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14. ABSTRACT
I discovered that cap-independent translation of oncogene mRNAs, e.g. c-*myc* or vascular endothelial growth factor (VEGF) transcripts, is regulated by affinity of the eukaryotic initiation factor (eIF4G) for sequences within the 5' untranslated region of such mRNAs. Our work contributes to understanding how translation initiation factors, phosphoproteins controlled by the major oncogenic signaling pathways in cancer, regulate gene expression at the translational level. My findings enable functional studies of eIF4G and how it responds to protein kinase inhibitors currently tested in cancer therapy, e.g. against breast cancer. Most importantly, my work contributes key empirical evidence in support of a novel experimental cancer treatment scheduled to enter clinical investigation within the next 6 months. Cell type specific cancer cell killing of the prototype oncolytic poliovirus, PVS-RIPO, depends on selective eIF4G:IRES binding in cancerous cells. My work provided the empirical basis behind this principle and constitutes a critical aspect of correlative studies demanded by FDA.

15. SUBJECT TERMS
Translation, eIF4E, eIF4G, IRES, Cancer, Poliovirus

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Introduction:

With support from the above-mentioned award, I recently (December, 2008) defended my thesis entitled '*Regulation of Translation by Eukaryotic Initiation Factor 4G*' and graduated with a Ph.D. degree from the Molecular Cancer Biology Graduate Studies Program at Duke University. My Ph.D. studies centered on investigations of protein synthesis control via the eukaryotic initiation factor (eIF) 4G, the central scaffolding factor in the cap-binding complex. eIF4G and its binding partner, the cap-binding protein eIF4E, are eminent determinants of gene control in cancer. Both are at the receiving end of oncogenic growth signaling via the phosphoinositide-3-kinase (PI3K)/mammalian target of rapamycin (mTOR) and the Ras/mitogen-activated protein kinase (MAPK) pathways. Thus, the principal growth signaling and nutrient sensing pathways in cancer converge on these translation initiation factors. A myriad of small molecule kinase inhibitors that influence protein synthesis via eIF4G (e.g. mTOR/PI3K inhibitors) are under evaluation for breast cancer treatment. Deplorably, there is almost no information about eIF4G's role in gene control in cancer, greatly limiting rational use of such drugs. My work has unraveled important principles of eIF4G's function in translation regulation. I began my studies by conducting ectopic eIF4G expression assays and evaluate its role in cap-independent translation initiation at viral RNAs (Dobrikova *et al*., 2006). I was further involved with studies of translation regulation via the eIF4G-binding partner poly-adenylate binding protein (PABP) (Bradrick *et al*., 2007). Most importantly, the bulk of my thesis work revealed eIF4G's role in regulating cap-independent translation initiation at oncogene mRNAs, e.g. c-*myc* and the vascular endothelial growth factor (VEGF) (Kaiser *et al*., 2008).

Body:

Activation of cap-independent translation by variant eukaryotic initiation factor 4G in vivo

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ABSTRACT

Protein synthesis is tightly controlled by assembly of an intricate ribonucleoprotein complex at the m^7GTP-cap on eukaryotic mRNAs. Ensuing linear scanning of the 5′ untranslated region (UTR) is believed to transfer the preinitiation complex to the initiation codon. Eukaryotic mRNAs are characterized by significant 5′ UTR heterogeneity, raising the possibility of differential control of translation initiation rate at individual mRNAs. Curiously, many mRNAs with unconventional, highly structured 5′ UTRs encode proteins with central biological roles in growth control, metabolism, or stress response. The 5′ UTRs of such mRNAs may influence protein synthesis rate in multiple ways, but most significantly they have been implicated in mediating alternative means of translation initiation. Cap-independent initiation bypasses strict control over the formation of initiation intermediates at the m^7GTP cap. However, the molecular mechanisms that favor alternative means of ribosome recruitment are not understood. Here we provide evidence that eukaryotic initiation factor (eIF) 4G controls cap-independent translation initiation at the c-*myc* and vascular endothelial growth factor (VEGF) 5′ UTRs in vivo. Cap-independent translation was investigated in tetracycline-inducible cell lines expressing either full-length eIF4G or a C-terminal fragment (Ct) lacking interaction with eIF4E and poly(A) binding protein. Expression of Ct, but not intact eIF4G, potently stimulated cap-independent initiation at the c-*myc*/VEGF 5′ UTRs. In vitro RNA-binding assays suggest that stimulation of cap-independent translation initiation by Ct is due to direct association with the c-*myc*/VEGF 5′ UTR, enabling 43S preinitiation complex recruitment. Our work demonstrates that variant translation initiation factors enable unconventional translation initiation at mRNA subsets with distinct structural features.

Keywords: C-*myc*; VEGF; translation; eIF4G; eIF4E; PABP

INTRODUCTION

Translation control provides a critical level of gene regulation, which has been implicated in the malignant phenotype (Bader and Vogt 2004; Mamane et al. 2004, 2006). It confers the cell's ability to integrate nutrient, stress, and mitogenic signals for global proteome control or for selective translation of specific mRNAs. In general, protein synthesis is modulated at the level of initiation (Gebauer and Hentze 2004). Eukaryotic initiation occurs when the 43S preinitiation complex, comprised of the 40S ribosomal subunit and ternary complex with eIF3, is recruited to the mRNA. Conventionally, this requires 5′ m^7GTP-cap inter-

action with the tripartite eIF4F (Gingras et al. 1999; Hershey and Merrick 2000). eIF4F constituents bind the cap (eIF4E), supply RNA helicase activity (eIF4A), and provide the central scaffold to engage eIF3 and the poly(A) binding protein (eIF4G). Once recruited to the mRNA, ribosomal scanning of the 5′ untranslated region (UTR) ensues until an initiation codon is encountered within a favorable context. Subsequent joining of the large (60S) ribosomal subunit produces an intact 80S particle that is primed for elongation.

A requirement for assembly of the canonical initiation apparatus at the cap tightly controls protein synthesis. However, this restraint is relaxed for certain mRNAs whose 5′ UTRs enable ribosome recruitment in a cap-independent manner. This mechanism is exemplified by uncapped positive-strand RNA virus genomes (Jang et al. 1988; Pelletier and Sonenberg 1988; Tsukiyama-Kohara et al. 1992). The principle of cap-independent translation initiation
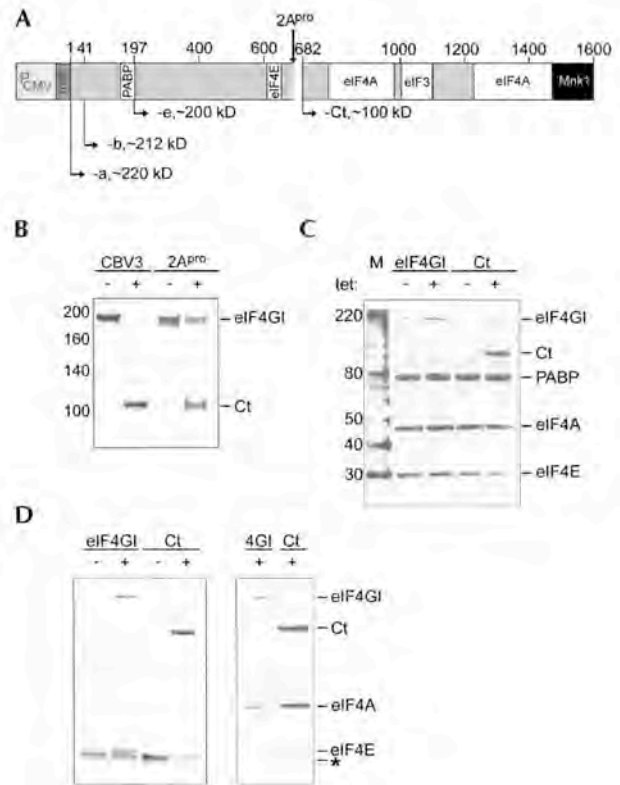
via an internal ribosomal entry site (IRES), first established with picornaviruses, has since been demonstrated with eukaryotic 5′ UTRs as well (Sachs et al. 1997; Stoneley and Willis 2004; Jackson 2005). There are no a priori structural predictors for IRES capacity, but generally these 5′ UTRs are uncommonly large, are predicted to form complex higher-order structures, and contain numerous upstream AUGs or CUGs.

Intriguingly, irregular 5′ UTRs with a potential for alternative initiation are common with potent regulators of fundamental biological processes, e.g., the c-myc oncogene (Nanbru et al. 1997; Stoneley et al. 1998). Alternative initiation may bypass the restraints of global translation repression and permit prompt selective induction of critical mRNAs with sudden onset of metabolic (Yaman et al. 2003), hypoxic (Stein et al. 1998), or thermal (Hernandez et al. 2004) stress as well as during apoptosis (Stoneley et al. 2000). Despite increasingly compelling evidence for a role of alternative translation initiation in cellular gene expression, less is known about the mechanisms facilitating this event.

In contrast, alternative initiation at viral IRESs has been more thoroughly characterized. Generally, translation of viral RNA occurs in the context of serious irreversible changes to the translation machinery and little regard for host cell survival (Lloyd 2006). For example, enterovirus 2A proteinase (2A^{pro}) cleavage of eIF4GI (Etchison et al. 1982) and -II (Gradi et al. 1998) separates the eIF4E/poly(A) binding protein (PABP)-binding motifs from the eIF3/-4A sites (Fig. 1A; Lamphear et al. 1995), blocking cap-dependent host protein synthesis without affecting viral IRES-mediated translation (Ohlmann et al. 1996). Analogous to eIF4G cleavage by viral proteases, eIF4G degradation and stimulation of m^{7}GTP-cap-independent translation has been observed in situations of cellular stress, e.g., that associated with the onset of apoptosis (Clemens et al. 1998; Bushell et al. 2006).

We examined the role of eIF4G and variants thereof in the induction of m^{7}GTP-cap-independent translation initiation at the 5′ UTRs of the c-myc oncogene and VEGF. We focused our studies mostly on the c-myc 5′ UTR, because c-myc mRNA remains polysome-bound in enterovirus-infected cells, demonstrating IRES competence (Johannes and Sarnow 1998). C-myc expression is abnormally upregulated in many cancers (Adhikary and Eilers 2005), its IRES has been directly implicated in aberrant expression in multiple myeloma (Chappell et al. 2000), and c-myc IRES-driven translation persists despite global translation repression in apoptosis (Nevins et al. 2003). Likewise the VEGF 5′ UTR has been shown to harbor IRES activity (Stein et al. 1998), which may operate in hypoxia-induced VEGF expression (Koritzinsky et al. 2006). Employing an inducible in vivo expression system, we found that the c-myc/VEGF 5′ UTRs, but not viral IRESs, are potently stimulated by a C-terminal eIF4G fragment. Trans-activation occurred despite the presence of endogenous eIF4G within an intact

FIGURE 1. eIF4G cleavage and tet-inducible *myc*-eIF4G-b and *myc*-Ct expressing cell lines. (*A*) Schematic of eIF4GI structure. Binding domains for PABP, eIF4E, -4A, -3, and Mnk1 are indicated. Numbering refers to amino acids in eIF4GI-a (Byrd et al. 2002). (*B*) eIF4GI immunoblot of lysates from untreated (−), CBV3-infected (CBV3), or 2A^{pro} transfected (2A^{pro}) HeLa cells. (*C*) eIF4GI-b and Ct expressing cell lines were tet-induced for 16 h and subjected to immunoblot using eIF4GI, -eIF4E-, eIF4A-, and PABP antibodies. (*D*) Lysates from eIF4GI-b or Ct expressing cell lines were subjected to cap-sepharose pull down (*left* panel) or immunoprecipitation (IP, *right* panel) using anti-myc antibody or mouse IgG control (data not shown). Cap-pull downs were probed with eIF4GI- and eIF4E antibodies, while IPs were probed with eIF4GI-, and eIF4A antibodies. The asterisk denotes a nonspecific background band.

translation environment. We demonstrated that induction of cap-independent translation initiation is due to the particular structure of eIF4GI fragments rather than absent interactions with other translation initiation factors. Finally, our experiments suggest that eIF4GI fragments stimulate cap-independent translation initiation by direct interaction with certain 5′ UTRs.

RESULTS

Stable cell lines with inducible expression of eIF4GI proteins

Enterovirus infection selectively favors cap-independent initiation (Lee and Sonenberg 1982; Buckley and Ehrenfeld
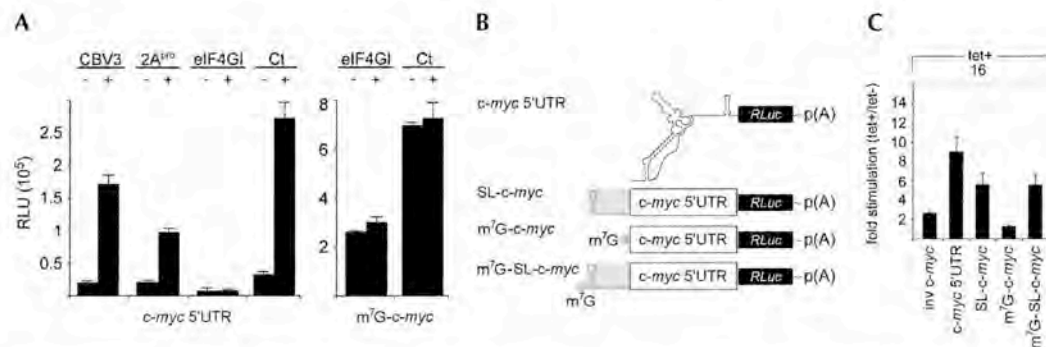
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1987). Despite a global shut-off of host protein synthesis in enterovirus-infected cells, mRNAs capable of cap-independent protein synthesis continue to be translated (Johannes and Sarnow 1998; Johannes et al. 1999). This scenario, thus, serves as a precedent for studies of the mechanisms inducing alternative translation initiation. Protein synthesis modulation by Enteroviruses has chiefly been attributed to $2A^{pro}$-mediated cleavage of eIF4GI and -II (Fig. 1B). Six hours after infection with Coxsackievirus B3 (CBV3) or 12 h after transfection with a $2A^{pro}$ expression RNA into HeLa cells, eIF4GI is efficiently degraded, giving rise to distinct N- and C-terminal cleavage fragments (Fig. 1B, Ct). Incomplete cleavage observed in transfected cells is likely due to transfection efficiency of <100%. The known effects of $2A^{pro}$ on cap-independent translation could be due to (1) diminished intact eIF4GI/-II; (2) distinct activity of fragments produced from intact eIF4G; (3) a combination of both; or (4) unrelated events, e.g., cleavage of other proteins by $2A^{pro}$ (Bovee et al. 1998; Zamora et al. 2002). To distinguish between these possibilities we generated stable HeLa cell lines with tet-inducible expression of myc-tagged eIF4GI-b or the Ct fragment (Fig. 1C). eIF4GI-b is produced by initiation 40 nucleotides (nt) downstream of the AUG for eIF4GI-a and yields more abundant protein than the latter, most likely because the 40 N-terminal amino acids contain 12 prolines (Fig. 1A; Byrd et al. 2002). We specifically investigated the effect of Ct in our approach because its appearance in CBV3-infected cells correlates with stimulation of cap-independent translation at the c-*myc* 5′ UTR, it contains the eIF3 binding site for 43S complex recruitment, and it can substitute for intact eIF4GI in c-*myc* 5′ UTR-mediated cap-independent translation in vitro (Hundsdoerfer et al. 2005).

Tet-induction for 16 h resulted in prominent expression of eIF4GI-b and Ct in the respective stable cell lines (Fig. 1C), although expression of the former was consistently less than Ct (Fig. 1C). Levels of other translation factors, e.g., PABP, eIF4A, and eIF4E, were not affected by ectopic expression of myc-eIF4GI-b or -Ct (Fig. 1C). Similarly, the interactions of exogenous eIF4GI and Ct with other translation initiation factors were intact (Fig. 1D). Coimmunoprecipitation using anti-myc-tag antibody confirmed binding of eIF4E to exogenous full-length eIF4GI-b, but not Ct, reflecting the lack of an eIF4E-binding domain in the latter (Fig. 1A). In contrast, eIF4A coimmunoprecipitated with both exogenous eIF4GI and Ct (Fig. 1D), as expected.

Ct is sufficient to stimulate the c-*myc*/VEGF 5′ UTRs in a cap-independent manner in vivo

To examine the effect of modulating eIF4GI expression or integrity on c-*myc* 5′ UTR-mediated translation in vivo, we conducted reporter RNA transfections in HeLa cells infected with CBV3, cotransfected with $2A^{pro}$ expression RNA, or with overexpression of myc-eIF4GI or myc-Ct (Fig. 2A). Translation of an uncapped c-*myc* 5′ UTR reporter (Fig. 2B) was stimulated significantly with all conditions producing Ct (Fig. 2A). Approximately sixfold induction in CBV3-infected cells exceeded approximately threefold stimulation with $2A^{pro}$, correlating with the extent of eIF4GI cleavage (Fig. 1B). Tet-induced expression of exogenous eIF4GI had no effect on cap-independent translation mediated by the c-*myc* 5′ UTR, but induced Ct expression elevated c-*myc* 5′ UTR-mediated translation substantially (Fig. 2A). Indeed, stimulation by Ct elevated

FIGURE 2. Effect of eIF4G cleavage and inducible eIF4GI-b and Ct expression on c-*myc* 5′ UTR-mediated translation. (*A, left* panel) Uncapped c-*myc* 5′ UTR-driven reporter translation in untreated (−), CBV3-infected (CBV3), $2A^{pro}$ co-transfected ($2A^{pro}$) HeLa cells, or stable tet-inducible eIF4GI-b and Ct expressing cell lines. Cells were infected with CBV3 30 min prior to reporter transfection or reporter RNA was cotransfected with $2A^{pro}$ expression RNA (Dobrikova et al. 2006). Tet induction was initiated 16 h prior to reporter transfection and all cells were lysed 6 h thereafter. (*A, right* panel) Capped c-*myc* 5′ UTR reporter translation in tet-inducible eIF4GI-b and Ct expressing cell lines. (*B*) Structure of c-*myc* 5′ UTR reporters. All constructs contained the c-*myc* 5′ UTR. Gray boxes symbolize a stable stem–loop structure followed by a spacer to block scanning (Thoma et al. 2004; Hundsdoerfer et al. 2005). m^7G indicates the presence of a cap structure. (*C*) Fold stimulation of translation upon tet induction of Ct-expressing cells transfected with the indicated reporter RNAs.

translation levels of uncapped c-*myc* 5′ UTR reporters within range of their capped equivalent (Fig. 2A). Since tet induction produced lower levels of full-length eIF4GI-b than Ct, lacking stimulation of cap-independent translation with the former might be a dose effect. Our findings do not support this possibility, because the eIF4GI-e isoform lacks stimulatory activity despite expression levels similar to Ct in transient transfection experiments (Fig. 5A, below). Supplying exogenous Ct in uninfected cells in the presence of intact native eIF4GI produced enhanced stimulation compared to virus- or 2A^{pro}-mediated eIF4G cleavage, approximately eightfold versus approximately six- and approximately threefold, respectively. Reducing intact eIF4G via CBV3 infection or 2A^{pro} expression in induced Ct-expressing cells did not produce incremental stimulation of c-*myc* 5′ UTR-mediated translation (data not shown), suggesting that Ct expression alone is sufficient for stimulation of cap-independent translation.

A critical factor in studies evaluating alternative translation initiation is the overall efficiency of cap-independent protein synthesis. Cap-independent translation at the c-*myc* 5′ UTR was substantially above background levels in all samples (Fig. 2A). To control for spurious m^{7}GTP-cap-independent initiation, we generated reporters containing the inverted c-*myc* 5′ UTR. Translation of un-capped mRNA containing the inverse c-*myc* 5′ UTR barely exceeded background levels (data not shown) and was not significantly stimulated in tet-induced Ct-expressing cells (Fig. 2C). Translation of mRNAs containing the correct c-*myc* 5′ UTR in Ct-expressing cells exceeded that at the negative control inverse construct 100-fold (data not shown). These observations suggest that stimulation of cap-independent translation by Ct requires the authentic c-*myc* 5′ UTR and is not due to random initiation at uncapped reporters.
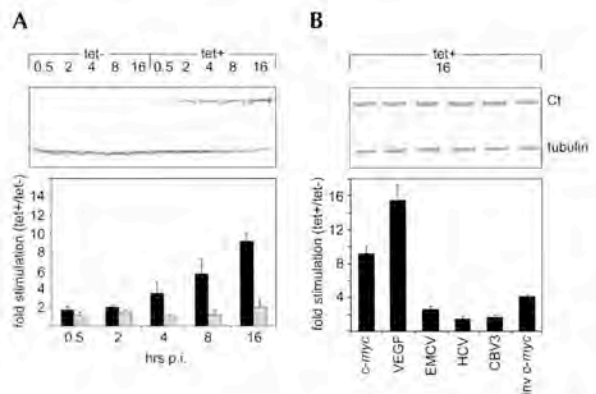
To unambiguously discern cap-dependent from cap-independent initiation, we generated additional reporter constructs. Induction of cap-independent translation initiation is likely to occur when cap-dependent initiation is repressed, for example, with eIF4G cleavage. Therefore, to discourage m^{7}GTP cap-mediated scanning while simultaneously allowing cap-independent translation, we engineered a stable stem–loop (SL) upstream of the c-*myc* 5′ UTR, following previously established approaches (Fig. 2B; Sherrill et al. 2004; Hundsdoerfer et al. 2005). Capped c-*myc* reporter translation was unaffected by Ct expression (Fig. 2A), but SL restored induction of cap-independent translation initiation at the c-*myc* 5′ UTR in the presence of an m^{7}GTP-cap (Fig. 2C). It also diminished the level of stimulation slightly (approximately sixfold versus approximately eightfold), most likely due to unintended effects on the structural arrangement of the c-*myc* 5′ UTR. Importantly, c-*myc* 5′ UTR reporters containing SL with or without an m^{7}GTP-cap were stimulated equally at approximately sixfold by Ct (Fig. 2C). Our data show that Ct-

mediated stimulation of translation initiation mediated by the c-*myc* 5′ UTR is operational under conditions of diminished 5′ UTR scanning.

To examine dose dependency and specificity of Ct-mediated stimulation of cap-independent translation, we compared diverse 5′ UTRs of cellular and viral origin in induced cells over time (Fig. 3). Stimulation of the uncapped c-*myc* 5′ UTR correlated with increasing Ct levels throughout 16 h post-induction (pi) without significant changes in m^{7}GTP-cap-mediated translation at the β-globin leader (Fig. 3A). Comparison of various uncapped reporters containing diverse 5′ UTRs demonstrated that maximum eightfold stimulation of translation at the c-*myc* 5′ UTR was exceeded only by the VEGF 5′ UTR (∼14-fold). Interestingly, the IRESs of encephalomyocarditis virus (EMCV), hepatitis C virus (HCV), or CBV3 were relatively unaffected (approximately twofold), as were reporters translating under control of the uncapped inverted c-*myc* 5′ UTR (Fig. 3B).

Effect of eIF4G interaction with PABP and eIF4E on cap-independent translation

Why does Ct but not full-length eIF4GI stimulate cap-independent translation via the c-*myc*/VEGF 5′ UTRs? Ct's stimulatory effect could be due to loss of interaction with PABP and eIF4E (Fig. 1A). We tested this possibility by transiently expressing myc-tagged eIF4GI-e (naturally lacking the PABP binding motif) (Byrd et al. 2002), an
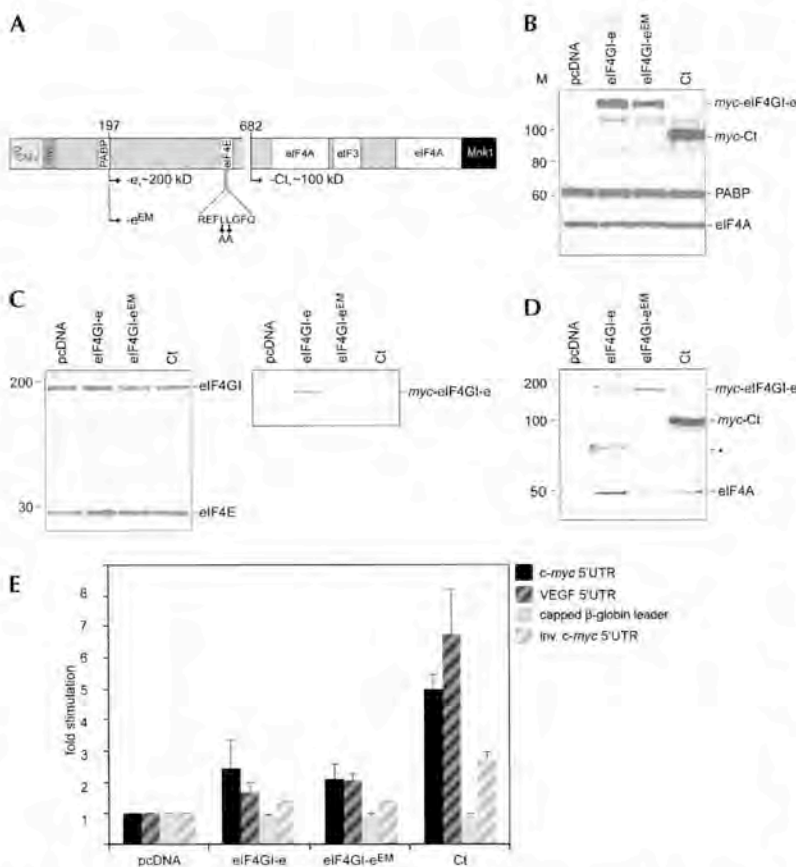
FIGURE 3. Dose dependency and specificity of Ct-mediated IRES stimulation. (*A*) Ct cells were tet-induced from 30 min up to 16 h. At given intervals, uncapped c-*myc* 5′ UTR (black bars) or m^{7}GTP-capped β-globin leader (gray bars) reporter RNAs were transfected and cells were lysed 6 h thereafter. The lysates were tested by immunoblot with myc-tag or tubulin antibodies (*top* panel). (*B*) Ct cells were tet-induced for 16 h and transfected with uncapped reporter RNAs under the control of the indicated sequences: the c-*myc*- or VEGF- 5′ UTRs; the EMCV-, HCV-, or CBV3 IRESs; the inverted c-*myc* 5′ UTR. RLuc activity was measured 6 h after transfection and lysates were tested by immunoblot with myc-tag and tubulin antibodies (*top* panel).

eIF4I-e variant with mutations in the eIF4E binding motif (eIF4I-e^{EM}) unable to bind to PABP and eIF4E (Mader et al. 1995), and Ct (Fig. 4A). Transient transfections of HeLa cells yielded approximately even levels of all eIF4GI variants (Fig. 4B).

We verified endogenous translation initiation factor interactions with exogenous eIF4GI variants using m^7-GTP cap-sepharose pull-down (Fig. 4C) or IP assays (Fig. 4D) from transfected cell lysates. As predicted, cap-sepharose pull-down revealed that endogenous eIF4GI and myc-eIF4GI-e interact with cap-eIF4E, while myc-eIF4GI-e^{EM} and myc-Ct failed to do so (Fig. 4C). Moreover, IP with myc-tag antibody confirmed eIF4A interaction with all

eIF4G variants (Fig. 4D). Next, we tested the effect of our eIF4GI variants on the translation efficiency of transfected reporter RNAs (Fig. 4E). None of the variants affected the capped β-globin leader reporter, and the inverted *c-myc* 5' UTR was only moderately stimulated by Ct (Fig. 4E). Similarly, eIF4GI-e/e^{EM} stimulated translation via the uncapped *c-myc*/VEGF 5' UTRs only minimally (approximately two-fold), while Ct significantly induced cap-independent translation (approximately sixfold) (Fig. 4E). Transient Ct transfection consistently yielded lower stimulation of the *c-myc*/VEGF 5' UTRs than stable inducible expression (approximately five- to eightfold versus approximately eight- to 14-fold, respectively).

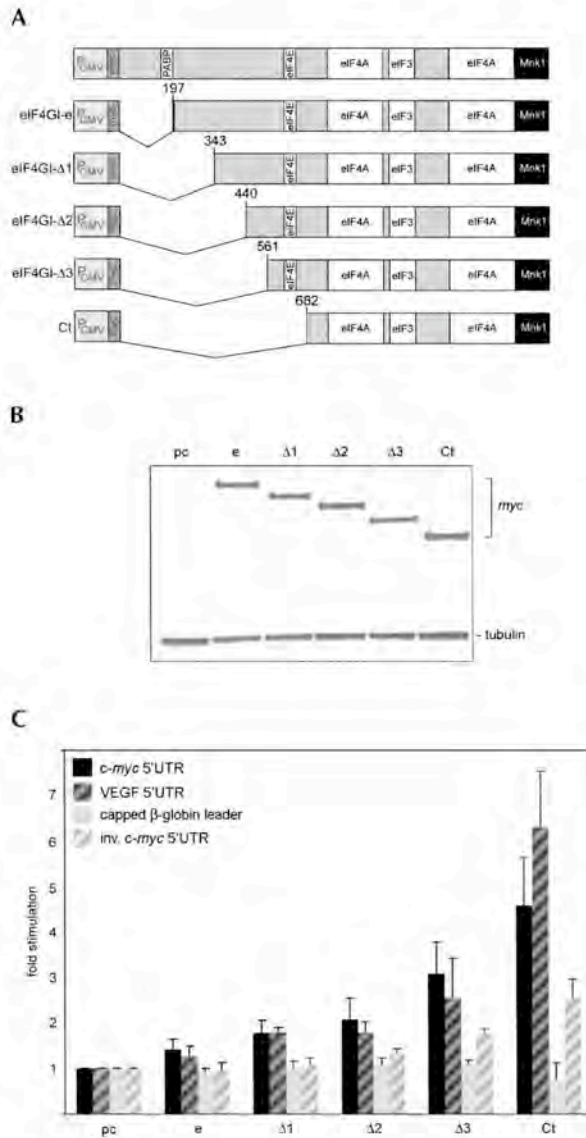## Role of the N terminus of eIF4G in cap-independent translation initiation

Since abolishing interaction with PABP or eIF4E itself did not convey stimulatory activity to eIF4GI-e, we evaluated if the 485 amino acids upstream of the 2A^{pro} cleavage site contain inhibitory activity. We generated three staggered N-terminal deletion constructs (Fig. 5A) for transient expression in HeLa cells (Fig. 5B). Stimulation of *c-myc*/VEGF 5' UTR-mediated translation increased with successive deletions. However, even eIF4GI-Δ3 (N-terminally extending Ct by 121 amino acids) only produced ~50% of the stimulation observed with Ct (Fig. 5C). Inverted *c-myc* 5' UTR-, and β-globin-leader-driven reporters were minimally affected (Fig. 5C).

Ct preferentially interacts with the c-myc and VEGF 5' UTRs

Since eIF4GI is an RNA-binding protein (Goyer et al. 1993) capable of recruiting the 43S preinitiation complex on its own, we speculated that Ct may induce cap-independent translation at the *c-myc* 5' UTR through direct association. All regions of eIF4G implicated in RNA binding are contained within Ct (Lomakin et al. 2000). First, to examine binding of Ct to specific RNAs in vivo, tet-induced, myc-Ct-expressing cells were transfected with the indicated reporter RNAs (Fig. 6A). Cell lysates generated from transfected cells were subjected to IP with myc-tag antibody

FIGURE 4. Effect on translation of eIF4GI-e and -4GI-e variants deficient in binding eIF4E. (A) Structure of eIF4GI-e and -4GI-e^{EM} (mutant amino acids in the eIF4E binding domain are indicated by arrows). (B) Hela cells were transfected with pcDNA, myc-eIF4GI-e, -4GI-e^{EM}, or Ct and lysates analyzed by immunoblot for exogenous myc-tagged proteins, PABP, and eIF4A. (C) m^{7}GTP-cap sepharose binding assays of lysates from B. Lysates were incubated with cap-sepharose, and bound proteins were eluted and probed with eIF4E, eIF4GI, or myc-tag antibodies. Endogenous eIF4E/eIF4G are bound by m^{7}GTP-cap sepharose in all samples. Only wild-type exogenous myc-eIF4G-e associates with the cap. (D) Co-IP assays of lysates from B. IP with myc-tag antibody reveals eIF4A binding to exogenous eIF4G variants in all samples. The asterisk indicates a nonspecific background band. (E) HeLa cells were transfected with *c-myc* (black bars) and VEGF (black hatched bars) IRES reporters or capped β-globin (gray bars) or uncapped inverse *c-myc* IRES (gray hatched bars) reporter constructs and lysed 6 h thereafter.

dogenous c-*myc* mRNA can bind to inducible Ct. Quantitative RT-PCR of the c-*myc* transcript and a GAPDH control revealed a sixfold enrichment of c-*myc* vs. GAPDH template in *myc* antibody IP (data not shown).

To further investigate Ct:RNA interactions, we examined binding of recombinant purified GST-tagged Ct to the c-*myc* 5' UTR biochemically by electrophoretic mobility shift assay (Fig. 6B–D, EMSA). Purified recombinant GST-Ct
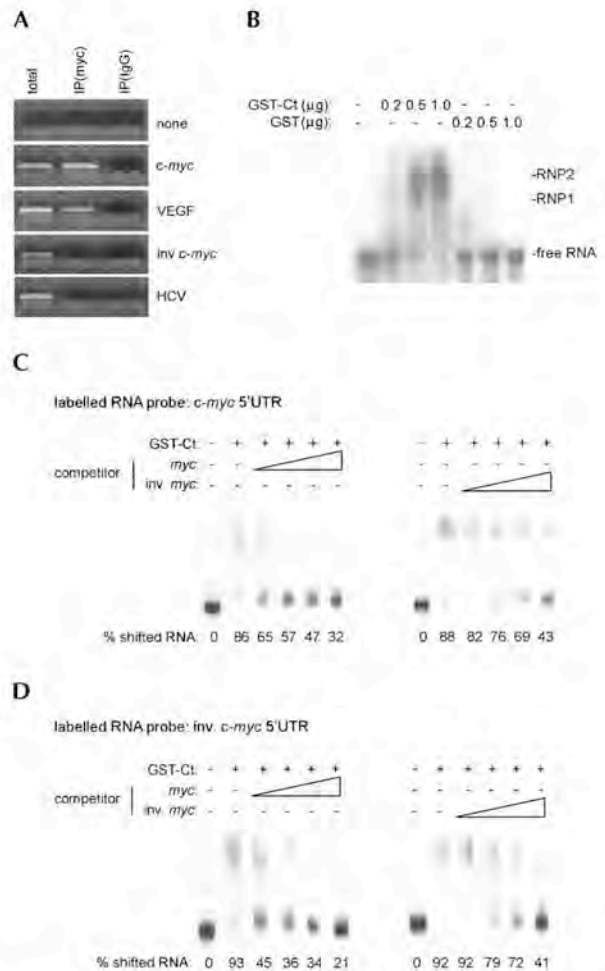
FIGURE 5. Effect on cap-independent translation of N-terminal eIF4GI deletion variants. (*A*) Structure of eIF4GI deletion variants. (*B*) Lysates from *C* were probed with myc-tag and tubulin antibodies. (*C*) Twenty-four hours after transfection with pcDNA, eIF4GI-e, eIF4GI-Δ1-3, or Ct expression plasmids, HeLa cells were transfected with uncapped c-*myc* (black bars) or VEGF (black hatched bars) IRES reporters or capped β-globin (gray bars) or uncapped inverse c-*myc* IRES (gray hatched bars) reporter constructs and lysed 6 h thereafter.

or preimmune serum. The immunoprecipitate was processed to isolate associated RNAs (see Materials and Methods). RT-PCR of RNA coimmunoprecipitated with *myc* antibody readily amplified a portion of the *RLuc* ORF from cells transfected with c-*myc* and VEGF 5' UTR reporters, but not the inverted c-*myc* 5' UTR construct or the HCV IRES (Fig. 6A). We also determined if en-

FIGURE 6. Interaction between Ct and the c-*myc* 5' UTR. (*A*) RT-PCR of RNA co-IPed with myc-tag antibody. RT-PCR from total cellular RNA, myc-tag IP, or isotype controlled nonspecific mouse IgG from lysed tet-induced Ct-expressing cells previously transfected with the indicated reporters. (*B*) Effect of GST-Ct or GST alone on radioactively labeled c-*myc* 5' UTR RNA in EMSA. Twenty nanograms of labeled c-*myc* 5' UTR (10^5 cpm) were incubated with varying amounts of GST-Ct or GST as indicated. (*C*) Effect of unlabeled competitors on the interaction between GST-Ct and labeled c-*myc* 5' UTR RNA and (*D*) inverse c-*myc* 5' UTR in EMSA. Twenty nanograms of labeled RNA were incubated with GST-Ct and increasing amounts of nonradioactive competitor RNA as indicated. Ratios of competitor RNA were 1:1, 1:2.5, 1:5, and 1:10.

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stimulated c-*myc* 5′ UTR-mediated translation 4.5-fold in HeLa in vitro translation extracts (data not shown). This confirmed the functionality of the purified GST-fusion protein. Then, we tested GST-Ct:c-*myc* 5′ UTR interactions by EMSA. GST-Ct, but not GST alone, formed ribonucleoprotein (RNP) complexes with the c-*myc* 5′ UTR, indicating that the GST-tag does not materially alter Ct's RNA-binding capacity (Fig. 6B).

To investigate the basis for Ct's selective binding to certain 5′ UTRs in vivo (Fig. 6A), we conducted competition assays comparing the authentic c-*myc* 5′ UTR with its inverse counterpart. As expected, RNP complex formation at the c-*myc* 5′ UTR was abolished by adding increasing amounts of unlabeled c-*myc* competitor (Fig. 6C, left panel). In contrast, unlabeled inverse c-*myc* competitor displayed inferior Ct binding, since higher concentrations were needed to compete for binding of the c-*myc* 5′ UTR to Ct (Fig. 6C, right panel). Conversely, although Ct shifted the inverse c-*myc* 5′ UTR, indicating the presence of general RNA-binding activity, RNP complex formation was substantially reduced even with the lowest concentration of unlabeled c-*myc* 5′ UTR competitor tested (Fig. 6D, left panel). Moreover, competition was more pronounced at all competitor RNA concentrations tested, when compared to the authentic c-*myc* 5′ UTR (Fig. 6, cf. C and D, left panels). Competition for binding to Ct of inverse c-*myc* 5′ UTR with unlabeled inverse competitor resembled the parallel experiment with the authentic c-*myc* 5′ UTR (Fig. 6, cf. C and D, right panels). These results suggest that Ct's capacity for interaction with a given 5′ UTR determines its effects on translation.

Ct mutants lacking RNA-binding activity fail to induce cap-independent translation

If Ct exerts stimulatory activity on cap-independent translation via direct association with the c-*myc* or VEGF 5′ UTRs, abolishing this binding should prevent translation stimulation. Although intrinsic RNA-binding ability of eIF4G has been reported (Goyer et al. 1993), the structural basis for this is not understood. With this in mind, we based our approach on previous speculation about a "classic" RNA recognition motif (RRM; Kenan et al. 1991; Burd and Dreyfuss 1994) in eIF4G. The two putative components of this motif have been altered before to study their influence on eIF4G binding to the EMCV IRES (Lomakin et al. 2000).

We reengineered these mutations into Ct (Fig. 7A), to investigate their effect on RNA binding and c-*myc*/VEGF 5′ UTR *trans*-activation. Since both Mut1 and -2 are situated within the binding site for eIF4A and close to the proposed footprint for eIF3 (Fig. 7A), we tested translation factor interactions with the mutant proteins (Fig. 7B). All Ct variants were expressed at even levels after transfection of cDNA expression plasmids. IP with myc-tag antibody from

lysates of transfected cells revealed efficient eIF4A binding to all Ct forms; curiously, Mut1 exhibited significantly less binding to eIF3 than Ct or Mut2 in this assay. It has been observed before that mutations in the proximal eIF4A binding site can affect eIF3 binding as well (Imataka and Sonenberg 1997).
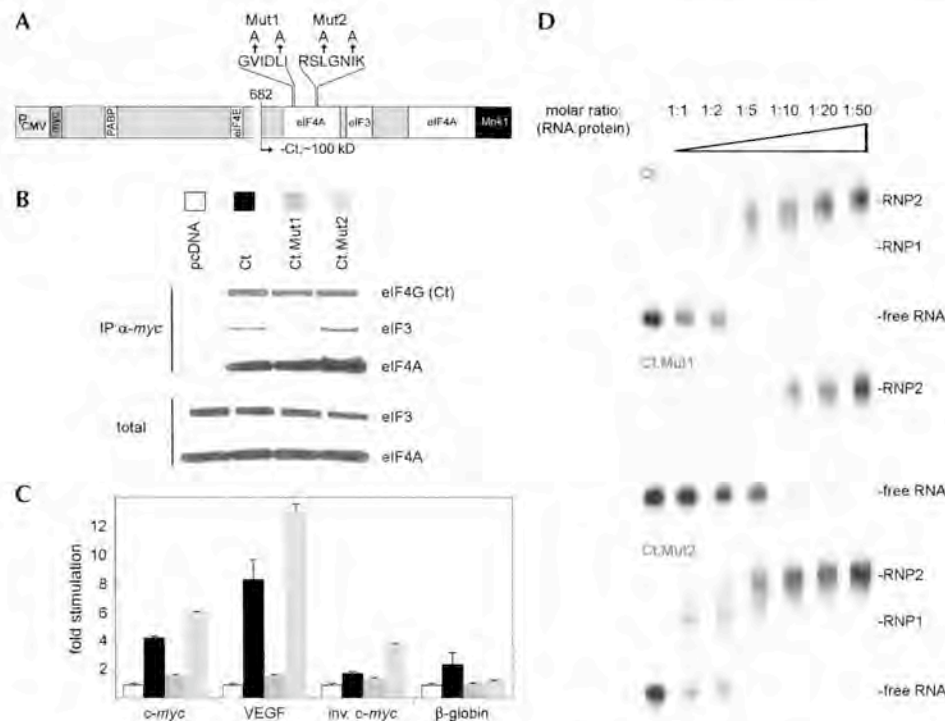
Transient expression of both Ct variants had opposing effects on cap-independent translation at the c-*myc*/VEGF 5′ UTRs. While Mut1 abolished stimulation altogether, Mut2 yielded significantly enhanced stimulation compared to parental Ct (Fig. 7C). There were marginal effects on the inverse c-*myc* 5′ UTR and capped β-globin reporters with Ct or Mut2. We generated recombinant GST-tagged Mut1 and Mut2 proteins to perform EMSAs. These studies revealed that, generally, stimulation of cap-independent translation via the c-*myc* 5′ UTR correlated with the ability of recombinant Ct variants to interact with RNA in vitro (Fig. 7D). Considerably higher concentrations (two- to fourfold) of Mut1 versus Ct protein were needed to generate the characteristic RNP complex observed upon interaction of Ct with the c-*myc* 5′ UTR (Fig. 7D). Notably, Mut2 formed complexes with the c-*myc* 5′ UTR more readily than either Mut1 or Ct (Fig. 7D). Mut1 displayed altered eIF3 binding activity, while both mutants showed differences in RNA affinity. These results further suggest a correlation between the intrinsic RNA-binding properties of Ct and its ability to stimulate translation initiation.

DISCUSSION

We show that an eIF4GI C-terminal fragment stimulates cap-independent translation initiation at the 5′ UTRs of c-*myc* and VEGF in vivo. Our data expand previous findings in eIF4GI-depleted HeLa cell extracts in vitro, which demonstrated that c-*myc* IRES-driven translation can occur independent of full-length eIF4GI and that Ct can functionally replace eIF4GI for translation initiation at the c-*myc* IRES, but not the m^{7}GTP-cap (Thoma et al. 2004; Hundsdoerfer et al. 2005). Our observations indicate that stimulation of cap-independent translation at the c-*myc* and VEGF 5′ UTRs is due to direct association with Ct, which occurred despite the presence of endogenous intact eIF4GI. Ct, because it contains all parts of eIF4G implicated in RNA-binding and the recognition motif for eIF3, retains the ability to recruit the 43S preinitiation complex. Translation stimulation at the c-*myc* 5′ UTR by Ct variants carrying mutations in putative RNA-binding motifs of eIF4G co-varied with the ability of recombinant protein to associate with this RNA element in vitro. This suggests that the level of stimulation of cap-independent translation at individual mRNAs correlates with the relative ability of Ct to associate with their 5′ UTR. Interestingly, Mut1, which was deficient in binding to the c-*myc* 5′ UTR (and, hence, devoid of stimulatory activity in vivo), also displayed reduced recruitment of eIF3. This suggests that

FIGURE 7. A Ct mutant with reduced RNA binding capacity fails to stimulate cap-independent translation. (*A*) Structure of eIF4G, Ct, and the location of two separate elements of a putative RNA recognition motif (Lomakin et al. 2000). Altered amino acids in Mut1 and -2 are indicated by arrows. (*B*) Co-IP of translation factors with myc-Ct or its variants. Immunoblots of eIF4A, eIF3a, and eIF4G from lysates transfected with the indicated expression plasmids. (*C*) Stimulation of the indicated 5′ UTRs after transfection of RNA reporters into cells expressing the diverse Ct variants (bars are labeled according to *B*). (*D*) EMSA of labeled *c-myc* 5′ UTR with recombinant GST-Ct or its variants as indicated.

RNA-binding and eIF3-association functions of eIF4G may overlap.

Interestingly, the effect of Ct expression on the *c-myc*/VEGF 5′ UTRs did not extend to the CBV3 IRES. This confirms earlier findings which show that CBV3 IRES stimulation in infected cells occurs independent of eIF4GI cleavage (Roberts et al. 1998; Dobrikova et al. 2006). This may appear counterintuitive since Ct is a by-product of CBV3 infection. Our observations are discordant with reports of 2A^{pro}-mediated stimulation of viral IRES-mediated translation in vitro (Liebig et al. 1993; Ziegler et al. 1995). However, similar in vitro translation studies yielded contradictory results regarding the magnitude and specificity of the effect of eIF4G cleavage on viral IRES-mediated translation (Liebig et al. 1993; Ziegler et al. 1995; Rifo et al. 2007). We attribute this variance to inconsistent empirical systems, variable structure of reporter constructs, and inherent differences of in vivo vs. in vitro assays. Moreover, we previously reported that correctly configured enteroviral IRES reporters translate as efficiently as capped conventional mRNAs in vivo in the absence of viral alterations of the host cell translation machinery (Dobrikova et al. 2006).

Lastly, CBV3 IRES-mediated translation is responsive to PABP (Bradrick et al. 2007), while translation at the *c-myc* IRES is not (Thoma et al. 2004), indicating divergent initiation factor involvement. We consistently observed more pronounced effects of Ct on the VEGF 5′ UTR than *c-myc*, suggesting that even among cellular 5′ UTRs, the response to eIF4GI variants may vary considerably.

What determines Ct's ability to induce cap-independent translation at specific mRNAs? IRESs are determined by their function, not their structure (Wimmer et al. 1993). Therefore, it is impossible to predict a capacity for cap-independent translation initiation a priori. Our findings imply that Ct may exhibit preferential interaction with certain 5′ UTRs in vivo. It will be interesting to decipher whether distinct subclasses of mRNAs can be defined by their capacity for direct interaction with eIF4G variants. If so, cap-independent translation of mRNA subsets may be controlled by the specific conditions regulating eIF4G. For example, in agreement with our findings, increased selective translation of IRES-containing mRNAs by eIF4G overexpression during hypoxia in breast cancer cells has been recently reported (Braunstein et al. 2007).

Interestingly, Ct-mediated stimulation of m^{7}GTP-capped c-*myc* reporter RNAs was contingent upon discouraging cap-dependent scanning by a stable stem–loop structure inserted upstream of the c-*myc* 5′ UTR. In agreement with previously published work on alternative translation initiation at eukaryotic mRNAs (Sherrill et al. 2004; Hundsdoerfer et al. 2005), our data show that m^{7}GTP-cap-independent initiation may be operational when conventional cap-dependent translation initiation is hindered.

Since all functional domains of Ct are present in eIF4G, the intact protein could in principle exert similar regulatory properties. For example, it is conceivable that loss of eIF4E interaction frees eIF4G from assembly at the m^{7}GTP-cap, thus increasing the amount of eIF4G available for cap-independent recruitment of ribosomes. However, our studies do not support this hypothesis because eIF4GI-e^{EM}, incapable of both PABP and eIF4E interaction, failed to stimulate cap-independent translation initiation. Alternatively, the N terminus might modulate functions of C-terminal eIF4G through integration of signaling stimuli. For example, binding of p21 activated protein kinase 2 (PAK2) to N-terminal eIF4G produces phosphorylation of Ser-896, which modulates its ability to support cap-dependent translation (Ling et al. 2005). Since successive N-terminal deletions of eIF4GI-e produced incremental c-*myc*/VEGF 5′ UTR stimulation, we speculate that Ct's unique properties may be due to altered structural arrangements, which may modify its inherent RNA-binding capacity (De Gregorio et al. 1998; Marcotrigiano et al. 2001).

Ct shares ~28% homology with p97/Dap5. Accordingly, recent reports have shown that p97 stimulates c-*myc* IRES activity in vitro (Hundsdoerfer et al. 2005), enhances cap-independent translation of pro-survival factors during mitosis (Marash et al. 2008), and modulates alternative translation initiation during endoplasmic reticulum stress (Lewis et al. 2008). Thus, p97 and Ct may exert similar effects on cap-independent translation of c-*myc* or VEGF mRNA in vivo.

Intuitively, the structural heterogeneity of 5′ UTRs suggests that the prevailing conditions for translation initiation in the cell do not equally apply to the entire transcript pool. Indeed, our studies show how a variant translation initiation factor selectively favors translation of a certain class of messages. While our studies focus on the c-*myc*/VEGF transcripts, two exemplar mRNAs known for their capacity for cap-independent initiation, this ability may be far more prevalent than currently recognized. Also, it is conceivable that 5′ UTRs that fail common tests for cap-independent initiation competence in vitro, e.g., translation of dicistronic reporters in translation extracts, are stimulated by Ct in vivo. We are currently evaluating these hypotheses. Cap-independent translation may be induced upon eIF4G degradation in tumor cells exposed to acute stress, e.g., ionizing radiation or metabolic crisis, pro-

moting tumor cell survival and treatment resistance. It is also possible that aberrantly generated eIF4G variants may lead to translational deregulation of specific messages in an otherwise intact translation environment.

MATERIALS AND METHODS

Stable cell lines and viruses

pcDNA5/TO/FRT/*myc*-eIF4GI-b and pcDNA5/TO/FRT/*myc*-Ct were constructed by subcloning NheI–NotI fragments of *myc*-tagged eIF4GI-b and Ct from corresponding pcDNA3.1 expression vectors (Dobrikova et al. 2006). A pcDNA5/TO/FRT/*luc* positive control vector was produced by subcloning an XhoI–NotI *Renilla Luciferase* (RLuc) fragment from pTNT (Dobrikova et al. 2006). Stable, inducible cell lines were established using the Flp-In T-Rex System (Invitrogen) according to the manufacturer's instructions. Briefly, HeLa R19 cells were transfected with ScaI-digested pFRT/lacZeo, and zeocin-resistant clones were screened by Southern blot for integrants containing a single FRT target site. Selected clones were transfected with BstZ17I-digested pcDNA6/TR, the tetracycline (tet) repressor expression vector. Individual zeocin/blasticidin-resistant clones were tested for tet-inducible RLuc expression after pcDNA5/TO/FRT/Luc transfection. We selected clones exhibiting the lowest basal levels:highest after-induction ratio for Flp-In T-Rex host cell lines. Finally, inducible eIF4GI-b or Ct cell lines were generated by transfecting host cell lines with corresponding vectors followed by blasticidin/hygromycinB selection. Propagation and use of CBV3 and construction of CBV3 2A^{pro} expression vectors are described elsewhere (Dobrikova et al. 2006).

RLuc reporter vectors, in vitro transription, RNA transfection, and *RLuc* assays

All reporter plasmids were generated by cloning diverse 5′ UTR sequences into the pSVN vector (Invitrogen) described before (Dobrikova et al. 2006), except c-*myc*/inverse c-*myc* 5′ UTR reporters, which were cloned into a pUC19 (NEB) backbone. Cloning cassettes consisted of the *RLuc* open reading frame (ORF) and an encoded 50-mer poly(A) tail; the T7 promoter was included in the forward 5′ UTR primer. The c-*myc* 5′ UTR was obtained by RT-PCR from HeLa cell total RNA with primers 1 and 2 (Table 1). We used a 5′ UTR segment corresponding to the P2 transcript, which constitutes ~75%–90% of the c-*myc* message (Stewart et al. 1984) and exhibits IRES activity (Le Quesne et al. 2001; Cencig et al. 2004; Thoma et al. 2004). The PCR-generated c-*myc* 5′ UTR fragment, or its inverse generated with primers 3 and 4, was inserted into the pUC19 cassette digested with BamHI/SfuI. To categorically exclude 5′ end-dependent translation at m^{7}GTP-capped or uncapped c-*myc* 5′ UTR reporters, we followed a strategy described previously (Thoma et al. 2004; Hundsdoerfer et al. 2005). Briefly, a predicted stem–loop structure was introduced upstream of the c-*myc* 5′ UTR by inserting synthetic oligonucleotides 5–6 into the EcoRI-KpnI sites of the pUC19 cassette. A c-*myc* 5′ UTR fragment lacking the T7 promoter was amplified with primers 7 and 2 and cloned into KpnI-SfuI sites of the stem–loop-containing reporter construct. To minimize interference of the stem–loop with cap-independent initiation at

TABLE 1. Primers used in this study

Number	Primer name	Sequence
1	5′ c-myc 5′ UTR	gcggatcctattacgactcactataggctcgctgtagtaattccagcgagaggcagagg
2	3′ c-myc 5′ UTR	ctttcgaagtcatcgtcgcgggagggtttccactacccg
3	5′ inverse c-myc 5′ UTR	gcggatcctaatacgactcactataggcgtcgcgggagggtttc
4	3′ inverse c-myc 5′ UTR	gctttcgaagtcatctcgctgtagtaattccagcgag
5	5′ Stemloop	aattctaatacgactcagtatagccccgagcgcccagatctgggcgctccggggtac
6	3′ Stemloop	cgtaccccggagcgcccagatctgggcgctccgggctatactgagtcgtattag
7	5′ myc-stemloop	ggggtaccctcgctgtagtaattccagcgagagg
8	5′ globin ORF	aaggtaccgcctggctcacctggacaacctcaagg
9	3′ globin ORF	aaggtacctagccacaccagccaccacttctgatag
10	5′ VEGF 5′ UTR	gcgcggccgctaatacgactcactatagggcttggggcagccgggtagctcggagg
11	3′ VEGF 5′ UTR	ctttcgaagtcatggtttcggaggcccgac
12	5′ eIF4GI-e^EM inner	cgttacgaccgtgagttcgcggccggttt
13	3′ eIF4GI-e^EM inner	caaagatgaactgaaaaccggccgcgaact
14	5′ eIF4GI-e^EM outer	ggatgccttcaaggaggcg
15	3′ eIF4GI-e^EM outer	cggcccacgggcagctc
16	5′ eIF4GI-delta1	ggaagcttagtcctctccaggctcccacc
17	5′ eIF4GI-delta2	ggaagcttgctactccagctacggctcc
18	5′ eIF4GI-delta3	cggcccacgggcagctc
19	5′ Ct.Mut1 inner	gggccattgacgccattttgagaaggc
20	3′ Ct.Mut1 inner	caaaaatggcgtcaatggcccctttgaggcg
21	5′ Ct.Mut2 inner	cgctctgcagggaatgccaagtttattgg
22	3′ Ct.Mut2 inner	ccaataaacttggcattccctgcagagcg
23	5′ Ct.outer	ttaagcttgggcccccaaggggtgg
24	3′ Ct.outer	cagcggccgctcagttgtggtcagactcc
25	5′ RLuc	cagtggtgggccagatgtaaacaa
26	3′ RLuc	taatacaccgcgctactggctcaa
27	5′ c-myc qPCR	acagcatacatcctgtccgtccaa
28	3′ c-myc qPCR	tgttctcgtcgtttccgcaacaag
29	5′ GAPDH qPCR	catgttcgtcatgggtgtgaacca
30	3′ GAPDH qPCR	agtgatggcatggactgtggtcat

the c-*myc* 5′ UTR, a 200-nt segment amplified from the human β-globin ORF (using primers 8, 9) was inserted into a KpnI site to separate the stem–loop from the IRES element as described previously (Thoma et al. 2004; Hundsdoerfer et al. 2005). The VEGF 5′ UTR was PCR-amplified using primers 10 and 11 from BAC RP11 (clone 710L16; Children's Hospital and Research Center, Oakland, CA) and cloned into the pSVN cassette digested with NotI/SfuI. The reporter constructs containing the CBV3 IRES and the β-globin leader (Dobríkova et al. 2006) as well as the EMCV and HCV IRESs (Bradrick et al. 2007) have been described elsewhere.

Reporter plasmids were linearized and used for in vitro transcription with T7 RNA polymerase (Ambion) to produce RNAs. Capped reporter RNAs were generated by adding m7G(5′)ppp(5′)G RNA cap structure analog (NEB) to in vitro transcription reactions as specified by the manufacturer. RNAs were purified by RNeasy (Quiagen), inspected for quality by agarose gel electrophoresis, and quantified by spectrometric analysis. In vivo *Rluc* expression assays were performed as described before (Bradrick et al. 2006; Dobríkova et al. 2006). Briefly, HeLa monolayers were transfected with equimolar amounts of reporter RNA using DMRIE-C reagent (Invitrogen). Six hours post-transfection, the cells were lysed with *Rluc* assay lysis buffer (Promega) and *Rluc* activity was assayed in a Berthold

LB9507 luminometer. At least three independent transfection experiments were carried out, and the data shown represent the average values and standard deviation.

Cloning of eIF4GI mutants and deletion variants, transient exogenous eIF4GI expression, and immunoblot

Myc-tagged eIF4GI-e was generated as described before (Dobríkova et al. 2006). The eIF4E binding site mutation in eIF4GI-e^EM was created by substituting Leu-617/Ala and Leu-618/Ala (Mader et al. 1995) by overlapping PCR using primers 12–15. The PCR product was inserted into the vector via PflMI/SbfI restriction sites. eIF4GI deletions, eIF4GI-Δ1-3, were generated by PCR with forward primers 16–18, respectively, and reverse primer 15. The PCR products were inserted into the vector cassette using PflMI and HindIII. Mutations in putative RNA-binding domains of Ct were introduced by overlapping PCR. Ct Mut1 (Val-797/Ala; Leu-800/Ala) and Mut2 (Leu-857/Ala; Ile-860/Ala) were generated using primers 19–20 and 23–24 or primers 21–22 and 23–24, respectively. The PCR products were inserted into the vector using HindIII/NotI. HeLa cells were transfected with the myc-eIF4GI-e, eIF4GI-e^EM, eIF4GI-Δ1-3, Ct Mut1/2, or wild-type Ct expression constructs or with pcDNA3.1 vector (1 μg per 60-mm dish) using

Lipofectamine 2000 (Invitrogen). Twenty-four hours after DNA transfection, cells were washed with serum-free medium, transfected with reporter RNA, and subjected to *RLuc* assay and analyzed by immunoblot. Myc-tag antibody (9E10; Sigma), biotinylated anti-mouse IgG (Vector Lab), streptavidin-peroxidase conjugate (Roche), and ECL Western blotting detection reagents (GE Healthcare) were used to detect exogenous eIF4GI.

Immunoprecipitation (IP), m^{7}GTP pull down and translation factor immunoblots

IP was carried out as described previously (Keene et al. 2006). Briefly, cells were scraped in PBS, pelleted by centrifugation, and resuspended in polysome lysis buffer (100 mM KCl, 5 mM MgCl$_2$, 10 mM HEPES at pH 7.4, 0.5% NP40, 2 mM DTT). Lysis was enhanced by rapid freezing in dry ice:ethanol, and cytoplasmic extract was collected by centrifugation. Prior to precipitation, protein G-sepharose beads (GE Healthcare) were washed in NT2 buffer (50 mM Tris at pH 7.4, 150 mM NaCl, 1 mM MgCl$_2$, 0.05% NP40) and coupled to myc-tag antibody or mouse IgG for 2 h at 4°C. Immuno-complexes were precipitated by incubating cell lysate with beads for 2 h at 4°C. The bound proteins were eluted in LDS buffer (Invitrogen), resolved by SDS-PAGE, and analyzed by immunoblot. The bound RNA was extracted with Trizol LS and stored at −80°C. m^{7}GTP pull downs were carried out with m^{7}GTP-sepharose beads (GE Healthcare) using the procedures described for IP. Cell lysates were resolved by SDS gel electrophoresis in precast 4%–12% Bis-Tris NuPAGE gels (Invitrogen) and transferred to PROTRAN nitrocellulose membrane (Whatman). Membranes were incubated with monoclonal antibodies against eIF4A and -4E (#31217 and #1126; Abcam), eIF3a (#2538, Cell Signaling), c-*myc* and tubulin (#9E10 and #T5168; Sigma), PABP (kindly provided by L. Penalva, University of Texas Health Science Center) or rabbit polyclonal eIF4GI antibody D279 raised against amino acids 1179–1196 of eIF4GI (Dobrikova et al. 2006).

Reverse transcription and quantitative PCR

Total cellular RNA or RNA coimmunoprecipitated with *myc* or nonspecific mouse IgG from tet-induced Ct-expressing cells was isolated using Trizol (Invitrogen) following the manufacturer's instructions. Reverse transcription was carried out with 500 ng of RNA using AMV-RT (Promega) followed by PCR with primers 25 and 26, to detect *Rluc* reporter RNA, or quantitative PCR with Platinum Sybr Green qPCR SuperMix-UDG (Invitrogen) using primers 27–28 and 29–30 to detect c-*myc* and GAPDH mRNA, respectively, in a Roche Light Cycler.

Recombinant proteins and EMSA

Ct or its variants were expressed as glutathione-S-transferase (GST) fusion proteins in BL21 bacteria. For this purpose, Ct was cloned into pGEX-4T1 (Amersham) using XmaI/HinDIII and NotI yielding pGEX-Ct. *Escherichia coli* BL21 cells transformed with pGEX-Ct or the vector backbone were induced with IPTG (0.1 mM) and cultured for 3 h. Cells were lyzed according to the manufacturer's instructions. Proteins were purified using a GSTrapFF column (Amersham) and dialyzed against hypotonic buffer (10 mM HEPES at pH 7.5, 10 mM KoAC, 0.5 mM MgOAc, 1 mM DTT, 5% glycerol). Recombinant proteins were used in EMSA as follows. Essentially, EMSAs were performed as described before (Gamarnik and Andino 1997). [α^{32}P]-UTP-labeled RNA probes were generated from c-*myc* or inverse c-*myc* 5′ UTR templates by in vitro transcription using T7 polymerase (Ambion). Unlabeled competitors were generated by in vitro transcription in the absence of [α^{32}P-UTP]. The binding reactions were carried out in binding buffer (40 mM KCl, 5 mM HEPES at pH 7.5, 2 mM MgCl$_2$, 4% glycerol, 2 mM DTT) with 20 ng [^{32}P]-labeled RNA in 20 μL final volume. The mix was incubated for 10 min at room temperature and analyzed by electrophoresis in 4% native polyacrylamide gels. Gels were prerun for 30 min at 4°C at 100 mV, then 15 μL of samples were loaded and electrophoresis was allowed to proceed for 2 h 20 min at constant voltage. Recombinant Gst-Ct, Gst alone, or RNA competitors were included in the preincubation reaction as described in each case. The gels were dried and visualized by autoradiography. The proportion of free RNA in each lane was determined by PhosphorImager quantification to calculate the percentage of shifted RNA in RNPs.

REFERENCES

Adhikary, S. and Eilers, M. 2005. Transcriptional regulation and transformation by Myc proteins. *Nat. Rev. Mol. Cell Biol.* **6:** 635–645.

Bader, A.G. and Vogt, P.K. 2004. An essential role for protein synthesis in oncogenic cellular transformation. *Oncogene* **23:** 3145–3150.

Bovee, M.L., Marissen, W.E., Zamora, M., and Lloyd, R.E. 1998. The predominant eIF4G-specific cleavage activity in poliovirus-infected HeLa cells is distinct from 2A protease. *Virology* **245:** 229–240.

Bradrick, S.S., Walters, R.W., and Gromeier, M. 2006. The hepatitis C virus 3′-untranslated region or a poly(A) tract promote efficient translation subsequent to the initiation phase. *Nucleic Acids Res.* **34:** 1293–1303.

Bradrick, S.S., Dobrikova, E., Kaiser, C., Shveygert, M., and Gromeier, M. 2007. Poly(A)-binding protein is differentially required for translation mediated by viral internal ribosomal entry sites. *RNA* **13:** 1582–1593.

Braunstein, S., Karpisheva, K., Pola, C., Goldberg, J., Hochman, T., Yee, H., Cangiarella, J., Arju, R., Formenti, S.C., and Schneider, R.J. 2007. A hypoxia-controlled cap-dependent to cap-independent translation switch in breast cancer. *Mol. Cell* **28:** 501–512.

Buckley, B. and Ehrenfeld, E. 1987. The cap-binding protein complex in uninfected and poliovirus-infected HeLa cells. *J. Biol. Chem.* **262:** 13599–13606.

Burd, C.G. and Dreyfuss, G. 1994. Conserved structures and diversity of functions of RNA-binding proteins. *Science* **265:** 615–621.

Bushell, M., Stoneley, M., Kong, Y.W., Hamilton, T.L., Spriggs, K.A., Dobbyn, H.C., Qin, X., Sarnow, P., and Willis, A.E. 2006. Polypyrimidine tract binding protein regulates IRES-mediated gene expression during apoptosis. *Mol. Cell* **23:** 401–412.

Byrd, M.P., Zamora, M., and Lloyd, R.E. 2002. Generation of multiple isoforms of eukaryotic translation initiation factor 4GI by use of alternate translation initiation codons. *Mol. Cell. Biol.* **22:** 4499–4511.

Cencig, S., Nanbru, C., Le, S.Y., Gueydan, C., Huez, G., and Kruys, V. 2004. Mapping and characterization of the minimal internal ribosome entry segment in the human c-myc mRNA 5′ untranslated region. *Oncogene* **23:** 267–277.

Chappell, S.A., LeQuesne, J.P., Paulin, F.E., deSchoolmeester, M.L., Stoneley, M., Soutar, R.L., Ralston, S.H., Helfrich, M.H., and Willis, A.E. 2000. A mutation in the c-myc-IRES leads to enhanced internal ribosome entry in multiple myeloma: A novel mechanism of oncogene de-regulation. *Oncogene* **19:** 4437–4440.

Clemens, M.J., Bushell, M., and Morley, S.J. 1998. Degradation of eukaryotic polypeptide chain initiation factor (eIF) 4G in response to induction of apoptosis in human lymphoma cell lines. *Oncogene* **17:** 2921–2931.

De Gregorio, E., Preiss, T., and Hentze, M.W. 1998. Translational activation of uncapped mRNAs by the central part of human eIF4G is 5′ end-dependent. *RNA* **4:** 828–836.

Dobrikova, E.Y., Grisham, R.N., Kaiser, C., Lin, J., and Gromeier, M. 2006. Competitive translation efficiency at the picornavirus type 1 internal ribosome entry site facilitated by viral *cis* and *trans* factors. *J. Virol.* **80:** 3310–3321.

Etchison, D., Milburn, S.C., Edery, I., Sonenberg, N., and Hershey, J.W. 1982. Inhibition of HeLa cell protein synthesis following poliovirus infection correlates with the proteolysis of a 220,000-dalton polypeptide associated with eucaryotic initiation factor 3 and a cap binding protein complex. *J. Biol. Chem.* **257:** 14806–14810.

Gamarnik, A.V. and Andino, R. 1997. Two functional complexes formed by KH domain containing proteins with the 5′ noncoding region of poliovirus RNA. *RNA* **3:** 882–892.

Gebauer, F. and Hentze, M.W. 2004. Molecular mechanisms of translational control. *Nat. Rev. Mol. Cell Biol.* **5:** 827–835.

Gingras, A.C., Raught, B., and Sonenberg, N. 1999. eIF4 initiation factors: Effectors of mRNA recruitment to ribosomes and regulators of translation. *Annu. Rev. Biochem.* **68:** 913–963.

Goyer, C., Altmann, M., Lee, H.S., Blanc, A., Deshmukh, M., Woolford Jr., J.L., Trachsel, H., and Sonenberg, N. 1993. TIF4631 and TIF4632: Two yeast genes encoding the high-molecular-weight subunits of the cap-binding protein complex (eukaryotic initiation factor 4F) contain an RNA recognition motif-like sequence and carry out an essential function. *Mol. Cell. Biol.* **13:** 4860–4874.

Gradi, A., Svitkin, Y.V., Imataka, H., and Sonenberg, N. 1998. Proteolysis of human eukaryotic translation initiation factor eIF4GII, but not eIF4GI, coincides with the shutoff of host protein synthesis after poliovirus infection. *Proc. Natl. Acad. Sci.* **95:** 11089–11094.

Hernandez, G., Vazquez-Pianzola, P., Sierra, J.M., and Rivera-Pomar, R. 2004. Internal ribosome entry site drives cap-independent translation of reaper and heat shock protein 70 mRNAs in *Drosophila* embryos. *RNA* **10:** 1783–1797.

Hershey, J.W. and Merrick, W.C. 2000. Pathway and mechanism of initiation of protein synthesis. In *Translational control of gene expression* (eds. N. Sonenberg et al.), pp. 637–654. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Hundsdoerfer, P., Thoma, C., and Hentze, M.W. 2005. Eukaryotic translation initiation factor 4GI and p97 promote cellular internal ribosome entry sequence-driven translation. *Proc. Natl. Acad. Sci.* **102:** 13421–13426.

Imataka, H. and Sonenberg, N. 1997. Human eukaryotic translation initiation factor 4G (eIF4G) possesses two separate and independent binding sites for eIF4A. *Mol. Cell. Biol.* **17:** 6940–6947.

Jackson, R.J. 2005. Alternative mechanisms of initiating translation of mammalian mRNAs. *Biochem. Soc. Trans.* **33:** 1231–1241.

Jang, S.K., Krausslich, H.G., Nicklin, M.J., Duke, G.M., Palmenberg, A.C., and Wimmer, E. 1988. A segment of the 5′ nontranslated region of encephalomyocarditis virus RNA directs internal entry of ribosomes during in vitro translation. *J. Virol.* **62:** 2636–2643.

Johannes, G. and Sarnow, P. 1998. Cap-independent polysomal association of natural mRNAs encoding c-myc, BiP, and eIF4G conferred by internal ribosome entry sites. *RNA* **4:** 1500–1513.

Johannes, G., Carter, M.S., Eisen, M.B., Brown, P.O., and Sarnow, P. 1999. Identification of eukaryotic mRNAs that are translated at reduced cap binding complex eIF4F concentrations using a cDNA microarray. *Proc. Natl. Acad. Sci.* **96:** 13118–13123.

Keene, J.D., Komisarow, J.M., and Friedersdorf, M.B. 2006. RIP-Chip: The isolation and identification of mRNAs, microRNAs and protein components of ribonucleoprotein complexes from cell extracts. *Nat. Protocols* **1:** 302–307.

Kenan, D.J., Query, C.C., and Keene, J.D. 1991. RNA recognition: Towards identifying determinants of specificity. *Trends Biochem. Sci.* **16:** 214–220.

Koritzinsky, M., Magagnin, M.G., van den Beucken, T., Seigneuric, R., Savelkouls, K., Dostie, J., Pyronnet, S., Kaufman, R.J., Weppler, S.A., Voncken, J.W., et al. 2006. Gene expression during acute and prolonged hypoxia is regulated by distinct mechanisms of translational control. *EMBO J.* **25:** 1114–1125.

Lamphear, B.J., Kirchweger, R., Skern, T., and Rhoads, R.E. 1995. Mapping of functional domains in eukaryotic protein synthesis initiation factor 4G (eIF4G) with picornaviral proteases. Implications for cap-dependent and cap-independent translational initiation. *J. Biol. Chem.* **270:** 21975–21983.

Le Quesne, J.P., Stoneley, M., Fraser, G.A., and Willis, A.E. 2001. Derivation of a structural model for the c-myc IRES. *J. Mol. Biol.* **310:** 111–126.

Lee, K.A. and Sonenberg, N. 1982. Inactivation of cap-binding proteins accompanies the shut-off of host protein synthesis by poliovirus. *Proc. Natl. Acad. Sci.* **79:** 3447–3451.

Lewis, S.M., Cerquozzi, S., Graber, T.E., Ungureanu, N.H., Andrews, M., and Holcik, M. 2008. The eIF4G homolog DAP5/p97 supports the translation of select mRNAs during endoplasmic reticulum stress. *Nucleic Acids Res.* **36:** 168–178.

Liebig, H.D., Ziegler, E., Yan, R., Hartmuth, K., Klump, H., Kowalski, H., Blaas, D., Sommergruber, W., Frasel, L., and Lamphear, B. 1993. Purification of two picornaviral 2A proteinases: Interaction with eIF-4 γ and influence on in vitro translation. *Biochemistry* **32:** 7581–7588.

Ling, J., Morley, S.J., and Traugh, J.A. 2005. Inhibition of cap-dependent translation via phosphorylation of eIF4G by protein kinase Pak2. *EMBO J.* **24:** 4094–4105.

Lloyd, R.E. 2006. Translational control by viral proteinases. *Virus Res.* **119:** 76–88.

Lomakin, I.B., Hellen, C.U., and Pestova, T.V. 2000. Physical association of eukaryotic initiation factor 4G (eIF4G) with eIF4A strongly enhances binding of eIF4G to the internal ribosomal entry site of encephalomyocarditis virus and is required for internal initiation of translation. *Mol. Cell. Biol.* **20:** 6019–6029.

Mader, S., Lee, H., Pause, A., and Sonenberg, N. 1995. The translation initiation factor eIF-4E binds to a common motif shared by the translation factor eIF-4 γ and the translational repressors 4E-binding proteins. *Mol. Cell. Biol.* **15:** 4990–4997.

Mamane, Y., Petroulakis, E., Rong, L., Yoshida, K., Ler, L.W., and Sonenberg, N. 2004. eIF4E—from translation to transformation. *Oncogene* **23:** 3172–3179.

Mamane, Y., Petroulakis, E., LeBacquer, O., and Sonenberg, N. 2006. mTOR, translation initiation and cancer. *Oncogene* **25:** 6416–6422.

Marash, L., Liberman, N., Henis-Korenblit, S., Sivan, G., Reem, E., Elroy-Stein, O., and Kimchi, A. 2008. DAP5 promotes cap-independent translation of Bcl-2 and CDK1 to facilitate cell survival during mitosis. *Mol. Cell* **30:** 447–459.

Marcotrigiano, J., Lomakin, I.B., Sonenberg, N., Pestova, T.V., Hellen, C.U., and Burley, S.K. 2001. A conserved HEAT domain within eIF4G directs assembly of the translation initiation machinery. *Mol. Cell* **7:** 193–203.

Nanbru, C., Lafon, I., Audigier, S., Gensac, M.C., Vagner, S., Huez, G., and Prats, A.C. 1997. Alternative translation of the proto-oncogene c-myc by an internal ribosome entry site. *J. Biol. Chem.* **272:** 32061–32066.

Nevins, T.A., Harder, Z.M., Korneluk, R.G., and Holcik, M. 2003. Distinct regulation of internal ribosome entry site-mediated translation following cellular stress is mediated by apoptotic fragments of eIF4G translation initiation factor family members eIF4GI and p97/DAP5/NAT1. *J. Biol. Chem.* **278:** 3572–3579.

Ohlmann, T., Rau, M., Pain, V.M., and Morley, S.J. 1996. The C-terminal domain of eukaryotic protein synthesis initiation factor (eIF) 4G is sufficient to support cap-independent translation in the absence of eIF4E. *EMBO J.* **15:** 1371–1382.

Pelletier, J. and Sonenberg, N. 1988. Internal initiation of translation of eukaryotic mRNA directed by a sequence derived from poliovirus RNA. *Nature* **334:** 320–325.

Rifo, R.S., Ricci, E.P., Décimo, D., Moncorgé, O., and Ohlmann, T. 2007. Back to basics: The untreated rabbit reticulocyte lysate as a competitive system to recapitulate cap/poly(A) synergy and the selective advantage of IRES-driven translation. *Nucleic Acids Res.* **35:** e121. doi: 10.1093/nar/gkm682.

Roberts, L.O., Seamons, R.A., and Belsham, G.J. 1998. Recognition of picornavirus internal ribosomal entry sites within cells; influence of cellular and viral proteins. *RNA* **4:** 520–529.

Sachs, A.B., Sarnow, P., and Hentze, M.W. 1997. Starting at the beginning, middle, and end: Translation initiation in eukaryotes. *Cell* **89:** 831–838.

Sherrill, K.W., Byrd, M.P., Van Eden, M.E., and Lloyd, R.E. 2004. BCL-2 translation is mediated via internal ribosome entry during cell stress. *J. Biol. Chem.* **279:** 29066–29074.

Stein, I., Itin, A., Einat, P., Skaliter, R., Grossman, Z., and Keshet, E. 1998. Translation of vascular endothelial growth factor mRNA by internal ribosome entry: Implications for translation under hypoxia. *Mol. Cell. Biol.* **18:** 3112–3119.

Stewart, T.A., Pattengale, P.K., and Leder, P. 1984. Spontaneous mammary adenocarcinomas in transgenic mice that carry and express MTV/myc fusion genes. *Cell* **38:** 627–637.

Stoneley, M. and Willis, A.E. 2004. Cellular internal ribosome entry segments: Structures, *trans*-acting factors and regulation of gene expression. *Oncogene* **23:** 3200–3207.

Stoneley, M., Paulin, F.E., Le Quesne, J.P., Chappell, S.A., and Willis, A.E. 1998. C-Myc 5′ untranslated region contains an internal ribosome entry segment. *Oncogene* **16:** 423–428.

Stoneley, M., Chappell, S.A., Jopling, C.L., Dickens, M., MacFarlane, M., and Willis, A.E. 2000. c-myc protein synthesis is initiated from the internal ribosome entry segment during apoptosis. *Mol. Cell. Biol.* **20:** 1162–1169.

Thoma, C., Bergamini, G., Galy, B., Hundsdoerfer, P., and Hentze, M.W. 2004. Enhancement of IRES-mediated translation of the c-myc and BiP mRNAs by the poly(A) tail is independent of intact eIF4G and PABP. *Mol. Cell* **15:** 925–935.

Tsukiyama-Kohara, K., Iizuka, N., Kohara, M., and Nomoto, A. 1992. Internal ribosome entry site within hepatitis C virus RNA. *J. Virol.* **66:** 1476–1483.

Wimmer, E., Hellen, C.U., and Cao, X. 1993. Genetics of poliovirus. *Annu. Rev. Genet.* **27:** 353–436.

Yaman, I., Fernandez, J., Liu, H., Caprara, M., Komar, A.A., Koromilas, A.E., Zhou, L., Snider, M.D., Scheuner, D., Kaufman, R.J., et al. 2003. The zipper model of translational control: A small upstream ORF is the switch that controls structural remodeling of an mRNA leader. *Cell* **113:** 519–531.

Zamora, M., Marissen, W.E., and Lloyd, R.E. 2002. Multiple eIF4GI-specific protease activities present in uninfected and poliovirus-infected cells. *J. Virol.* **76:** 165–177.

Ziegler, E., Borman, A.M., Kirchweger, R., Skern, T., and Kean, K.M. 1995. Foot-and-mouth disease virus Lb proteinase can stimulate rhinovirus and enterovirus IRES-driven translation and cleave several proteins of cellular and viral origin. *J. Virol.* **69:** 3465–3474.

Key Research Accomplishments:

- Elucidation of cis-acting elements contributing to c-*myc* mRNA translation control
- Establishment of tet-inducible cell lines/reporter systems to study cellular IRES regulation
- Discovery of *trans* stimulation of c-*myc* IRES function by eIF4G
- Discovery of enhanced IRES function with a C-terminal eIF4G (Ct) cleavage fragment
- Discovery of control over cellular IRES function via eIF4G/Ct:IRES interaction
- Comprehensive elucidation of translation regulation via eIF4G/interaction with fellow translation factors
- Generation of phospho- eIF4G mutants
- Discovery of eIF4G phosphorylation controlling Mnk1 binding/eIF4E phosphorylation

Reportable Outcomes:

Manuscripts:
Dobrikova EY, Grisham RN, Kaiser C, Lin J, Gromeier M (2006) Competitive translation efficiency at the picornavirus type 1 internal ribosome entry site facilitated by viral *cis* and *trans* factors. *J Virol* 80:3310-21. **PMID: 16537598**

Bradrick SS, Dobrikova EY, Kaiser C, Shveygert M, Gromeier M (2007) Poly(A)-binding protein is differentially required for translation mediated by viral internal ribosome entry sites. *RNA* 9:1582-93. **PMID: 17652408**

Kaiser C, Dobrikova EY, Bradrick SS, Shveygert M, Herbert JT, Gromeier M (2008) Activation of cap-independent translation by variant eukaryotic initiation factor 4G *in vivo*. *RNA* 14:2170-82. **PMID: 18755839**

Presentations:
'Activation of cap-independent translation by variant eukaryotic initiation factor 4G *in vivo*.'
EMBO Meeting: Translation Control. 2007. Heidelberg, Germany.

Degrees obtained:
PhD in Molecular Cancer Biology, Duke University, Dec. 2008.

Cell lines:
A series of cell lines with tetracycline-inducible expression of eIF4G or its variants. These have been made available to numerous researchers.

Employment:
Post-doctoral position in the laboratory of Dr. Matthias Hentze, EMBO, Heidelberg, Germany, as of 03/2009.

Conclusions:

Control of protein synthesis is profoundly unhinged in breast cancer. This is due to abnormal or inappropriate activity of PI3K/mTOR and Ras/MAPK oncogenic signaling pathways that convey pro-growth and -invasion signals to the protein synthesis machinery. All oncogenic signaling converges on two translation initiation factors, eIF4E and -4G, which jointly control all ribosome recruitment to mRNAs. Despite a stampede of drug discovery targeting protein kinases in the PI3K or Ras pathways, little is known about the control of protein synthesis in cancer, how it responds to currently used kinase inhibitor regimens and what measures are required for effective therapeutic targeting of protein synthesis in cancer.

My work has contributed key information to better understand translation control in cancer and aid in drug testing and development. I discovered that an important, alternative mode of translation initiation, which occurs independent of a 5' m^7G cap and the cap-binding protein eIF4E, is mediated by direct interation of eIF4G with cellular mRNAs. This mechanism is used by principal oncogenic mRNAs, e.g. the c-*myc* oncogene or VEGF. My work has helped to unravel the fundamental principles of translation regulation that produce uncontrolled expression of such oncogenes. Based on my thesis work, ongoing empirical studies are using information gathered for correlative studies of a novel experimental cancer therapy based on genetically recombinant poliovirus. Moreover, my work has laid the groundwork for correlative testing and efficacy studies of a vast array of protein kinase inhibitors employed in cancer treatment.

References:

Dobrikova EY, Grisham RN, Kaiser C, Lin J, Gromeier M (2006) Competitive translation efficiency at the picornavirus type 1 internal ribosome entry site facilitated by viral *cis* and *trans* factors. *J Virol* 80:3310-21.

Bradrick SS, Dobrikova EY, Kaiser C, Shveygert M, Gromeier M (2007) Poly(A)-binding protein is differentially required for translation mediated by viral internal ribosome entry sites. *RNA* 9:1582-93.

Kaiser C, Dobrikova EY, Bradrick SS, Shveygert M, Herbert JT, Gromeier M (2008) Activation of cap-independent translation by variant eukaryotic initiation factor 4G *in vivo*. *RNA* 14:2170-82.