regulation of protein degradation also controls protein levels within ESCs and ESC maintenance. Human ESCs express high levels of the 19S proteasome subunit PSMD11 and exhibit increased proteasome capacity relative to a variety of differentiated progeny (Vilchez et al., 2012). Overexpression of PSMD11 increases proteasome capacity while decreased expression of PSMD11 reduces ubiquitin-dependent protein degradation (Vilchez et al., 2012). Reduced proteasome activity reduces the expression of pluripotency markers (Vilchez et al., 2012), suggesting that ESCs depend on elevated proteasome activity for their maintenance. Given that protein synthesis increases during ESC differentiation (Ingolia et al., 2011; Sampath et al., 2008), these findings suggest that low protein levels, achieved through reduced translation and increased degradation, promote ESC maintenance (Figure 1A).

Dynamic Protein Synthesis among Stem/Progenitor Cells

To understand the extent to which differences in protein synthesis among cells regulate normal tissue development and homeostasis, it is necessary to study fetal and adult tissues *in vivo*. One limitation has been the dependence upon pulsed metabolic labeling to mark the synthesis of nascent polypeptides. Amino acid analogs, such as methionine analogs, do not compete with endogenous amino acids, limiting this approach to cells that survive and proliferate in culture media depleted for the relevant amino acid (Beatty et al., 2006). This has impeded efforts to directly measure differences in protein synthesis among cells *in vivo*. Moreover, the tissue culture environment differs from the *in vivo* environment in many ways that influence cellular properties (Joseph and Morrison, 2005), including protein synthesis, at least in hematopoietic stem cells (HSCs) (Signer et al., 2014). Other techniques for measuring protein synthesis can be applied to uncultured cells, but depend upon stable isotope labeling *in vivo* followed by mass spectrometry (Krüger et al., 2008; Xiao et al., 2008) or on ribosome profiling (Li et al., 2014). These techniques have so far only been used when cells are abundant and have not been scaled down to analyze purified rare cell populations, such as somatic stem cells. Single-cell assays to measure protein synthesis have been developed to look at the translation of mRNAs containing specific translation regulatory motifs (Han et al., 2014), but not at global protein synthesis rates. Consequently, there is a general need to develop more assays that enable quantitation of protein synthesis in small numbers of cells *in vivo*.

A recent advance that facilitates quantitation of protein synthesis in individual cells *in vivo* is the synthesis of O-propargyl-puromycin (OP-Puro) (Liu et al., 2012). OP-Puro enters the acceptor site of ribosomes and is covalently incorporated into nascent polypeptide chains. Isolation and fixation of OP-Puro-treated cells, followed by exposure to an azide-conjugated fluorophore, leads to the fluorosination of all polypeptides that incorporate OP-Puro via a click chemistry reaction (Liu et al., 2012). The amount of protein synthesis per hour in individual cells *in vivo* can then be quantitated by flow cytometry (Signer et al., 2014).

Administration of OP-Puro *in vivo* shows that cells within somatic stem cell lineages exhibit dynamic regulation of protein synthesis. Adult HSCs exhibit less protein synthesis than

restricted hematopoietic progenitors (Signer et al., 2014). We observed up to 10-fold differences in the amount of protein synthesized per cell per hour in different hematopoietic stem/progenitor cell populations. Although most adult HSCs are quiescent, their lower levels of protein synthesis are independent of cell-cycle status, cell size, and RNA content (Signer et al., 2014). Genetic perturbations that modestly increase or decrease protein synthesis both impair HSC function. A 30% decrease in global protein synthesis in *Rpl24^{Bst/+}* mutant HSCs impairs their ability to reconstitute the hematopoietic system of irradiated mice. Likewise, increasing protein synthesis by deleting the PI3K pathway inhibitor *Pten* depletes HSCs and promotes leukemia development (Signer et al., 2014; Yilmaz et al., 2006). Introducing the *Rpl24^{Bst/+}* mutation onto the *Pten*-deficient background rescues both of these phenotypes, demonstrating that PTEN maintains HSCs and suppresses leukemia mainly by attenuating protein synthesis. Thus, HSCs and restricted hematopoietic progenitors synthesize different amounts of protein per hour, and these differences are necessary for normal HSC function.

Experimental interventions or mutations that reduce protein synthesis extend lifespan in an evolutionarily conserved manner (reviewed in Taylor and Dillin, 2011). This has been proposed to occur at least partly because reduced protein synthesis increases protein quality and reduces the burden imposed on the protein-folding chaperone system by misfolded protein aggregates. Certain long-lived mitotic cells, including some stem cells, may maintain proteome quality by limiting protein synthesis (Signer et al., 2014) or increasing proteasome activity (Vilchez et al., 2012, 2014). The increased longevity associated with decreased protein synthesis may partly reflect the increased fitness of certain mitotic cells or increased tissue regenerative capacity during aging as a consequence of reduced proteotoxic stress, though this has not yet been tested.

Proteotoxic stress occurs under physiological conditions, such as in pancreatic β cells that increase protein synthesis to produce insulin in response to blood glucose spikes (Back et al., 2009). Proteotoxic stress reduces protein synthesis by inhibiting mRNA translation, typically at the level of initiation. However, proteotoxic stress can also pause ribosomes during elongation (Liu et al., 2013). This pausing typically occurs at sites near where the nascent polypeptide chain emerges from the ribosome and depends on the activity of various chaperone proteins. Given the cell-specific differences in the expression and activity of various chaperones, the response to proteotoxic stress likely varies from cell to cell (Morimoto, 2008). Human cord blood HSCs are particularly sensitive to proteotoxic stress, undergoing apoptosis in response to a PERK-mediated unfolded protein response (van Galen et al., 2014). The data suggest that protein misfolding influences HSC function under physiological conditions and support the idea that hematopoietic cells vary in their sensitivity to proteotoxic stress.

TORC1 Regulates Stem Cells through Protein Synthesis

mTORC1 signaling represents a major mechanism by which cells integrate nutrient availability, growth factor signaling, and developmental cues to regulate protein synthesis (Figure 2A) (Laplanche and Sabatini, 2012). mTORC1 activation promotes

protein synthesis through inhibition of 4E-BPs and activation of S6K1, which acts on a number of translation initiation proteins and other factors involved in translational elongation and ribosome biogenesis (Browne and Proud, 2002; Ma and Blenis, 2009; Mayer et al., 2004; Shahbazian et al., 2006) (Figure 2B).

The importance of mTORC1-mediated regulation of translation initiation during development has recently begun to emerge. For example, increases in protein synthesis that occur during early sea urchin embryogenesis correlate with decreased inhibition of eIF4E by 4E-BP as a consequence of increased 4E-BP degradation (Cormier et al., 2001; Salaün et al., 2003). The increase in 4E-BP degradation is blocked by rapamycin treatment, suggesting that it reflects mTORC1 signaling. Phosphorylated 4E-BP1 levels increase as ESCs differentiate into EBs, potentially explaining the increase in protein synthesis in EBs (Sampath et al., 2008). While loss of S6K1 has little or no effect on the viability and proliferation of ESCs, activation of S6K1 promotes the differentiation of these cells (Easley et al., 2010; Kawasome et al., 1998). mTORC1 signaling likely promotes increased protein synthesis during ESC differentiation.

Multiple adult tissues depend upon appropriate mTORC1 signaling to regulate stem cell maintenance, proliferation, and tumor suppression (Gan et al., 2008; Groszer et al., 2001; Yilmaz et al., 2006; Zhang et al., 2006b). Increased activation of mTORC1 in vivo quickly depletes adult neural stem cells (Bonaquidi et al., 2011), epidermal stem cells (Castilho et al., 2009), and HSCs (Yilmaz et al., 2006; Zhang et al., 2006b). The stem cell depletion that occurs after *Pten* deletion occurs largely due to a deleterious increase in protein synthesis, at least in the hematopoietic system (Signer et al., 2014). Decreasing mTORC1 activity or constitutively expressing 4E-BP1 in neonatal neural stem cells enhances self-renewal and prevents differentiation (Hartman et al., 2013). While mTORC1 signaling regulates the growth of many cells, the level of mTORC1 activation varies among different cells (Betschinger et al., 2013; Sampath et al., 2008; Signer et al., 2014) and in the same cells over time (Chen et al., 2009; Magee et al., 2012). This raises the possibility that changes in mTORC1 signaling, and modulation of global protein synthesis rates, may determine developmental changes, rather than simply arising as a consequence of those changes.

Ribosome Assembly Influences Tissue Homeostasis

Given that differences among cells in protein synthesis, even among lineally related cells in the same tissue, are required for normal tissue homeostasis (e.g., Signer et al., 2014), one key question is how these differences among cells are regulated. Beyond differences in mTORC1 signaling, additional mechanisms involving modulation of ribosomal subunit expression and ribosome assembly also contribute to differences in protein synthesis among cells. The heterogeneity in these mechanisms among cells is illustrated by the cell- and tissue-specific defects that arise as a consequence of defects in ribosome assembly.

Genes that encode individual ribosome components exhibit great differences in expression among tissues (Kondrashov et al., 2011) and even among cell types within the same tissue (Signer et al., 2014). Consistent with this, mutations in genes that encode ribosome components often have phenotypes that are surprisingly cell type specific. In zebrafish, mutations in the

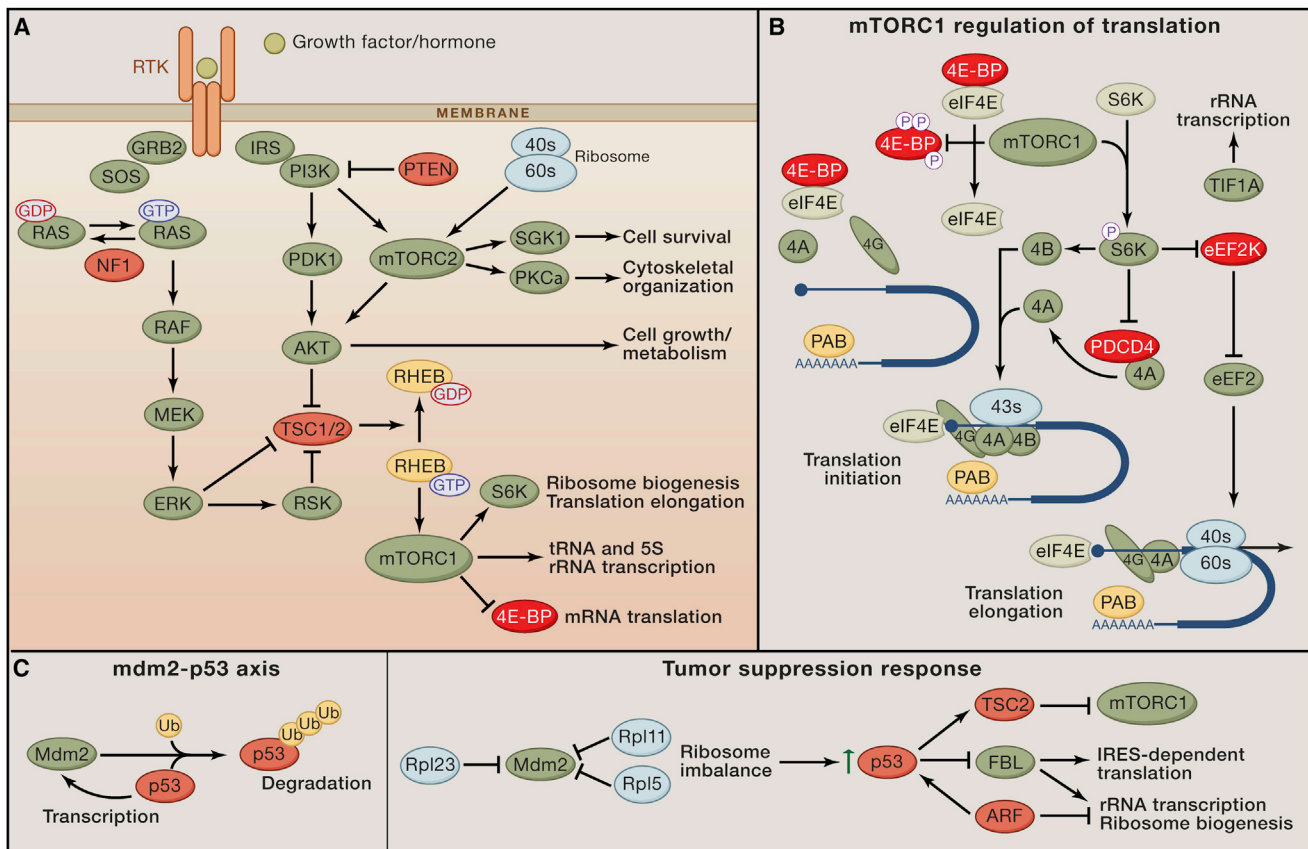


Figure 2. Signaling Pathways that Regulate Protein Synthesis

(A) Growth factors, hormones, nutrients, and stress all influence protein synthesis through complex signaling pathways (Laplane and Sabatini, 2012; Topisirovic and Sonenberg, 2011). mTORC1 preferentially promotes the translation of a subset of mRNAs that contain complex 5' UTRs and clusters of 5' terminal oligopyrimidines (TOP) or similar pyrimidine-rich sequences (Hsieh et al., 2012; Jefferies et al., 1994; Thoreen et al., 2012). These TOP genes primarily encode proteins that promote translation, including ribosomal proteins, initiation factors, and elongation factors (Thoreen et al., 2012). While many proteins have been proposed to regulate the translation of TOP mRNAs (Damgaard and Lykke-Andersen, 2011; Tcherkezian et al., 2014) and the translation of specific TOP containing mRNAs is regulated in a cell-specific manner (Avni et al., 1997; Ivanov et al., 2011), the roles of these elements in stem cell maintenance and tissue homeostasis remain unclear.

(B) The regulation of translation initiation represents the major mechanism by which cells regulate protein synthesis. The eIF4F complex, which includes eIF4E, eIF4G, and eIF4A, promotes the translational initiation of virtually all cellular mRNAs (Sonenberg and Hinnebusch, 2009) and mRNAs with highly structured 5' UTRs are particularly sensitive to eIF4E activity (Feoktistova et al., 2013; Koromilas et al., 1992). Activated mTORC1 phosphorylates 4E-BPs, preventing their inhibition of eIF4E. In the absence of 4E-BPs, eIF4E binds to the m7G mRNA cap and recruits eIF4G, which in turn acts as a scaffold for the RNA helicase eIF4A. The formation of this eIF4F complex on the 5'-end of mRNA promotes the initiation of cap-dependent translation. Activated mTORC1 also phosphorylates and activates S6K, which phosphorylates eIF4B, eEF2K, and TIF1A. Phosphorylated eIF4B enhances the helicase activity of eIF4A to unwind secondary structures in mRNA, which promotes translational initiation. Phosphorylation of eEF2K by S6K interferes with its ability to block the activity of the translational elongation factor eEF2. Activation of TIF1A promotes ribosomal RNA transcription.

(C) The E3 ubiquitin ligase Mdm2 promotes the degradation of the tumor suppressor p53. p53 acts in a feedback loop to promote the transcription of the *Mdm2* gene. Thus under normal conditions, p53 levels remain low. Imbalances in ribosome biogenesis induce a tumor suppression response in which free ribosomal proteins, particularly Rpl5 and Rpl11, bind and inhibit Mdm2 (Bhat et al., 2004; Dai and Lu, 2004; Dai et al., 2006; Zhang et al., 2003). This results in the accumulation of p53, which promotes the activity of TSC2, thereby repressing the activity of mTORC1. p53 and other tumor suppressors, such as ARF, inhibit rRNA transcription and ribosome biogenesis (Lessard et al., 2010; Sloan et al., 2013; Sugimoto et al., 2003).

ubiquitously expressed ribosome proteins, Rpl22 and Rpl22-like (Rpl22l), have distinct developmental phenotypes, affecting T lineage progenitors and HSCs, respectively (Zhang et al., 2013). Both Rpl22 and Rpl22l compete for binding to *smad1* mRNA and have opposing effects on its translation, which partially accounts for their distinct phenotypes. Similarly, *rps19*-deficient zebrafish embryos display specific defects in erythropoiesis and cartilage development (Danilova et al., 2008), while reduced expression of Rps29 results in erythropoietic defects and ectopic cell death in the head (Taylor et al., 2012). The *Tail short* (*Ts*) mu-

tation in mouse *Rpl38* causes specific skeletal defects marked by reduced expression of 8 of 39 Hox proteins (Kondrashov et al., 2011). *Rpl38^{Ts}* mutations reduce the levels of 80S monosome formation on specific Hox gene transcripts. By contrast, mutations in multiple genes that encode other ribosome components (*Rps19^{DSK3/+}*, *Rps20^{Dsk4/+}*, *Rpl29^{+/-}*, *Rpl29^{-/-}*, *Rpl24^{Bst/+}*) do not cause similar skeletal phenotypes or effects on Hox gene expression (Kondrashov et al., 2011). Thus, Rpl38 plays a specialized role in regulating the translation of specific mRNAs, perhaps in a tissue-specific manner.

Human ribosome-related diseases, collectively referred to as ribosomopathies, further reflect the preferential dependence of specific cells on specific ribosome components. Human ribosomopathies include Diamond-Blackfan anemia, 5q syndrome, Treacher Collins syndrome, Blooms and Werner syndromes, dyskeratosis congenita, and cartilage hair hypoplasia (Armistead and Triggs-Raine, 2014). These disorders are caused by mutations in genes that encode ribosome components or genes that affect Pol I transcription or ribosomal RNA processing (Armistead and Triggs-Raine, 2014). Each ribosomopathy exhibits different spectrums of defects in tissue homeostasis, though hematopoietic and craniofacial/neural crest defects are commonly observed (Narla and Ebert, 2010). It remains unclear why hematopoietic cells and neural crest cells exhibit particular sensitivity to loss-of-function mutations in ribosome components or factors required for their synthesis.

The hematopoietic defects observed as a result of mutations in certain ribosomal components likely reflect a combination of defects in stem cells and restricted hematopoietic progenitors. For example, HSC function is impaired in dyskeratosis congenita (Friedland et al., 1985; Marsh et al., 1992) and in 5Q syndrome (Nilsson et al., 2000), whereas Diamond-Blackfan anemia appears to more strongly reflect defects in erythroid restricted progenitors (Abkowitz et al., 1991; Lipton et al., 1986).

Phenotypes caused by ribosomal defects reflect a variable combination of tumor suppressor induction along with changes in the synthesis of key proteins (Armistead and Triggs-Raine, 2014; Barkić et al., 2009; Bellodi et al., 2010; Danilova et al., 2008; Ludwig et al., 2014; Narla and Ebert, 2010; Taylor et al., 2012; Zhang and Lu, 2009). Ribosome proteins can also exhibit important functions outside the ribosome, complicating the interpretation of at least some mutant phenotypes (Xue and Barna, 2012). For example, Rps13 regulates the splicing of its own message (Malygin et al., 2007) and ribosomes can bind mTORC2 and promote its signaling in a translation-independent manner (Zinzalla et al., 2011). It remains unclear whether extraribosomal functions are unusual properties pertaining to a minority of ribosome components or whether many ribosome components have unrecognized extraribosomal functions, including cell-type-specific functions.

Ribosome assembly is also regulated differently in different cells. Disruption of UTP4 causes North American Indian childhood cirrhosis (Armistead and Triggs-Raine, 2014). UTP4 is a component of the small subunit processome, a large ribonucleoprotein complex that promotes the maturation of 18S rRNA (Freed and Baserga, 2010). Unique among ribosomopathies in only affecting a single tissue, North American Indian childhood cirrhosis first presents as childhood jaundice and progresses to biliary cirrhosis. Knockdown of Utp4 in zebrafish also leads to specific defects in the biliary system (Wilkins et al., 2013), suggesting that the function of Utp4 in this tissue has been conserved across species. By contrast, disruption of Notchless, a murine ortholog of the yeast 60S subunit maturation factor Rsa4, depletes HSCs but not more mature hematopoietic cells (Le Bouteiller et al., 2013).

Asymmetric segregation of ribosome processing and rRNA transcription factors influence fate determination in stem cells and their daughter cells. The ribosomal RNA processing factor

Wicked, a member of the conserved rRNA processing U3 snoRNP complex, exhibits asymmetric segregation during *Drosophila* germline stem cell divisions (Fichelson et al., 2009). The asymmetric distribution of Wicked during mitosis ensures that presumptive germline stem cells inherit more of this factor than daughters fated to differentiate. This same mode of inheritance is also observed in several other stem cell populations (Fichelson et al., 2009). More recent work shows that a *Drosophila* Pol I regulatory factor, Under-developed (Udd), also becomes enriched in germline stem cells relative to their differentiating daughters (Zhang et al., 2014a). Loss of both *wicked* and *udd* compromises germline stem cell maintenance (Fichelson et al., 2009; Zhang et al., 2014a). The drop in Pol I activity in *udd* mutants promotes some aspects of differentiation while expanded rRNA transcription as a result of increased TIF-1A expression delays differentiation (Zhang et al., 2014a). Interestingly, down-regulation of rRNA synthesis in cultured mammalian hematopoietic progenitors can also promote differentiation (Hayashi et al., 2014). Further work will be needed to explore whether mammalian lineages also exhibit asymmetric inheritance of Pol I regulatory and rRNA processing factors.

It will be important to compare ribosome biogenesis and translational regulation among stem cells and their daughters at different stages of development, in different tissues, and in different stem cell activation states. It remains unclear whether stem cells exhibit consistent differences relative to their differentiating progeny or whether there is as much diversity in regulatory mechanisms among stem cells as among differentiated cells.

Regulated Protein Synthesis Control Suppresses Neoplastic Proliferation

The regulation of protein synthesis promotes tissue homeostasis partly by preventing inappropriate proliferation. Most cells increase their rate of protein synthesis during cell division. Cancer cells generally synthesize protein more rapidly and more efficiently than normal cells (Ruggero, 2013). Translational control mechanisms regulate the expression and function of a variety of oncogenes and tumor suppressors through diverse mechanisms (Topisirovic and Sonenberg, 2011) and cancer cells dysregulate translation initiation to increase protein synthesis and sustain neoplastic proliferation (Ruggero, 2013). Mechanisms that increase or deregulate protein synthesis can thus promote the development of cancer.

Mechanisms that negatively regulate protein synthesis often suppress tumorigenesis. Key oncogenic signals, such as Myc or PI3K pathway activation, increase protein synthesis and change the efficiency with which subsets of mRNAs are translated (Barna et al., 2008; Hsieh et al., 2012). This is critically important because the regulation of translation initiation, the efficiency with which mRNAs are recruited to active ribosomes, represents the major mechanism by which cells regulate protein synthesis. Based on studies of cancer cells (Hsieh et al., 2012), it seems likely that there are differences among stem cells and restricted progenitors in terms of how they regulate translation initiation, though this has not yet been studied.

Genetic changes or pharmacological agents that reduce protein synthesis can impede cancer development and progression (Barna et al., 2008; Hsieh et al., 2010; Hsieh et al., 2012; Signer

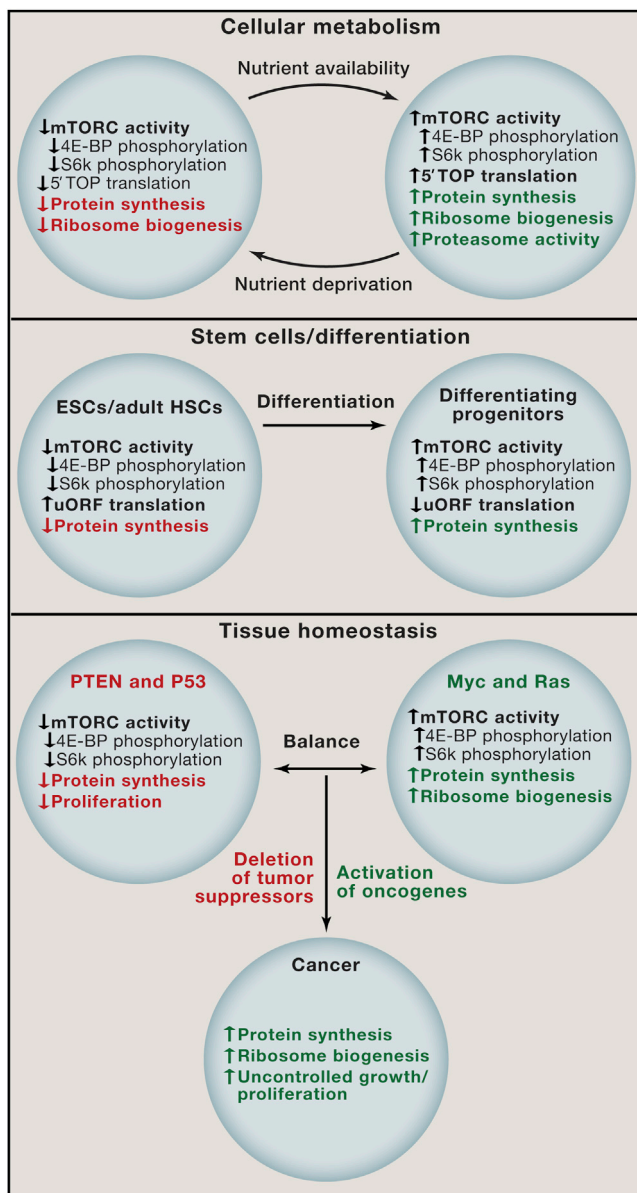


Figure 3. Differences in the Regulation of Protein Synthesis among Cells

(A) Cells respond to changes in nutrient levels by altering protein synthesis, ribosome biogenesis (Laplante and Sabatini, 2012), and protein degradation (Zhang et al., 2014b) by modulating mTORC1 signaling.

(B) Embryonic stem cells (ESCs) and adult hematopoietic stem cells (HSCs) exhibit lower levels of protein synthesis than their differentiating progeny. Whether other stem cell lineages display similar changes in protein synthesis upon differentiation will require further analysis. One intriguing possibility is that this property may vary among stem cells, depending on their cell-cycle kinetics and whether they are long-lived or short-lived *in vivo*.

(C) Tissue homeostasis depends on a balance between proto-oncogenes and tumor suppressors. Tumor suppressors such as PTEN and p53 reduce cellular growth and proliferation through a number of different mechanisms, including decreasing mTORC1 activity and reducing global protein synthesis (Laplante and Sabatini, 2012). Proto-oncogenes, including Myc and Ras, increase protein translation and ribosome biogenesis, fueling growth and proliferation. Cells transform to cancer when mutations reduce tumor suppressor function or increase oncogene function (van Riggelen et al., 2010).

et al., 2014). Moreover, a number of tumor suppressors negatively regulate protein synthesis, for example by negatively regulating mTORC1 signaling (Laplante and Sabatini, 2012). Tumor suppressors can thus regulate protein synthesis through a variety of mechanisms.

Mutations in ribosome biogenesis factors or ribosome components impair tissue homeostasis and promote the development of cancer through diverse mechanisms. For example, in X-linked dyskeratosis congenita, disruption of dyskerin (DKC1), an enzyme that pseudouridylates rRNA (Ni et al., 1997), leads to bone marrow failure, skin abnormalities, various carcinomas, and acute myeloid leukemia (Heiss et al., 1998; Ruggero et al., 2003). DKC1 mutations do not affect global protein synthesis but do reduce the translation of a subset of mRNAs with IRES elements in their 5' UTRs (Yoon et al., 2006), including the p53 and p27 tumor suppressors (Bellodi et al., 2010). Thus, one mechanism by which mutations in ribosome components and biogenesis factors promote the development of cancer is through effects on the translation of tumor suppressors. To the extent that ribosomopathies induce proteotoxic stress or a tumor suppressor response, they may also create a selective pressure to inactivate tumor suppressors in normal cells that are engaged in tissue regeneration, increasing the probability of subsequent transformation.

Tumor suppressor responses may be induced by sustained oncogenic stimuli as a consequence of their effects on protein synthesis. Diverse oncogenic stimuli induce tumor suppressor responses in a wide range of normal cells (Collado and Serrano, 2010). A series of studies suggest that cells may sense oncogenic stimuli based on their effects on protein synthesis. Multiple distinct mutations in ribosomal proteins induce the accumulation of p53, as do defects in ribosomal biogenesis and nucleolar stress (Armistead and Triggs-Raine, 2014; Barkić et al., 2009; Bellodi et al., 2010; Danilova et al., 2008; Narla and Ebert, 2010; Taylor et al., 2012; Zhang and Lu, 2009). For example, disruption of Rps14 activates p53 and loss of p53 rescues the hematopoietic progenitor cell depletion observed in these mutants (Barlow et al., 2010; Dutt et al., 2011). p53 expression increases in response to defects in ribosome biogenesis, partly as a consequence of Mdm2 binding by ribosomal components (such as Rpl5 and Rpl11) (Bhat et al., 2004; Dai and Lu, 2004; Dai et al., 2006; Zhang et al., 2003), suggesting that disruption of the normal stoichiometry of ribosome assembly leads to the sequestration of Mdm2 by free ribosomal proteins (Figure 2C).

The efficiency of reprogramming of somatic cells to pluripotency by Sox2, Myc, Klf4, and Oct4 (all of which are oncogenes) is limited partly by p53 and p16^{Ink4a}/p19^{Arf} tumor suppressor expression (Kawamura et al., 2009; Li et al., 2009; Marión et al., 2009; Utikal et al., 2009). An important recent paper by Sonnenberg and colleagues demonstrates that loss of 4E-BP1/2 impairs the reprogramming of fibroblasts to pluripotent stem cells, partly by inducing the expression of the p21^{ciP1} tumor suppressor (Tahmasebi et al., 2014). However, in the absence of p53, loss of 4E-BP1/2 promotes reprogramming. Since 4E-BPs are negative regulators of translation, these data suggest protein synthesis must increase for successful reprogramming, but that this induces a tumor suppressor response that reduces reprogramming efficiency.

Tumor suppressors can also be induced in response to changes in the quality of the proteome, such as in an unfolded protein response. Increased protein synthesis can overwhelm the chaperone system and lead to the accumulation of misfolded proteins that aggregate and impede diverse cellular processes (Back et al., 2009; Schröder and Kaufman, 2005). Induction of an unfolded protein response reduces protein synthesis and increases proteasome activity and tumor suppressor expression. The unfolded protein response reduces the number of active polysomes through eIF2 α phosphorylation, allowing free ribosomal proteins to associate with Mdm2, stabilize p53, and induce cell-cycle arrest (Zhang et al., 2006a). Thus, cellular surveillance of protein synthesis and protein quality is achieved through diverse mechanisms that induce tumor suppressors, influencing tissue homeostasis and neoplastic proliferation.

Perspective

Stem cell function and tissue homeostasis are regulated by networks of proto-oncogenes and tumor suppressors that feature key transcriptional regulators that distinguish stem cells from their progeny (He et al., 2009). While these networks have been understood to regulate cell identity, signal transduction, transcription, and cell-cycle progression, other areas of cellular physiology continue to be of uncertain relationship to cellular identity. It is likely that many of these aspects of cellular physiology exhibit much more cellular specificity in their regulation than currently appreciated. Consistent with this, protein synthesis is regulated differently in different kinds of cells and these differences are critical for fate determination and the maintenance of tissue homeostasis (Figure 3). These functions are necessarily and intimately intertwined with the role of translational regulation in tumor suppression. The ability of translational regulation to suppress the development of cancer reflects its role in preventing inappropriate proliferation by stem cells and other cells within normal tissues. As our understanding of translational regulation matures, new biology will emerge, marked by unanticipated regulatory mechanisms.

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