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Review

Homeodomain proteins and eukaryotic translation initiation factor 4E (eIF4E): an unexpected relationship

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Summary. The central role of post-transcriptional modification of the expression of several genes involved in tumorigenesis implicates eIF4E as a pivotal factor in the regulation of cell survival, growth and proliferation. Overexpression of eIF4E leads to malignant transformation *in vitro* and induces tumor formation *in vivo*. Furthermore, upregulated expression of eIF4E has been reported in a variety of human malignancies. Consequently, studies over the last ten years have sought to better characterize the molecular mechanisms and cellular factors that control eIF4E activity. These efforts have revealed a role for eIF4E in diverse biological processes including embryonic development, cell cycle progression, synaptic plasticity and cancer. In this review we focus on several members of the homeodomain protein family, which have recently been identified as a novel class of eIF4E regulators.

Key words: eIF4E, Homeoproteins, HOXA9, OPRH/HEX

Introduction

The translation initiation factor 4E (eIF4E) plays an important role in the regulation of gene expression at the post-transcriptional level (reviewed in von der Haar et al., 2004). In the cytoplasm, during the initiation step of cap-dependent translation, eIF4E binds the 5' terminal cap structure (m7GpppN, where N is the first transcribed nucleotide) of the mRNA molecule and forms a heterotrimeric complex (referred to as eIF4F), with the scaffolding protein eIF4G and the mRNA helicase eIF4A. The eIF4F complex allows the recruitment of given transcripts to the 40S subunit of the ribosome, scanning of 5'UTRs for the initiation codon, recognition of the initiation codon, and the establishment of the codon-anticodon interaction (Kozak, 1989; Dever, 1999, 2002; Gingras et al., 1999; Pestova and Hellen, 2000).

A substantial fraction of eIF4E (33-68%) is found in the nucleus, where it participates in nuclear export of a specific subset of transcripts (Lejbkowicz et al., 1992; Rousseau et al., 1996; Lai and Borden, 2000; Iborra et al., 2001; Topisirovic et al., 2002). It is presently unknown whether eIF4E directly transports mRNA molecules across the nuclear membrane or if it indirectly participates in these events. A significant portion of nuclear eIF4E is organized into discrete multiprotein domains, a substantial subset of which overlaps with PML nuclear bodies (reviewed in Strudwick and Borden, 2002). The integrity of these discrete nuclear domains, known as eIF4E nuclear bodies, appears necessary for efficient eIF4E dependent nuclear export of mRNA (Cohen et al., 2001; Topisirovic et al., 2002, 2003a; Kentsis et al., 2004).

There is a substantial body of data indicating that eIF4E is a central regulator of cell growth, proliferation and survival. Accordingly, its overexpression transforms immortalized cell lines, contributes to malignant transformation of primary cells and promotes tumorigenesis in transgenic mice (reviewed in Sonenberg and Gingras, 1998; Montanaro and Pandolfi, 2004; Rosenwald, 2004). Furthermore, increased levels of eIF4E have been reported in a variety of human malignancies (notably breast cancer, head and neck squamous cell carcinoma, non-Hodgkin lymphomas and several types of myeloid leukemia), where eIF4E levels apparently correlate with increased tumor invasiveness and metastatic potential (Topisirovic et al., 2003b; De Benedetti and Graff, 2004).

The biological effects of eIF4E are achieved through the selective upregulation of several proto-oncogenes as well as growth and survival promoting proteins including cyclin D1, VEGF, ODC, c-myc and Bcl-xl (Rousseau et al., 1996; Sonenberg and Gingras, 1998; Topisirovic et al., 2002; Topisirovic et al., 2003a; De Benedetti and Graff, 2004; von der Haar et al., 2004). This is due to the selective increase in translational efficacy and/or nuclear export of the corresponding transcripts that are usually referred to as eIF4E sensitive (Rousseau et al., 1996; Sonenberg and Gingras, 1998;

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Topisirovic et al., 2002; Topisirovic et al., 2003a; De Benedetti and Graff, 2004; von der Haar et al., 2004). However, factors that determine eIF4E sensitivity of the transcripts are still largely unknown. Loss of the cap-binding activity of eIF4E results in its inability either to promote nuclear export or to increase translation efficiency of eIF4E sensitive transcripts (Sonenberg and Gingras, 1998; Culjkovic et al., 2005). Thus, the cap binding activity is necessary for both the translational and transport activities of eIF4E. Interestingly, recent findings suggest that distinctive elements in the mRNA molecule are responsible for determining the eIF4E sensitivity of transcripts at the level of translation versus the level of mRNA transport.

The translational efficiency of a given transcript is dependent on the eIF4E levels and also on the presence of long and highly structured 5'UTRs that are present in most eIF4E sensitive transcripts (reviewed in Sonenberg and Gingras, 1998; Gingras et al., 1999). Although the precise mechanism of eIF4E dependent mRNA transport is still largely unknown, it seems that the recently identified 100 nucleotide element (4ESE) in the 3'UTR of cyclin D1 mRNA confers sensitivity of the transcripts to eIF4E dependent mRNA transport (Culjkovic et al., 2005). These findings suggest that RNA elements controlling eIF4E-mediated mRNA transport are distinct and separate from elements that determine the level of translation efficiency. This implies that the two processes are autonomous, and therefore may be independently regulated. In support of this prediction, results from eIF4E overexpression experiments indicate that "eIF4E sensitive" targets can be upregulated at the level of translation (e.g. VEGF), at the level of mRNA transport (e.g. cyclin D1) or at both levels (e.g. ODC) (Kevil et al., 1996; Rousseau et al., 1996).

Regulation of eIF4E activity and the eIF4E binding motif

Since eIF4E plays an important role in growth control, cell proliferation and cell survival in adult and developing tissues, its activity must be tightly regulated. One possible mechanism of eIF4E regulation is its phosphorylation at the single serine residue by members of the Mnk group of kinases (Lachance et al., 2002; Morley and Naegele, 2002; Scheper and Proud, 2002; Topisirovic et al., 2004; Ueda et al., 2004). Furthermore, several inhibitory proteins that bind eIF4E have been identified including 4E-BPs, PML, Maskin and Cup. In order to suppress eIF4E activity, these proteins must directly bind to eIF4E (Strudwick and Borden, 2002; Clemens, 2004; Richter and Sonenberg, 2005). The details of the molecular contacts involved in these interactions have been the subject of many recent studies. With the structural data available for the eIF4E:eIF4G and eIF4E:4E-BP complexes, it is now clear that all characterized protein-binding partners of eIF4E bind to the same region on the eIF4E molecule. This region, centered around the W73 residue, is characterized by the convex surface that is distal from the cap-binding pocket and does not contain any known residues involved in cap binding (Marcotrigiano et al., 1997; Matsuo et al., 1997; Gross et al., 2003). Furthermore, all protein-binding partners of eIF4E, except PML which uses its RING domain, utilize an evolutionarily conserved eIF4E motif to bind the convex dorsal surface of eIF4E (Strudwick and Borden, 2002). Thus, the eIF4E binding motif is present in eIF4G, eIF4E-binding proteins (4E-BPs), Maskin, Cup and eIF4E-Transporter (4E-T), and is defined by the minimal consensus sequence $Y(X)_4L\Phi$ (where X is any amino acid and Φ is any hydrophobic amino acid; Fig. 1., panel A.) (Sonenberg and Gingras, 1998; Dostie et al., 2000; Richter and Sonenberg, 2005).

The emerging roles of eIF4E in embryonic development and differentiation

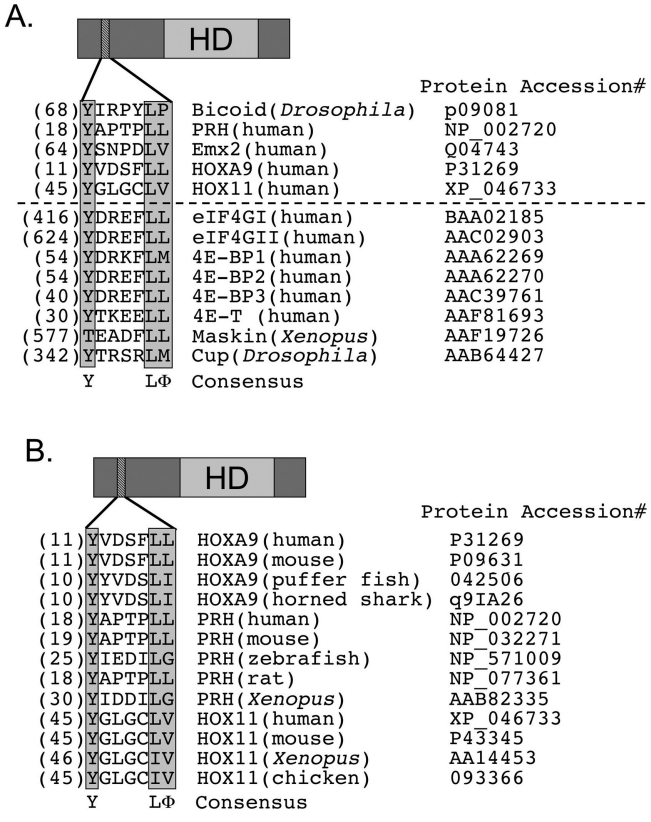

Fig. 1. ~200 homeoproteins contain evolutionarily conserved eIF4E binding motifs. **A.** Sequence alignment of eIF4E binding motifs in several homeoproteins (above dashed line) and known eIF4E interacting proteins (below dashed line). The relative positions of the eIF4E binding motif (hatched box) and homeodomain (HD) in homeoproteins are indicated on the diagram. **B.** Sequence alignment of the eIF4E binding motif from HOXA9, PRH and HOX11 proteins from different species indicating high evolutionary conservation of this domain. Residues that are part of the eIF4E binding motif consensus sequence [$Y(X)_4L\Phi$] are highlighted.

Several recent studies indicate that eIF4E plays an important role during embryonic development and tissue patterning. For example, eIF4E injection in *Xenopus* oocytes preferentially enhances translational efficiency of activin, a mesoderm inducing member of the TGFß superfamily, without affecting the rates of global protein synthesis. The elevation of activin levels, and possibly other mesoderm inducing factors, induces mesoderm formation in ectodermal explants of eIF4E injected embryos. Furthermore, these explants elongate in a way similar to those treated with activin or FGF. When injected at a later stage, eIF4E induces the expression of mesodermal markers along with the formation of mesenchyme and coelomic cavities in explants that are normally differentiating towards ectodermal tissues (Klein and Melton, 1994). During zebrafish development, eIF4E expression shows dynamic and asymmetric patterns. This fluctuation of eIF4E concentration may determine the spatio-temporal patterns of gene expression in a tissue and/or mRNA specific manner (Fahrenkrug et al., 1999). It is well established that the spatio-temporal control of gene expression is essential for a number of processes in embryonic development including specification of cell fates, embryonic induction, and establishment of the body axis.

In addition to its emerging roles in embryogenesis, eIF4E seems to play an active role in differentiation and maintenance of cell-type specific functions in adult tissues. For example, changes in 4E-BPs expression and/or phosphorylation that are specific to the monocytic or to the granulocytic pathway suggest that eIF4E activity is differentially regulated during myelogenesis (Grolleau et al., 1999). The levels of eIF4E decrease during BrdU-induced differentiation of the HL-60 promyelocytic leukemia cells and overexpression of eIF4E blocks vitamin D and ATRA induced differentiation of U937 human monoblastic leukemia cells (Topisirovic et al., 2003b; Walsh et al., 2003). Conversely, overexpression of eIF4E along with BrdU treatment induces differentiation in the keratin-negative human lung cell line, DLKP (Walsh et al., 2003).

Thus, in addition to its general role, eIF4E also acts as a tissue-specific translation and/or mRNA transport enhancer in developing and adult tissues. This tissue-specific mode of eIF4E activity seems to be crucial for normal development and differentiation, and necessitates the presence of tissue-specific regulation mechanisms. Recent studies indicate that several members of the homeodomain family regulate eIF4E activity in a tissue-specific manner (Niessing et al., 2002; Topisirovic et al., 2003a, 2005; Nedelec et al., 2004). The expression of these regulatory proteins is confined to a limited number of tissues, with the members of this family of proteins playing a central role in development and differentiation.

Homeodomain proteins as regulators of eIF4E

Homeodomain proteins, also referred to as homeoproteins, represent a family of transcription factors that share an evolutionarily conserved DNA-binding domain composed of ~60 amino acids (Laughon, 1991). In addition to mediating sequence-specific DNA binding, the homeodomain can be involved in protein-protein interactions and RNA binding (Gehring et al., 1994). The homeoproteins are encoded by homeobox genes that are located in four clusters designated as HOX A through D, while the others are known as divergent homeobox genes (McGinnis and Krumlauf, 1992). The members of homeodomain family of proteins play essential roles in the regulation of embryonic development and morphogenesis (McGinnis and Krumlauf, 1992; Kmita and Duboule, 2003). Furthermore, many of these proteins are expressed in adult tissues, suggesting that they regulate basic and cell type-specific activities throughout life (Cillo et al., 2001). Recently, it was shown that some members of the homeoprotein family are involved in the regulation of cellular growth and proliferation. Further, disruption of homeoprotein function has been linked to tumorigenesis (Coqueret et al., 1998; Cillo et al., 2001; Abate-Shen, 2003).

Based on the identification of Bicoid and PRH proteins as modulators of eIF4E activity, we decided to carry out database searches of the 803 homeoproteins present in the Swissprot database, looking for the Y(X)4LF consensus sequence. (The results of the database searches are given on the following website: http://icb.med.cornell.edu/borden/hd_tbl.htlm). Interestingly, at least one copy of this sequence was present in 199 of the examined homeoproteins. In 100 of these, the eIF4E binding motif was found to be located N-terminal to the homeodomain as is the case for the known eIF4E binding partners Bicoid and PRH (Fig. 1, panel A.) (Niessing et al., 2002; Topisirovic et al., 2003a). Subsequent studies showed that several homeoproteins containing the eIF4E binding motif (i.e. HOXA9, HOX11 and Emx2) directly interact with eIF4E (Topisirovic et al., 2003a, 2005; Nedelec et al., 2004). Notably, the $Y(X)_4L\Phi$ sequence is highly conserved amongst homeoproteins from different species ranging from zebrafish to humans (Fig. 1, panel B). The evolutionary conservation of this sequence further highlights the functional importance of the eIF4E binding motif in these homeoproteins.

Thus, homeoproteins are positioned to potentially modulate both nuclear and cytoplasmic activities of eIF4E independently of transcription. This implies that at least some of their biological functions are achieved through the modulation of the expression of "eIF4E-sensitive" transcripts at the post-transcriptional level. Furthermore, the disruption of the homeoprotein mediated regulation of eIF4E activity could play a major role in eIF4E dependent oncogenesis.

In the following sections, we will discuss how four specific homeoproteins affect the activity of eIF4E and correlate this with their biological effects. Also, we will summarize the possible molecular mechanisms that

underlie this novel mode of eIF4E regulation.

Bicoid

Bicoid (BCD) is a homeoprotein that acts as the anterior determinant in pattern formation during early *Drosophila* embryogenesis (reviewed in Rivera-Pomar and Jackle, 1996). It acts as a transcriptional activator of segmentation genes and causes specific translational repression of caudal (cad) mRNA in the anterior region of the embryo (Rivera-Pomar and Jackle, 1996; Rivera-Pomar et al., 1996).

Recent studies revealed that BCD induced repression of cad mRNA translation is mediated through binding of BCD to the 3' UTR, thus interfering with formation of the eIF4F complex (Fig. 2, panel A). Specifically, BCD utilizes the arginine-rich RNA binding motif in its homeodomain to bind a region in the 3'UTR of *cad* mRNA denoted as the BCD binding region (BBR). At the same time, BCD interacts with 5' cap-bound eIF4E via the conserved N-terminal eIF4E binding motif (Niessing et al., 2002). BCD binds the same dorsal surface of eIF4E as eIF4G and thereby, in a fashion similar to 4E-BPs, blocks the eIF4E:eIF4G interaction and the subsequent assembly of eIF4F complex on cad mRNA (Fig. 2, panel A.) Therefore, similarly to Maskin and Cup, BCD acts as a target specific repressor of eIF4E, where the target specificity is determined by the presence of the BBR in the 3'UTR of *cad* mRNA (Cao and Richter, 2002; Niessing et al., 2002; Nakamura et al., 2004; Nelson et al., 2004). It is worth noting that although BCD, Maskin and Cup induce mRNA-specific translational repression, they utilize different molecular strategies. As mentioned above, BCD utilizes its RNA-binding motif to directly interact with cad mRNA. In contrast, the interaction of Maskin and Cup with target mRNAs is mediated by CPEB and Bruno/Smaug respectively (Cao and Richter, 2002; Nakamura et al., 2004; Nelson et al., 2004). The integrity of the BCD homedomain and the presence of the functional eIF4E binding motif are necessary for the efficient inhibition of cad mRNA translation (Niessing et al., 2002).

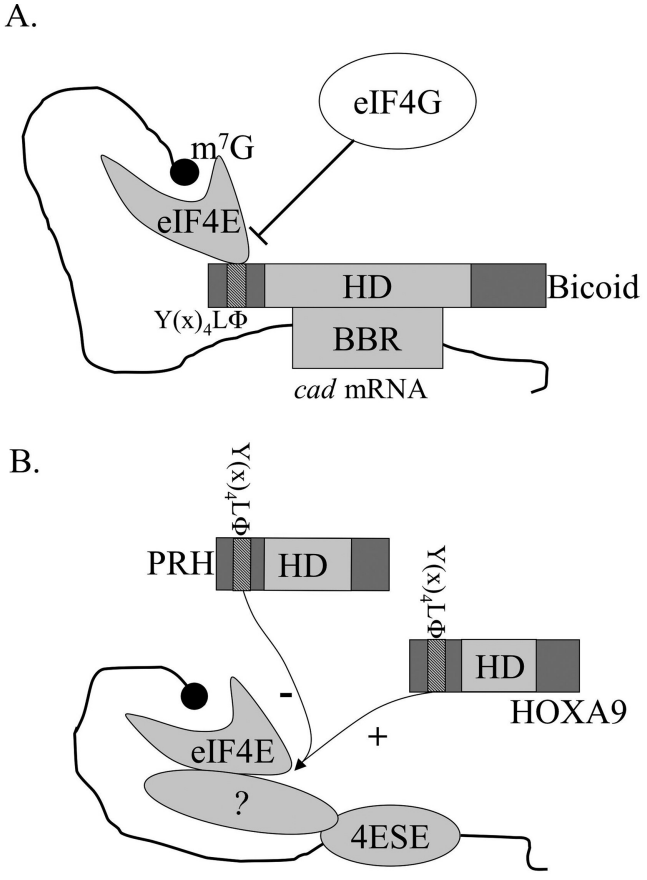

Fig. 2. Homeoproteins utilize different molecular strategies to alter the activity of eIF4E. **A.** Bicoid (BCD) selectively inhibits translation of *caudal* (*cad*) mRNA by interfering with eIF4F complex formation. BCD binds the Bicoid binding region (BBR) in the 3'UTR of cad mRNA and utilizes the eIF4E binding motif [$Y(X)_4L\Phi$] to bind the dorsal surface of eIF4E, thereby blocking eIF4G binding. **B.** Homeoproteins that stimulate (e.g. HOXA9) or repress (e.g. PRH) the transport activity of eIF4E alter the nuclear export of transcripts that are transported in an eIF4E dependent manner. HOXA9 and PRH stimulate and suppress eIF4E mediated mRNA transport, respectively. These proteins utilize their eIF4E binding motifs [$Y(X)_4L\Phi$] to bind the dorsal surface of eIF4E.

Emx2

The Emx2 homeoprotein is involved in the control of developmental functions in adult tissues. For example, it participates in regulating neurogenesis in the subventricular zone and dentate gyrus (Gangemi et al., 2001; Nakatomi et al., 2002). Nedlec and colleagues recently showed that Emx2 is also expressed in the adult olfactory epithelium (Nedelec et al., 2004). This region is the site of permanent turnover of the olfactory sensory neuron (OSN) axons in the olfactory nerve and of the synapses between the OSN axon terminals and postsynaptic targets (Mackay-Sim and Kittel, 1991). The Emx2 protein is present in the adult OSN nuclei, axons and axon terminals. The same authors showed that Emx2 directly interacts with eIF4E (Nedelec et al., 2004). Similar to Bicoid, Emx2 contains a conserved eIF4E binding motif in its N-terminal region, and thus it appears that the formation of Emx2:eIF4E complex is achieved through interaction of the eIF4E binding motif and the dorsal surface of eIF4E. Furthermore, Emx2 and eIF4E co-immunoprecipitate and co-sediment in high density synaptosomal subfractions. This interaction is resistant to treatment with nonionic detergents or RNase. The co-sedimentation of eIF4E and Emx2 in these fractions, which are enriched in vesicles and granular structures, suggests that Emx2 is associated and co-transported with eIF4E within high density particles (Nedelec et al., 2004). The interaction of Emx2 and eIF4E in the OSN axons indicates that Emx2 locally regulates the expression of certain mRNAs by

modulating the translational activity of eIF4E. The nuclear fraction of Emx2, in addition to its function as a transcriptional regulator, is positioned to modulate eIF4E dependent mRNA transport. Thus, like Bicoid, Emx2 is another homeoprotein that can alter the expression of its target genes not only at the transcriptional level, but also at the post-transcriptional level by modulating eIF4E activity. Two additional homeoproteins that are expressed in the developing and adult central nervous system, Otx2 and Engrailed 2, directly bind eIF4E, suggesting that this mode of eIF4E dependent modulation of gene expression could be shared by homeoproteins that regulate neurogenesis (Nedelec et al., 2004).

PRH

The Proline-Rich Homeodomain protein (PRH), also known as hematopoietically expressed homeodomain (Hex) is a divergent homeoprotein, classified as a member of the tinman family of homeoproteins (Crompton et al., 1992). It was identified in avian hematopoietic cells and is highly conserved between amphibian, mammalian and avian species (Crompton et al., 1992; Bedford et al., 1993; Newman et al., 1997; Tanaka et al., 1999a). PRH plays an important role in early embryonic patterning and hematopoiesis. During embryogenesis, its function is essential for the formation of the forebrain, liver and thyroid gland. The homozygous deletion of *prh* gene results in an embryonic lethal phenotype in mice that is characterized by defects in the development of these organs (Martinez Barbera et al., 2000). In hematopoietic cells, PRH is strongly expressed in pluripotent erythromyeloid and B-cell progenitors, and is generally downregulated during differentiation of most hematopoietic lineages (Manfioletti et al., 1995; Jayaraman et al., 2000). Outside of the hematopoietic system, PRH is expressed in only a limited number of adult tissues - liver, lung, thymus, and endothelial cells (Crompton et al., 1992; Bedford et al., 1993; Hromas et al., 1993; D'Elia et al., 2002).

Forced expression of PRH in bone marrow cells of C57BL6 mice results in increased frequency of T cell lymphomas, and its upregulation has been linked with certain types of lymphoid leukemia (Hansen and Justice, 1999; George et al., 2003). On the other hand, PRH is downregulated in certain types of myeloid leukemia (Topisirovic et al., 2003b). These findings suggest that PRH can act as both tumor suppressor and an oncogene, depending on the cellular context.

The PRH protein consists of three separate domains: a proline rich N-terminal domain, a central homeodomain and an acidic C-terminal domain. PRH acts as a transcriptional repressor in hematopoietic, liver, thyroid and embryonic stem cells (Tanaka et al., 1999b; Brickman et al., 2000; Pellizzari et al., 2000; Guiral et al., 2001). In hematopoietic cells, PRH represses transcription by utilizing a homeodomain (which *in vitro* binds to TATA box sequences and the TATA box binding protein, TBP), and a separate N-terminal proline-rich region (Guiral et al., 2001). It was recently reported that PRH binds members of the Groucho/transducin-like enhancer of split (TLE) family of proteins, which act as transcriptional co-repressors and play multiple roles in embryonic development. The binding of TLE protein family members was mapped to the N-terminal proline-rich region of PRH, suggesting that this domain allows recruitment of transcriptional co-repressors to target genes in hematopoietic cells (Swingler et al., 2004).

Our recent findings indicate that in addition to its transcriptional function, PRH regulates gene expression at the post-transcriptional level by modulating eIF4E activity (Topisirovic et al., 2003a). In its N-terminal proline rich region, PRH contains a conserved eIF4E binding motif that does not overlap with the binding site for Groucho/TLE family members. PRH utilizes the eIF4E binding motif to directly bind the dorsal surface of eIF4E. PRH co-immunoprecipitates with eIF4E in both the cytoplasmic and the nuclear compartment of U937, K562 and primary human hematopoietic cells (Topisirovic et al., 2003a,b). In all these cases, a substantial fraction of nuclear PRH co-localized with eIF4E nuclear bodies. Overexpression of PRH results in the selective repression of eIF4E dependent mRNA transport, and when expressed ectopically in NIH 3T3 cells, the inhibition of eIF4E mRNA transport activity coincides with the suppression of eIF4E induced malignant transformation. These effects require both the direct interaction of eIF4E with PRH (i.e. via an intact eIF4E binding site at the N-terminus of PRH and the presence of the W73 residue on the dorsal surface of eIF4E) and the presence of PRH in the nucleus. The disruption of the PRH NLS causes its exclusion from the nucleus, and abolishes the ability of PRH to inhibit eIF4E mediated transformation of NIH3T3 cells. This finding suggests that, although the role of PRH in the modulation of eIF4E translational activity remains elusive, the PRH protein primarily regulates the nuclear activity of eIF4E (i.e. its transport activity). In support of this hypothesis, alterations of the subcellular localization of PRH have been reported in certain types of thyroid tumors and myeloid leukemia. In these specimens, PRH is almost completely excluded from the nucleus, which correlates with the loss of its regulatory function (D'Elia et al., 2002; Topisirovic et al., 2003b). In myeloid leukemia specimens, inhibition of NFkB activity leads to the restoration of PRH activity and correlates with its re-location to the nucleus and co-localization with eIF4E bodies (Topisirovic et al., 2003b). Conversely, forced expression of PRH leads to the disruption of eIF4E nuclear bodies, and subsequent dislocation of eIF4E to the cytoplasm accompanied by abrogation of eIF4E dependent mRNA transport (Topisirovic et al., 2003a). Mechanistically, PRH only modestly affects the cap binding affinity of eIF4E. Thus it seems likely that PRH induced suppression of eIF4E dependent mRNA transport arises from its ability to block the binding of

positive regulators (e.g. HOXA9) to the dorsal surface of eIF4E (Fig. 2, panel B). This mechanism will be elaborated in detail below.

HOXA9

HOXA9 is a member of the *HOX* homeobox gene family. In addition to regulating body pattern formation and tissue identity during embryogenesis, many members of this family, including HOXA9, play an important role in adult hematopoiesis (Gehring, 1987; McGinnis and Krumlauf, 1992; Lawrence et al., 1996; Magli et al., 1997; Kmita and Duboule, 2003). Accordingly, HOXA9 deficient mice show mild defects in hematopoiesis which affect myeloid, erythroid and lymphoid compartments (Lawrence et al., 1997; Izon et al., 1998). The overexpression of HOXA9 blocks differentiation and immortalizes growth factor-dependent myeloid progenitors, which, after a latency period, results in malignant transformation (Kroon et al., 1998; Thorsteinsdottir et al., 2001). HOXA9 is also frequently upregulated in human acute myeloid leukemias (AML) where its elevated levels correlate with poor patient prognosis (Golub et al., 1999). Thus, there is substantial evidence that the HOXA9 protein is implicated in both normal hematopoiesis and leukemic transformation. At the molecular level, it has been reported that HOXA9 acts as a transcription factor (Shen et al., 1999; Dorsam et al., 2004). In support of this, putative HOXA9 gene targets were recently identified by microarray analysis (Dorsam et al., 2004).

Like all of the aforementioned homeoproteins, HOXA9 contains an eIF4E binding motif that is located in its N-terminal region, outside of its homeodomain. Like other regulators of eIF4E activity, it utilizes this site to directly bind the dorsal surface of the eIF4E protein. When overexpressed in U937 cells, HOXA9 co-immunoprecipitates and co-localizes with eIF4E in both the nucleus and the cytoplasm of the cell. This coincides with the disruption of the eIF4E:PRH interaction and is accompanied by substantial increases in the efficiency of nuclear export and translation of "eIF4E-sensitive" transcripts (Topisirovic et al., 2005). The same effect is seen in a subset of myeloid leukemia specimens with upregulated eIF4E and downregulated PRH levels (Topisirovic et al., 2003b, 2005). These specimens also show almost complete exclusion of the PRH protein from the nucleus. The latter changes seem to depend on NFkB activity, where molecular genetic inhibition of NFkB expression results in apparent reconstitution of the co-localization of PRH with eIF4E nuclear bodies and disruption of HOXA9:eIF4E interaction. These changes are accompanied by the normalization of eIF4E dependent mRNA transport (Topisirovic et al., 2003b).

Recent findings demonstrate that HOXA9 stimulates eIF4E activities in both translation and mRNA transport. These effects are distinct from its role in transcription. For instance, the overexpression of a mutated form of HOXA9 with a disrupted eIF4E binding site, which is still active in transcription, fails to produce the latter effects, indicating that HOXA9 must directly interact with eIF4E in order to modulate its activity.

Similarly to PRH, HOXA9 only modestly affects the binding of eIF4E to the mRNA cap structure (Topisirovic et al., 2005). However, these proteins show similar affinity for eIF4E binding *in vitro*. Since both proteins bind the same surface of eIF4E, it is plausible to speculate that the competition between PRH and HOXA9 could contribute to the molecular mechanism underlying homeoprotein mediated modulation of eIF4E dependent mRNA transport (Fig. 2, panel B). This hypothesis will be discussed in detail below.

One question that remains elusive is how HOXA9 stimulates the translational activity of eIF4E? The question, with regard to translation, is especially puzzling because HOXA9 binds to the same surface of eIF4E as eIF4G (Sonenberg and Gingras, 1998). Thus, the interaction of eIF4E and HOXA9 could interfere with eIF4F complex formation and subsequently lead to the inhibition of translation. One possible explanation for this somewhat paradoxical observation is that HOXA9 acts prior to the assembly of the eIF4F complex. This theory is supported by the finding that HOXA9 is not found bound to polysomes, thereby allowing eIF4G access to eIF4E. Furthermore, the affinity of eIF4G for eIF4E is approximately 1000 fold higher than the affinity of HOXA9 (i.e. Kd ~1nM for the eIF4G:eIF4E complex and Kd~1μM for the HOXA9:eIF4E complex) (Gross et al., 2003; Topisirovic et al., 2005). Thus, HOXA9 could easily be displaced by eIF4G allowing the translation of "eIF4E-sensitive" transcripts. A similar "displacement" mechanism was proposed for Maskin, the protein that, like HOXA9, has a substantially lower binding affinity for eIF4E than eIF4G. Maskin suppresses the translation of cytoplasmic polyadenylation element (CPE) containing mRNAs in *Xenopus* oocytes arrested at prophase (Stebbins-Boaz et al., 1999). When oocytes are induced to complete meiosis, CPE binding protein (CPEB) stimulates growth of the poly(A) tail, which stimulates the binding of poly(A) binding protein (PABP). PABP then interacts with eIF4G, which in turn displaces Maskin from eIF4E, thereby inducing translation (Cao and Richter, 2002).

The interplay between homeoproteins as a possible mechanism of regulation of eIF4E dependent mRNA transport

The molecular mechanism of PRH and HOXA9 mediated modulation of eIF4E activity remains largely unknown. PRH and HOXA9 only modestly affect the affinity of eIF4E for the cap (less than 10 fold, comparing with >100 fold reduction of eIF4E cap binding activity by PML) (Cohen et al., 2001; Kentsis et al., 2001; Topisirovic et al., 2003a, 2005). These findings indicate the mechanism(s) that PRH and HOXA9 utilize to modulate eIF4E activity do not involve changes in cap binding affinity. We recently

reported that PRH binds eIF4E with approximately the same affinity as HOXA9. Indeed, the apparent affinity of PRH for eIF4E is only ~ 2.5 fold higher than HOXA9 binding (Topisirovic et al., 2005). This suggests that PRH and HOXA9 compete for eIF4E binding, since they bind the same site on the dorsal surface of eIF4E (Fig. 1, panel B). Ignoring other unknown factors, the relative ratio of PRH and HOXA9 in the cell would therefore be the major determinant controlling binding to eIF4E. This hypothesis is consistent with the loss of the PRH:eIF4E interaction in HOXA9 overexpressing U937 cells and implies that disruption of the eIF4E interaction with inhibitory proteins could be a molecular mechanism underlying HOXA9 induced stimulation of eIF4E activity (Topisirovic et al., 2005). Furthermore, in myeloid leukemia specimens that show elevated levels of eIF4E and HOXA9, and downregulated expression of PRH, the PRH:eIF4E interaction is virtually abolished. In the same specimens, there is a substantial increase in HOXA9:eIF4E interaction that is accompanied by upregulation of several eIF4E sensitive targets (Topisirovic et al., 2003b).

Thus, PRH mediated suppression of eIF4E dependent mRNA transport could arise from its ability to outcompete positive regulators (e.g. HOXA9) of eIF4E transport. Conversely, HOXA9 could block the binding of negative regulators (e.g. PRH) to the dorsal surface of eIF4E, thereby promoting the nuclear export of eIF4E sensitive transcripts. There is a substantial amount of data indicating that the homeodomain is involved in RNA binding and protein-protein interactions (Laughon, 1991; Gehring et al., 1994). Based on the results of these studies, it is plausible to speculate that homeoproteins which stimulate nuclear export of eIF4E sensitive mRNAs, recruit specific transcripts through homeodomain:mRNA binding or proteins through homeodomain mediated protein-protein interactions. This activity would promote the assembly of transport competent eIF4E containing mRNPs. Alternatively, the homeoproteins that suppress nuclear activity of eIF4E could interfere with the formation of these mRNPs, thereby lowering the efficiency of eIF4E dependent nuclear export. This implies that homeoproteins could be the major factors determining the specificity of transcripts that are transported in an eIF4E dependent manner (Fig. 2, panel B).

Conclusions

Homeoproteins, the pivotal factors that govern embryonic development, morphogenesis and differentiation in adult tissues, are considered to primarily act as transcription factors. However, there is substantial body of data emerging that suggests some of these proteins affect the expression of certain subsets of genes at the post-transcriptional level through modulation of eIF4E activity. These data also indicate that the homeoprotein mediated alteration of eIF4E activity can affect expression of specific proteins, as is the case for Bicoid induced repression of Caudal mRNA translation. In addition, more general effects, as observed for PRH and HOXA9 modulation of eIF4E sensitive targets is also an important mechanism regulating the activity eIF4E.

Experimental evidence indicates that the modulation of eIF4E by homeoproteins (e.g. BCD, PRH, HOXA9) is independent of their transcriptional activity. Hence in addition to their function in transcription, which is thought to be responsible for long-term changes in the proteome, homeoproteins can be involved in short-term changes in expression of growth and survival factors by modulating eIF4E activity. The latter mechanism could be activated by both intra- and extra-cellular stimuli, which is supported by the recent finding that homeoproteins are involved in intercellular signaling (Prochiantz and Joliot, 2003). Therefore, homeoproteins are poised to integrate proliferation and differentiation signals in adult and embryonic tissues as a response to various intra- and extra-cellular challenges.

As mentioned above, there are ~200 members of the homeoprotein family that contain putative evolutionarily conserved eIF4E binding motif. Thus, it is possible that modulation of eIF4E activity is a feature commonly shared between homeoproteins, and that these proteins utilize this function in order to establish spatio-temporal patterns of gene expression that are necessary for normal embryonic development and morphogenesis. Most of the homeoproteins are expressed in a limited number of tissues, suggesting that these proteins act as tissue-specific modulators of eIF4E activity. This mode of regulation indicates that homeoproteins are positioned to modulate expression of eIF4E sensitive targets in a tissue specific manner, thereby regulating the growth and survival of cells in developing and adult tissues. Furthermore, the competition between inhibitory and stimulatory homeoproteins (i.e PRH and HOXA9) for eIF4E binding suggests the existence of a novel regulatory network that controls the activity of eIF4E. Elucidation of the precise molecular mechanisms that govern this process will help us to understand the role of this unexpected relationship in development and tumorigenesis.

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