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Translational Control in Myeloid Disease

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1. Introduction

Over the years a wealth of information on the role played by transcription factors in myeloid biology has contributed to our understanding of both normal and abnormal myeloid development. However, the regulation of mRNA translation in myeloid cell maturation has, in comparison, been a neglected area of study. A better understanding of the translational control of myeloid gene expression will undoubtedly provide important insights into both normal and abnormal myeloid maturation. This chapter summarizes our current understanding of the regulation of myeloid gene expression at the mRNA translational level and delineates levels of disruption in myeloid disease, with an emphasis on leukemias.

2. Myeloid differentiation and neutrophil development

During hematopoiesis, granulocytes and monocytes arise from a common progenitor cell in the bone marrow and differentiate in response to cytokines and transcription factors ultimately giving rise to mature neutrophils and monocytes in the circulation. The granulocyte progenitor cells pass through several identifiable maturational stages, during which they acquire the morphologic appearance and granule contents that characterize the mature granulocyte(Rev in(Berliner, 1998)). The earliest identifiable granulocyte precursor is the myeloblast, which is characterized by very few granules, little cytoplasm and a prominent nucleolus. Transition to the promyelocyte stage is associated with the acquisition of primary granules. Primary granules are found in both granulocytes and monocytes and contain many of the proteins necessary for intracellular microbicidal activity (Bainton, 1975). The transition to the myelocyte stage is associated with the acquisition of secondary or "specific" granules(Bainton, 1971). Myelocytes further mature to give rise to bands and mature neutrophils. The appearance of secondary granules and their content proteins, provides a unique marker of commitment to terminal neutrophil differentiation (reviewed in (Borregaard *et al.*, 2001)). Several lines of evidence from our laboratory have established that the expression of the secondary granule protein (SGP) genes, which are functionally diverse and physically unlinked, is coordinately regulated at the level of mRNA transcription (reviewed in(Berliner, 1998)). Absence of SGP gene expression is a consistent abnormality in Myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML).

2.1 Transcriptional control of myeloid maturation

Maturation of myeloid progenitor cells into specialized blood cells that play a vital role in innate immunity, is regulated by a well-orchestrated interplay of transcription factors (Tenen, 2003). Recent studies have delineated transcription factors that contribute to the process of maturation. These include a category of factors termed "master regulators" of lineage development and include PU.1, and CCAAT enhancer-binding protein-α (C/EBPα) (Tenen, 2003). These factors not only promote lineage-specific gene expression but also suppress alternative lineage pathways. For example, Laslo et al (Laslo *et al.*, 2006) elegantly demonstrated that cell fate determination is dependent upon subtle changes in expression levels of transcription factors, which regulate differential lineage maturation. Levels of PU.1 expression are increased by Egr-1/Nab-2 in developing monocytes/macrophages; while Egr-1 simultaneously represses the expression of the neutrophil specific Gfi-1 transcription factor (see below), thereby repressing the neutrophil development program.

2.1.1 C/EBPα

C/EBPα has been recognized to be a master regulator of the granulopoietic developmental program. It is expressed at high levels throughout myeloid differentiation (Tsukada *et al.*, 2011). C/EBPα binds to the promoters of multiple myeloid-specific genes, thereby regulating gene expression at many different stages of myeloid maturation. C/EBPα$^{-/-}$ mice die perinatally due to defects in gluconeogenesis that result in fatal hypoglycemia, and demonstrate an early block in the differentiation of granulocytes without affecting either monocyte/macrophage maturation or the differentiation of other hematopoietic lineages. The expression of C/EBPα is associated with growth arrest and differentiation of granulocyte precursor cells. This block in proliferation is thought to occur via the interaction of C/EBPα with cyclin-dependent protein kinases (cdk2 and cdk4), resulting in a block in cell proliferation. In addition, C/EBPα inhibits E2F-dependent transcription, which also contributes to inhibition of cell proliferation and induction of differentiation associated with C/EBPα-induced granulopoiesis (Timchenko *et al.*, 1997; Timchenko *et al.*, 1999)

2.1.2 PU.1

A second master myeloid regulator PU.1, is a member of the Ets family of transcription factors and is expressed in both B cells and monocyte/macrophages (Chen *et al.*, 1995). PU.1 is also expressed at lower levels in granulocytes and eosinophils as well as in CD34^{+} hematopoietic progenitor cells. High levels of PU.1 expression in fetal livers of mice preferentially directs monocyte/macrophage development, whereas low levels of PU.1 result in B-cell development (DeKoter and Singh, 2000). Studies have revealed that downregulation of c-Jun, a coactivator of PU.1, by C/EBPα is necessary for granulocytic maturation and is the mechanism through which C/EBPα blocks macrophage development (Rangatia *et al.*, 2002). Gene knockout studies of PU.1 in mice resulted in perinatal lethality accompanied by the absence of mature monocytes/macrophages, B cells as well as and delayed and reduced granulopoiesis (Scott *et al.*, 1994). Following *in vitro* differentiation, embryonic stem (ES) cells derived from PU.1$^{-/-}$ blastocysts fail to express mature myeloid cell markers, suggesting that PU.1 is not essential for the initial events associated with myeloid lineage commitment but is necessary for the later stages of development.

2.1.3 Growth Factor Independence-1 (Gfi-1)

Gfi-1 is a highly conserved transcriptional repressor that encodes a 55kD nuclear proto-oncogene that is composed of six zinc finger domains at the carboxy terminus and a N-terminal SNAG or repression domain (rev in (van der Meer *et al.*, 2010). Gfi-1 is expressed at high levels in the thymus and bone marrow, while its paralog Gfi1B, is expressed in the bone marrow and spleen. An essential role of Gfi-1 in neutrophil differentiation became apparent following reports of gene disruption in mice (Hock *et al.*, 2003). Gfi1-null mice are severely neutropenic and eventually succumb to bacterial infections. These mice lack mature neutrophils and their granulocyte precursors are unable to differentiate into mature neutrophils and also lack expression of specific granule proteins (SGPs). In addition, Gfi-1–/– bone marrow expresses atypical $Gr1^{+}Mac1^{+}$ myeloid precursor cells that appear to have characteristics of both granulocyte and macrophage precursors. These observations confirm a critical role for Gfi-1 in the neutrophil maturation program. Work from our laboratory has shown that Gfi-1 synergizes with another member of the CCAAT enhancer binding protein family of transcription factors, C/EBPε to transactivate the promoters of late myeloid genes. This synergy is lost in a patient with specific granule deficiency (SGD), who has a heterozygous substitution mutation in the C/EBPε gene as well as decreased levels of Gfi-1 in the bone marrow (Khanna-Gupta *et al.*, 2007). Heterozygous dominant negative mutations in the Gfi-1 gene have been described in two patients with severe congenital neutropenia (SCN) (Person *et al.*, 2003), thus emphasizing the role of Gfi-1 in the neutrophil maturation pathway.

Over the years a great deal of information pertaining to the transcriptional regulation of myeloid development has become available and has aided in our understanding of the process of granulopoiesis and how it goes awry in myeloid leukemias. In contrast, as outlined below, the role of mRNA translation in the process of normal myeloid development is only just beginning to be understood (rev in (Khanna-Gupta, 2011))

3. An overview of the process of mRNA translation in eukaryotic cells

Eukaryotic protein synthesis involves the coordinated interplay of hundreds of macromolecules such as mRNAs, tRNAs, activating enzymes, protein factors and ribosomes. Ribosomes are the protein synthetic factories upon which protein synthesis proceeds and are composed of a large (60S) and a small (40S) subunit. Each of these subunits is composed of two-thirds RNA and one-third protein. Protein synthesis occurs on the ribosome in three phases: translation initiation, elongation and termination. Regulation of gene expression takes place primarily at the initiation stage which therefore is the rate-limiting step in protein synthesis. The delicate balance of events leading to protein expression is critical for cellular growth, proliferation, differentiation and apoptosis (Rev in (Van Der Kelen *et al.*, 2009)). In eukaryotes, translation initiation factors (eIFs) play a crucial role in the dissociation of 40S and 60S ribosomal subunits thus enabling recruitment of mRNA and initiator tRNAs to the 40S subunit followed by interaction with the 60S subunit resulting in the reformation of the 80S ribosome allowing for elongation and termination of the polypeptide chain to ensue (rev in (Van Der Kelen *et al.*, 2009)).

3.1 Control of translation initiation

The first step in the translation of mRNA in eukaryotic cells begins with the binding of the 40S small ribosomal subunit to the 5′ end of the mRNA to be translated in the process of 5′

cap-dependent translation. Cap-binding protein eukaryotic initiation factor 4E (eIF4E) recognizes and binds to the m^7GpppN cap (where m is a methyl group and N any nucleotide) structure at the 5'end of the mRNA to be translated. Under normal physiologic conditions, eIF4E strongly associates with 4E-binding proteins (4E-BPs) thus preventing eIF4E from initiating protein synthesis, this represents the first rate limiting step in the process of protein synthesis. This inhibition is overcome by the phosphorylation of 4E-BPs via signal transduction pathways (involving PI3K and mTOR among others, see below) that are regulated by growth factors and nutrient status of the cell, thus causing 4E-BPs to be phosphorylated and to dissociate from eIF4E (Figure 1). This enables a competing adapter molecule eIF4G, to bind to eIF4E. eIF4G then recruits the ATP-dependent RNA helicase eIF4A (eIF4E, 4G and 4A are collectively referred to as eIF4F in the literature), the ubiquitously expressed cofactor eIF4B as well as eIF3, a multisubunit initiation factor, all of which bind to the 5' cap region of the mRNA, thus setting the stage for mRNA translation to begin (rev in(Sonenberg and AG., 2009)).

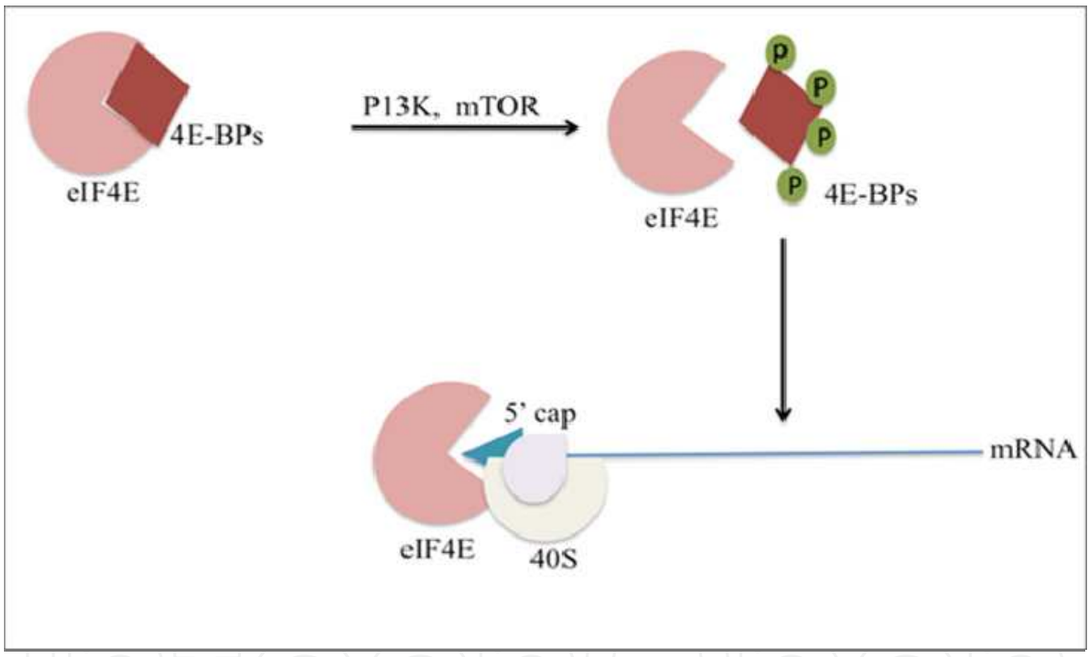

Fig. 1. Under basal conditions eIF4E is tightly bound to 4E-BPs. When cells are under stress or nutrient deprived, Phosphorylation of 4E-BPs is triggered by P13K (Phospho-inositol Kinase) and mTOR, which causes the 4E-BPs to be phosphorylated and/or to have reduced affinity for eIF4E. eIF4E is now free to bind eIF4G to initiate mRNA translation.

Translation initiation however requires the small ribosomal subunit 40S to be complexed with the so called ternary complex before it associates with the mRNA (Figure 2). The assembly of the ternary complex, consisting of the G protein eIF2B, eIF2, the initiator tRNA, $tRNAi^{met}$ and GTP, is the second rate-limiting step in mRNA translation. Recognition of the first AUG codon in the mRNA to be translated followed by initiation of protein synthesis is dependent on this process. Upon recognition of the first in-frame AUG, eIF2-GTP is hydrolyzed and the resulting eIF2-GDP is restored to eIF2-GTP by the guanine-nucleotide-exchange factor known as eIF2B to continue another round of translation initiation (Figure

2). This process is inbibited when the α subunit (S51 residue) of eIF2 is phosphorylated. This is brought about by the activation of a number of eIF2α kinases which are activated under conditions of cellular stress and aid in the reduction of protein synthesis until the stressful circumstance has passed (Figure 2). If stress remains unabated, apoptosis ensues. EIF2α kinases include the Heme-regulated inhibitor kinase (HRI), RNA-dependent protein kinase (PKR), PKR-like endoplasmic-reticulum kinase (PERK) and mGCN2(mammalian general control non-derepressing) (rev in (Raven and AE., 2008) and (Chen, 2007) and references therein). Since phosphorylated eIF2α has a higher affinity for eIF2B than eIF2α, phosphorylation of even a small percentage of eIF2α can lead to a reduction in protein synthesis. Phosphorylation of eIF2α can be reversed upon removal of the phosphate group by specific phosphatases thus restoring the cell to homeostasis (Harding *et al.*, 2009).

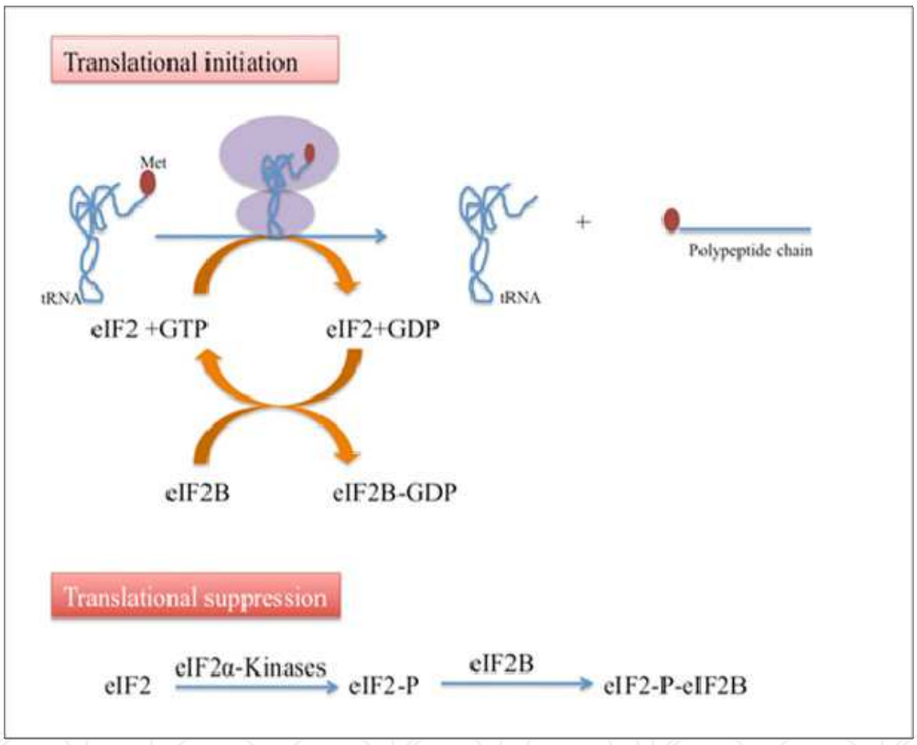

Fig. 2. During initiation, a GTP molecule is hydrolyzed to GDP which is then recycled by eIF2B, making eIF2α-GTP available for the continuation of the synthesis cycle. However, under cellular stress conditions, alterations in nutrient status, growth factors, mitogens and inducers of differentiation a variety of a eIF2α kinases are activated which causes the phosphorylation of eIF2α which has an increased affinity towards eIF2B. This traps available eIF2α and eIF2B resulting in a block in protein synthesis.

Formation of the 43S preinitiation complex occurs once the ternary complex binds to the 40S subunit complexed with eIF3 and eIF1A. The pre-initiation complex further associates with the mRNA through eIF3 and eIF4G, thus forming the 48S initiation complex. mRNA scanning begins when the 40S subunit moves along the mRNA in a 5′ to 3′ direction. Upon encountering the first in-frame translation initiation codon AUG, a codon (mRNA) – anticodon (tRNA) recognition is established resulting in the dissociation of initiation factors

from the 40S subunit. This enables the binding of the 60S subunit resulting in the assembly of the functional ribosome (80S) and for peptide elongation to proceed.

The 3′ end of the mRNA together with a number of the initiation factors ensure the stabilization of ribosome-mRNA interaction. Poly (A) tail and polyA binding protein (PABP) interact with eIF4G to form a pseudo-circular mRNA. This structure is thought to reduce the translational error rate as translation from intact RNAs alone would be permitted. This mRNA configuration also gives the initiation factors the necessary spatial proximity that ensures efficient dissociation and re-association capabilities to permit another round of protein synthesis to occur.

3.2 The mTOR pathway

mTOR (mammalian/mechanistic target of rapamycin) is involved in an evolutionarily conserved pathway that is critical for cellular responses to environmental cues. mTOR is a serine/threonine protein kinase belonging to the phospho inositide 3-kinase (PI3K)-related protein kinases (PIKK) family of protein kinases, which consists of a protein complex that enable organisms to cope with metabolic, environmental and genetic stresses (rev in (Sengupta *et al.*, 2010)). Mammalian TOR forms two structurally and functionally distinct multiprotein complexes, mTORC1: in which mTOR is complexed with Raptor (the regulatory protein of mTOR), LST8 (also called GβL) and PRAS40 (proline-rich Akt/PKB substrate 40kDa), and mTORC2, harboring both LST8 and Rictor (rapamycin-insensitive companion of mTOR). Only mTORC1 is responsive to the inhibitory effects of the antibiotic rapamycin (Figure 3) (Wullschieger *et al.*, 2006). When activated, mTORC1 functions to regulate protein synthetic pathways in response to nutritional, environmental and growth factor mediated signals. The TCS1 and TCS2 (tuberous sclerosis 1 and 2) proteins form a tumor suppressor complex that transmits signals to mTORC1 by regulating the activation of Rheb (Ras homolog enriched in brain; a GTP-GDP exchange protein). The TCS1 and TSC2 complex regulates the GTP-loading state of Rheb. GTP-bound Rheb interacts with mTORC1 and renders it active. The two major targets of mTOR are the 4E-BPs (see Figure 1) and the 40S ribosomal protein S6 kinase (S6K1), both important components of the translational machinery (Figure 3).

Upon activation, mTORC1 regulates the phosphorylation/activation of p70 S6 kinase (S6K1) and the phosphorylation/deactivation of 4E-BP1(Platanias, 2005). Activation of S6 kinase modulates ribosome biogenesis through the activation of ribosomal protein S6 (rpS6)(Lee-Fruman *et al.*, 1999) (Figure 3). S6K1 also phosphorylates eIF2B, SKAR (S6K1 Aly/REF-like target) and eukaryotic elongation factor 2 kinase, thus affecting both the initiation and elongation stages of mRNA translation.

As described above, hypo-phosphorylation of the 4E-BPs increases their affinity for eIF4E, thus blocking the interaction of eIF4G and eIF4A thereby hampering translation initiation of mRNA from proceeding (Figure 1). However, phosphorylation of the 4E-BPs by activation of the mTORC1 signaling pathway (Figure 3) results in lowered affinity of these proteins for eIF4E, thus allowing for the formation of the competing eIF4E-eIF4G-eIF4A (eIF4F)-mRNA complex that permits mRNA translation to proceed(Gingras *et al.*, 2001). Thus inhibition of mTORC1 activity results in the down regulation of the activity of several components of the translational machinery resulting in a block in cell proliferation and eventually to cell death.

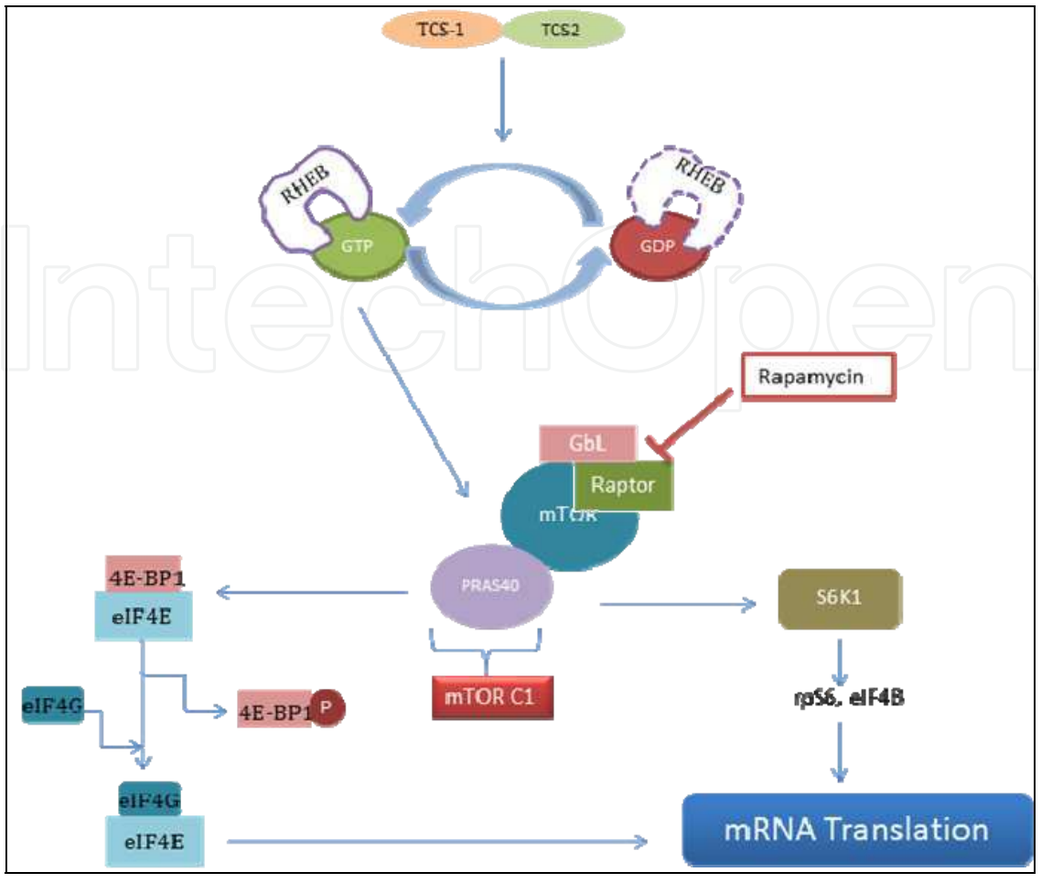

Fig. 3. mTOR pathway. See text for details

4. Translation control in Myeloid cells

While the general principles of mRNA translation hold in myeloid cells, it has been shown that when *4E-BP1* and *4E-BP2* genes were knocked out in mice an impairment of myelopoiesis was observed with no obvious effect on thymocyte maturation(Olson *et al.*, 2008). An increase in the number of immature granulocytic precursors and a decrease in the numbers of mature granulocytes was observed in these mice compared to their wild type littermates. Other studies have shown that expression of the 4E-BPs is significantly increased during granulopoiesis(Grolleau *et al.*, 1999). It was thus concluded that 4E-BP1 and 4E-BP2 play an important role in the early phases of granulo-monocytic differentiation thereby highlighting a critical role for translation initiation during granulopoiesis. It should be noted that 4E-BP1 has been shown to be constitutively phosphorylated in both chronic myeloid leukemia (CML) and in acute myeloid leukemia (AML) due to the constitutive activation of mTOR and Bcr-Abl in CML(Ly *et al.*, 2003) and PI3K-Akt in AML(Xu *et al.*, 2003).

4.1 Translational defects in myeloid leukemias

Activation of aberrant transcriptional and signaling pathways leading to enhanced survival and proliferation of leukemic progenitors is a hallmark of acute myeloid leukemia (AML)

(Scholl *et al.*, 2008; Tenen, 2003). The recent past has seen marked changes for the better in our understanding of the biology underlying AML. Based on these advances, targeting overactive signaling pathways in transformed cells has become an active area of research with the ultimate goal of finding molecules that specifically target only the transformed cell.

4.1.1 mTOR

The mTOR pathway has been shown to be activated in a number of cancers including AML (rev in (Tamburini *et al.*, 2009a)). In fact, mTORC1 has been shown to be activated in over 90% of primary AML samples (Tamburini *et al.*, 2009b). There has thus been a great effort to demonstrate the efficacy and use of Rapalogs, a class of drugs that include the mTORC1 inhibitor Rapamycin (see Figure 3) and RAD001, as anti-cancer agents. However, despite the anticipated cell death that blocking the mTORC1 pathway should result in, it has been demonstrated that the anti-leukemic effects of rapalogs are merely cytostatic. This is likely due to the fact that mTORC1 inhibition by rapalogs results in the activation of a number of feedback loops involving leukemogenic kinases such as P13K and ERK, thus limiting the anti-leukemic effects of this class of drugs.(Wang *et al.*, 2008)

4.1.2 Oncogenic mRNAs

The translation efficiencies of different mRNAs are dependent partly on the structural complexity of their 5'UTR (untranslated region). mRNAs with simple or short 5'UTRs, such as in the actin mRNA, are translated with high efficiency even in untransformed cells. However, mRNAs harboring long and complex 5'UTRs are generally translated with low efficiency and are referred to as "weak" mRNAs because of weak interactions with the eIF4F translation initiation complex (see above). In transformed cells on the other hand, this interaction improves due to the increased activity of eIF4F leading to increased translation of the weak mRNAs. It is noteworthy that a number of these weak mRNAs have oncogenic potential because they encode proteins involved in cell cycle regulation (e.g. cyclin D1) DNA replication (ornithine decarboxylase) and other pathways (c-myc, VEGF, Bcl-2, survin), all of which contribute to cell survival and proliferation. Expression of such mRNAs is regulated at the translational level and their overexpression likely contributes to the transformation process in AML ((Tamburini *et al.*, 2009a) and references therein). Since these "oncogenic" mRNAs have been found to be more sensitive to translation inhibition, there has been an effort to identify compounds that block mRNA translation. An example of such a compound is 4EGI-1 which is a 4E-BP1 mimetic and potently blocks the interaction of eIF4E and eIF4G during translation initiation (see above). 4EGI-1 has been shown to abrogate the expression of c-Myc and Bcl-x_L, both proteins with oncogenic potential. Additionally, this compound has shown therapeutic potential as it induces selective apoptosis in AML blast while allowing normal CD34+ hematopoietic progenitors to survive (Tamburini *et al.*, 2009b).

Overexpression of the initiation factor eIF4E has been described in primary cells derived from M4/M5 AML compared to bone marrow mononuclear cells (Topisirovic *et al.*, 2003). EIF4E expression has been known to increase protein synthesis and to transform cells (Wendel *et al.*, 2007). It should be noted that the antiviral compound ribavarin has been shown to inhibit the activity of eIF4E (Kentsis *et al.*, 2004). In a small study involving 11 M4/M5 AML patients who were given ribavarin, three patients responded with one in complete remission and two in partial remission (Assouline *et al.*, 2009). This study

demonstrates that blocking the translational engine could prove to be a very important tool in the development of future AML therapeutics.

5. Translational control of the myeloid master regulator C/EBPα

C/EBPα is the founding member of a family of basic region/leucine zipper (bzip) transcription factors many of which contribute to granulopoiesis and are regulated at the translation level. (Rev in(Fuchs, 2007; Koschmieder *et al.*, 2009; Muller and Pabst, 2006; Schuster and Porse, 2006)). For the sake of simplicity only the translational control of the master myeloid regulator C/EBPα will be discussed here.

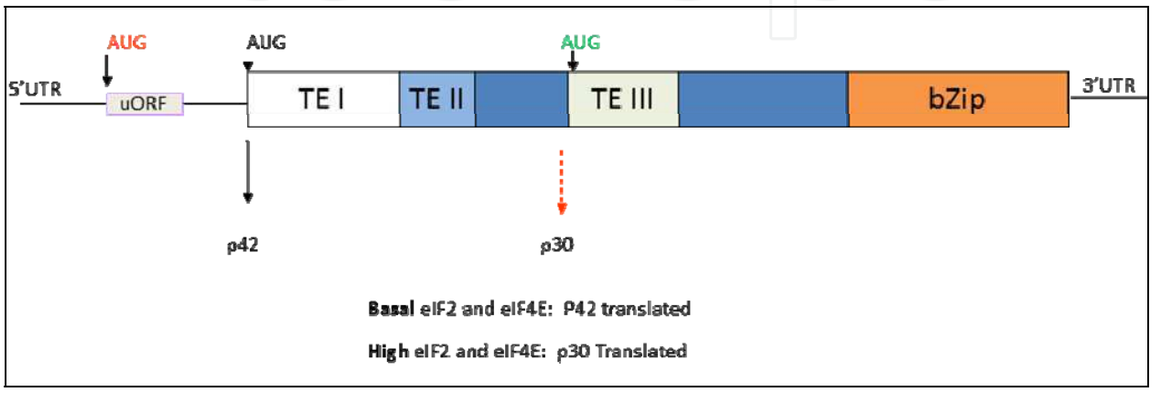

Fig. 4. Structure of the C/EBPα mRNA. TE I, II and III are activation domains. bZip is the basic leucine-zipper DNA binding domain.

Profound hematopoietic abnormalities have been reported for mice in which C/EBPα is ablated(Zhang *et al.*, 1997). The C/EBPα gene is intronless and generates two isoforms as a result of the differential utilization of alternate translation start codons. The resultant p42kD (full length) and p30kD (truncated) C/EBPα proteins differ from each other at the N-terminus, which is shorter in the p30kD protein (Figure 4). Translational control of C/EBPα-isoform expression occurs via a conserved cis-regulatory uORF (upstream open reading frame) in the 5'UTR (untranslated region) that is out of frame with the coding region of C/EBPα and is thought to be responsive to the activities of the translation initiation factors eIF4E and eIF2 (Figure 4 and see above). Thus, an increase in the activity of eIF2 or eIF4E, results in the increase in expression of the shorter p30 isoform (rev in(Calkhoven *et al.*, 2000)). An uORF monitors the site of translation initiation by sensing the activity of the eIF2 and eIF4E. When levels of these factors are high, the out-of-frame uORF (Figure 4, red AUG) is translated, but termination of its translation very close to the translational start site (black AUG) for p42 is thought to prevent reinitiation at the p42 AUG. Instead, ribosomes continue to scan and reinitiate at a downstream AUG (Figure 4, green AUG), resulting in the expression of C/EBPα p30. In contrast, under basal conditions, when levels of the initiation factors are relatively low, most ribosomes do not initiate translation at the uORF but instead initiate translation at the p42 AUG by a process involving "leaky ribosome scanning", resulting in translation of the full length C/EBPα p42 isoform(Calkhoven *et al.*, 2000). This mechanism of translational control appears to be conserved among key regulatory proteins which govern differentiation and proliferation (rev in (Khanna-Gupta, 2011)).

High levels of expression of the p30 C/EBPα protein have been shown to interfere with the DNA binding ability of the full length p42 C/EBPα, thus inhibiting transactivation of key granulocytic target genes in a dominant-negative manner (Pabst *et al.*, 2001), In addition, p30 transactivates the expression of a distinct subset of target genes different from that of the full length p42 C/EBPα thereby altering their expression (Geletu *et al.*, 2007). Mice engineered to express only the p30 C/EBPα isoform resulted in the development of AML with complete penetrance (Kirstetter *et al.*, 2008). Thus changes in the ratio of p42:p30 isoforms of C/EBPα play a critical role in contributing to AML(Fu *et al.*, 2010).

Suppression of C/EBPα translation has also been observed in the leukemic blasts of patients with CML (chronic myelogenous leukemia). This occurs via an RNA binding protein, hnRNP-E2, which binds to the uORF of the C/EBPα mRNA, thereby inhibiting translation. Expression of hnRNP-E2 is thought to be upregulated by the activity of the oncogenic BCR-ABL fusion protein in CML patients, and downregulation of hnRNP-E2 by the BCR-ABL inhibitor Imatinib results in restoration of C/EBPα protein expression and granulocytic differentiation of the CML blasts(Perrotti *et al.*, 2002).

6. Role of microRNAs in translation control

6.1 General principles

MicroRNAs (miRNAs) are 18-24 nucleotides long non-coding RNAs that regulate eukaryotic gene expression influencing cellular functions as diverse as cell proliferation, differentiation and apoptosis. miRNAs are non-coding RNAs which silence target genes post-transcriptionally by binding to complementary sequences in the 3'UTR (untranslated region) of the target mRNA resulting in either mRNA degradation or translation repression (rev in (Ambros, 2004)). More than 100 miRNAs are expressed in the hematopoieteic compartment (rev in (Gazzar and McCall, 2011)). MiRNAs are encoded in the genome and are initially transcribed by RNA polymerase II as long primary transcripts referred to as primary miRNAs (pri-miRNAs). These hairpin transcripts are recognized and processed by RNase III enzymes called Drosha and DGCR8 into 60-80 nucleotide intermediates called precursor miRNAs (pre-miRNAs) which are then exported to the cytoplasm by Exportin 5 where a second ribonuclease termed Dicer cleaves the pre-miRNAs to generate double stranded 18-24 nucleotide long miRNAs. One strand of the miRNA is next destroyed and the remaining strand, the guide strand, is then incorporated into the RNA-induced silencing complex or RISC, a large protein complex that also contains the Argonaute 2 or mRNA cleaving proteins. The miRNA guides the RISC complex to target complementary regions in the 3'UTRs of mRNAs, leading to repression of translation or destabilization of the mRNA by deadenylation. (rev in (Manikandan *et al.*, 2008)). In mammalian cells, 6-8 nucleotides of the miRNA (known as the seed region) base pair with the cognate recognition sequence in the 3'UTR of the target mRNA. In general, a perfectly matched sequence between the seed region and mRNA target results in degradation of the mRNA target. An imperfect match however, results in translation repression (rev in (Gazzar and McCall, 2011)). MiRNAs are thought to repress translation by blocking translation initiation of the target mRNA (Filipowicz *et al.*, 2008).

6.2 miRNAs in myeloid biology

An increasing body of evidence implicates miRNA activity in mediating both normal and abmormal myelopoiesis (rev in (Pelosi *et al.*, 2009) and (Gazzar and McCall, 2011)).

Granulocytes arise from the GMPs (granulocyte monocyte progenitors) which are capable of developing into granulocytes or monocytes as a result of cytokines and thus transcription factor activation (Dahl R *et al.*, 2003). MiRNAs have been shown to activate or be activated by myeloid specific transcription factors such as PU.1, C/EBPα and Gfi-1. Mir-223, for example, is thought to be a direct target of C/EBPα and its expression increases during granulopoiesis. C/EBPα is a master regulator of granulopoiesis (Tenen, 2003). Complete loss of mir-223 in mice results in the expansion of granulocyte precursor cells resulting from a cell autonomous increase in the number of granulocytic progenitors (Johnnidis *et al.*, 2008). In contrast, overexpression of mir-223 in acute promyelocytic leukemia (APL) cells results in an enhanced capacity for granulocytic differentiation (Fazi *et al.*, 2005). Mir-223 is thus thought to be a positive regulator of granulopoietic differentiation. It has also been shown that mir-223 targets E2F1, a master cell cycle regulator, by inhibiting translation of its mRNA. Thus, granulopoiesis appears to be regulated by a C/EBPα–miR-223–E2F1 axis, where miR-223 functions as a key regulator of myeloid cell proliferation associated with E2F1 in a negative feedback loop(Pulikkan *et al.*, 2010).

In a recent study, Eiring et al demonstrated a new "decoy" role for miRNAs involving the master reugator C/EBPα. They showed that mir-328 is down regulated in myeloid cells of chronic myelogenous leukemia (CML) patients in blast crisis. Restoration of mir-328 expression however rekindles differentiation in the CML blast cells by a mechanism involving the simultaneous interaction of mir-328 with the C/EBPα translational inhibitor hnRNP-E2 (see above), as well as with the mRNA for PIM1, a survival factor. Since, the interaction with hnRNP-E2 occurs independently of mir-328's seed sequence, this miRNA acts as a "sink" for hnRNA-E2 binding allowing for the release of C/EBPα mRNA from the negative effects of hnRNA-E2-mediated translational inhibition. Thus mir-328 appears to control cell fate by its ability to base pair with the 3'UTR of target mRNAs (PIM1) as well as by acting as a decoy for hnRNP binding resulting in the release of C/EBPα from translational inhibition, thereby altering cell fate(Eiring *et al.*, 2010).

Gfi-1 (growth factor independence-1), a transcriptional repressor that promotes granulocytic differention, has been shown to regulate the expression of mir-196b (Velu *et al.*, 2009). The expression of mir-196b is high in the common myeloid progenitors (CMPs) which can differentiate either along the granulocytic or monocytic lineages. Gfi-1 has been shown to bind to the promoter of mir-196b thereby repressing its expression and promoting granulopoiesis. Additionally, overexpression of mir-196b was shown to block granulopoieis, confirming the importance of low level expression of this miRNA in contributing to granulopoietic maturation (Hock *et al.*, 2003) .

PU.1, a master transcriptional regulator of monocyte/macrophage differentiation, regulates the expression of mir-424, which in turn inhibits the expression of the transcription factor NFI-A. Mir-424 expression has been shown to promote monocyte/macrophage differentiation. Thus the two primary transcriptional determinants of myeloid differentiation, PU.1 and C/EBPα, both involve the activity of microRNAs for lineage maturation (Rosa *et al.*, 2007). Thus, miRNAs and myeloid specific transcription factors play a critical role in lineage maturation pathways by forming lineage-specific regulatory loops (rev in (Gazzar and McCall, 2011)), which if disrupted can lead to the development of leukemias.

Numerous studies have analyzed the expression of miRNAs in acute myeloid leukemias and the resulting miR signatures generated have proved to be helpful in classifying sub-types of AML and hence the choice of treatment options to be used, as well as in determining the efficacy of targeted therapies against AML. For example, Pelosi et al analyzed the expression of 12 selected granulocytic signature miRNAs and the impact of all *trans*-retinoic acid (ATRA)-based therapy in a cohort of acute promyelocytic leukemia (APL) patients (Pelosi *et al.*, 2009). APL is a subtype of acute leukemia and is characterized by the accumulation of promyelocytes as a result of a chromosomal translocation, most commonly involving the retinoic acid alpha receptor and the PML gene in a t(15;17) configuration. APL patients respond well to treatment with retinoic acid, a vitamin A derivative. The authors demonstrated using a quantitative real time PCR approach, that 9 miRNAs were overexpressed while three (mir-107, -342 and Let-7c) were downregulated in the blasts of APL patients, compared to normal promyelocytes. They showed in addition, that patients successfully treated with ATRA showed down regulation of mir-181b and upregulation of mir-15b, -16,-107, -223, -342 and let-7c. Thus, a small subset of miRNAs appeared to be differentially regulated in APL and could be modulated by treatment with ATRA. This approach is fast becoming a paradigm in diagnosing and determining the efficacy of treatment regimens used in acute myeloid leukemias.

7. Concluding remarks and perspectives

Inspite of the fact that there has been a surge of interest in the role of mRNA translational regulation in mediating gene expression in myeloid cells in recent times, a great deal of work has yet to be done. A general interest in this subject derives its source from the fact that cellular pathways commonly deregulated in AML including cell cycle progression, proliferation and differentiation are mechanistically tied to mRNA translation. For example, several upstream (AKT, TSC1/2) and downstream (eIF4E) mediators of the mTORC1 pathway are either mutated or activated in AML. Although there has been an intense search for therapeutic strategies targeting the mTOR pathway in myeloid cells, much work is yet to be done to gain a fundamental understanding of the role of the key players that contribute to translation initiation and control in normal and abnormal myeloid cells.

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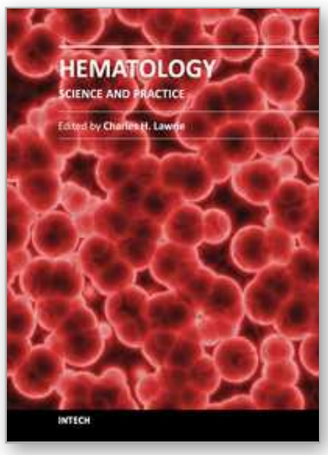

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Hematology encompasses the physiology and pathology of blood and of the blood-forming organs. In common with other areas of medicine, the pace of change in hematology has been breathtaking over recent years. There are now many treatment options available to the modern hematologist and, happily, a greatly improved outlook for the vast majority of patients with blood disorders and malignancies. Improvements in the clinic reflect, and in many respects are driven by, advances in our scientific understanding of hematological processes under both normal and disease conditions. Hematology - Science and Practice consists of a selection of essays which aim to inform both specialist and non-specialist readers about some of the latest advances in hematology, in both laboratory and clinic.

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