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Control of Cell Survival and Proliferation by Mammalian Eukaryotic Initiation Factor 4B▽†

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Translation initiation plays an important role in cell growth, proliferation, and survival. The translation initiation factor eIF4B (eukaryotic initiation factor 4B) stimulates the RNA helicase activity of eIF4A in unwinding secondary structures in the 5′ untranslated region (5′UTR) of the mRNA *in vitro*. Here, we studied the effects of eIF4B depletion in cells using RNA interference (RNAi). In agreement with the role of eIF4B in translation initiation, its depletion resulted in inhibition of this step. Selective reduction of translation was observed for mRNAs harboring strong to moderate secondary structures in their 5′UTRs. These mRNAs encode proteins, which function in cell proliferation (Cdc25C, c-myc, and ODC [ornithine decarboxylase]) and survival (Bcl-2 and XIAP [X-linked inhibitor of apoptosis]). Furthermore, eIF4B silencing led to decreased proliferation rates, promoted caspase-dependent apoptosis, and further sensitized cells to camptothecin-induced cell death. These results demonstrate that eIF4B is required for cell proliferation and survival by regulating the translation of proliferative and prosurvival mRNAs.

Targeting the translation initiation pathway is emerging as a potential therapy for inhibiting cancer cell growth (35, 38). Ribosome recruitment to the 5′ ends of eukaryotic mRNAs proceeds via translation initiation mechanisms that are dependent either on the 5′ cap structure (m^7GpppN, where N is any nucleotide) or an internal ribosome entry site (IRES). The majority of translation initiation events in eukaryotes are mediated through cap-dependent translation whereby the 40S ribosomal subunit is recruited to the vicinity of the mRNA 5′ cap structure by the eukaryotic initiation factor 4F (eIF4F) complex. eIF4F is comprised of eIF4E (the cap-binding subunit), eIF4A (an RNA helicase), and eIF4G (a large scaffolding protein for eIF4E, eIF4A, and other initiation factors). Once assembled at the 5′ cap, the 40S ribosomal subunit in association with several initiation factors scans the 5′ untranslated region (5′UTR) of the mRNA until it encounters a start codon in a favorable context, followed by polypeptide synthesis (37).

Early *in vitro* studies have shown that the initiation factor eIF4B acts to potentiate ribosome recruitment to the mRNA (3, 45). eIF4B stimulates translation of both capped and uncapped mRNAs *in vitro* (1, 36). This function is exerted through stimulation of the helicase activity of eIF4A (43), possibly through direct interactions with eIF4A (44) or with mRNA, the ribosome-associated eIF3, and 18S rRNA (28, 29, 44). Thus, eIF4B is thought to form auxiliary bridges between the mRNA and the 40S ribosomal subunit. Toeprinting studies using mammalian eIF4B underscored its importance in the assembly of the 48S initiation complex, especially on mRNAs harboring secondary structures in the 5′UTRs (11).

In vivo studies of eIF4B are limited. Ectopic expression of eIF4B in cultured *Drosophila melanogaster* cells and in developing eye imaginal discs stimulated cell proliferation (16). Enhanced cell proliferation is most likely mediated by increased translation of a subset of mRNAs, since knockdown of *Drosophila* eIF4B by RNA interference (RNAi) caused a modest reduction in global translation but compromised the survival of insect cells grown under low serum conditions (16). Studies of eIF4B in mammalian cells yielded contradictory results. Transient overexpression of eIF4B stimulated translation initiation in a phosphorylation-dependent manner in some cells (18, 49) while inhibiting translation in others (30, 31, 41). These differences might be attributed to disparate levels of eIF4B overexpression.

To address the physiological role of eIF4B in mRNA translation in the cell, RNAi knockdown of eIF4B was used here. We demonstrate that eIF4B is required for optimal translation. Importantly, the translation of mRNAs bearing structured 5′UTRs, such as the cell cycle regulators Cdc25C, c-myc, and ODC (ornithine decarboxylase), and the antiapoptotic factors Bcl-2 and XIAP (X-linked inhibitor of apoptosis), was reduced as a result of eIF4B silencing by RNAi. Furthermore, eIF4B silencing promoted caspase-dependent apoptosis. Thus, we show that mammalian eIF4B is required for cell proliferation and survival, whereby it acts by regulating the translation of a functionally related subset of mRNAs.

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MATERIALS AND METHODS

Cells and treatments. HeLa S3 cells were from the ATCC and maintained in Dulbecco modified Eagle medium (DMEM) (Sigma-Aldrich) containing 10% fetal bovine serum (FBS). For G_2/M arrest experiments, cells were treated with 100 ng/ml nocodazole (Sigma-Aldrich) overnight. Caspase activity was inhibited with the pancaspase inhibitor zVAD-fmk (benzyloxycarbonyl-Val-Ala-DL-Asp [zVAD] conjugated to fluoromethylketone [fmk]) according to the manufacturer's instructions (MP Biomedicals). For cytotoxicity experiments, cells were treated with 0.8, 4, and 20 μM camptothecin (Sigma-Aldrich).

Transfections. A human eIF4B small interfering RNA (siRNA) (5′GGACA GGAAGUGAGUCAUC3′) and nontargeting control siRNA (5′GCAACAGA GGUUUGUAGUA3′) were purchased from Dharmacon. Plasmid expressing Bcl-2 with influenza virus hemagglutinin epitope (HA-Bcl-2) has been described elsewhere (32). Transfections were performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

Propidium iodide staining and annexin V labeling. Cell cycle analysis with propidium iodide (PI) staining was performed as previously described (23). Apoptosis in eIF4B-silenced HeLa cells was measured using annexin V-FITC (fluorescein isothiocyanate) apoptosis kit (Invitrogen) according to the manufacturer's instructions. Analysis was performed using a FACScan (Becton-Dickinson).

Western blotting and antibodies. SDS-PAGE and Western blot analysis were performed as described previously (47). The antibodies were as follows: eIF4B (29); β-actin (Sigma-Aldrich); cleaved poly(ADP-ribose) polymerase (PARP), caspase 9, XIAP (Cell Signaling Technology); Bcl-2, Cdc25C, c-myc, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Santa Cruz); and ODC (BioMol).

Sucrose gradient fractionation and polysome analysis. Mock-silenced and eIF4B-silenced HeLa cells were grown to 60 to 70% confluence. The cells were washed once in cold phosphate-buffered saline (PBS) containing 100 μg/ml cycloheximide and scraped off the plate using a rubber policeman into 1 ml of the same solution. The cells were centrifuged for 10 min at 1,200 rpm and resuspended in 425 μl hypotonic lysis buffer (5 mM Tris-HCl [pH 7.5], 2.5 mM $MgCl_2$, 1.5 mM KCl). The lysates were transferred to a prechilled tube, incubated with 100 μg/ml cycloheximide, 2 mM dithiothreitol (DTT), and 2 μl RNasin (40 U/μl; Stratagene), kept on ice for 5 min, and vortexed. To each 425 μl of lysate, 25 μl of 10% Triton X-100 and 25 μl of 10% sodium deoxycholate were added. The samples were vortexed again and incubated on ice for 5 min. Cell extracts were centrifuged for 5 min at 14,000 rpm; the supernatant was collected, and protein concentration was determined. Equal amounts of protein were loaded onto prechilled 10 to 50% sucrose gradients. Each gradient was formed in a Beckman centrifuge tube (14 by 89 mm) (catalog no. 3311372; Beckman Instruments, CA) using 11 1-ml steps starting with 50% sucrose at the bottom and ending with 10% sucrose (4% steps) at the top. The tubes were centrifuged in a Beckman SW40Ti rotor at 35,000 rpm for 3 h at 4°C. Fractions were collected (24 fractions of 12 drops or 12 fractions of 24 drops each, depending on the experiment) using a Foxy JR ISCO collector and UV optical unit type 11 (Lincoln, NE). The data were acquired using an analog-to-digital converter USB-1208 device and TracerDAQ software (Measurement Computing Inc.).

RT-PCR. Sucrose gradient fractions were subjected to RNA extraction using TRIzol (Invitrogen). Reverse transcription was performed using a SuperScript III reverse transcriptase kit (Invitrogen) and random hexamers (Invitrogen) according to the manufacturer's instructions. PCRs were carried out in a Mastercycler Realplex^2 (Eppendorf) or a CFX96 (Bio-Rad) reverse transcription-PCR (RT-PCR) system using iQ Sybr green Supermix (Bio-Rad) according to the manufacturer's instructions. PCR was performed using 1 μl of RT reaction mixture in a total reaction mixture volume of 10 μl containing reverse and forward primers (0.5 μl of 10 μM solutions). The amplification conditions consisted of an initial denaturation step of 2 min at 95°C, followed by up to 40 cycles of 15 s at 95°C, 15 s at 55°C, and 20 s at 68°C. PCR products were loaded and analyzed in 1.8% agarose gels. Primers for Cdc25C, c-myc, ODC, Bcl-2, XIAP, GAPDH, and β-actin are listed in Table S1 in the supplemental material.

[^{35}S]methionine/cysteine metabolic labeling. The cells were seeded in 24-well plates, and [^{35}S]methionine/cysteine labeling was performed as described previously (53).

MTT assay. The cells were seeded in 96-well plates. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) substrate solution was used according to the manufacturer's instructions (Sigma-Aldrich). Briefly, MTT was added to the cell cultures for 3 h. Formed formazan crystals were dissolved in dimethyl sulfoxide (DMSO), and the plates were analyzed using a Varioskan Flash microplate reader and Scanit software at 570 nm (Thermo Fisher Scientific).

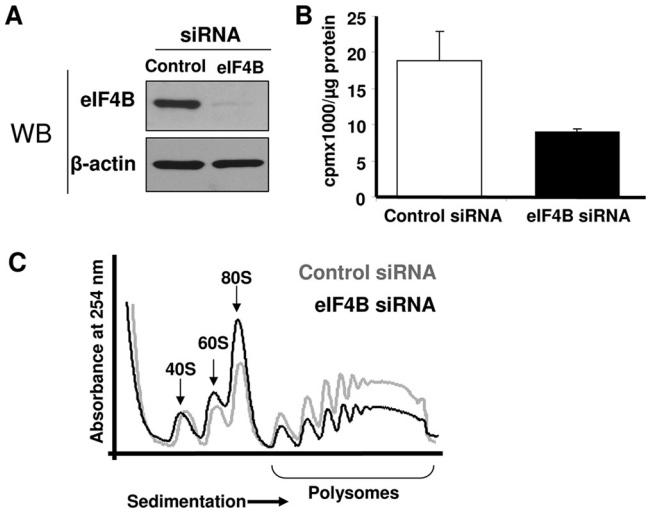

FIG. 1. Translation initiation is inhibited in eIF4B-silenced cells. (A) Lysates from eIF4B siRNA-silenced HeLa cells were subjected to Western blotting (WB) for eIF4B and β-actin. (B) Control and eIF4B-silenced cells were labeled with [^{35}S]methionine/cysteine. (C) Polysomes from eIF4B-silenced and control siRNA-treated cells were analyzed on 10 to 50% sucrose density gradients. All procedures are described in Materials and Methods.

RESULTS

eIF4B silencing inhibits translation initiation and protein synthesis. Because *in vitro* studies documented the requirement of eIF4B for translation initiation, but *in vivo* data were inconsistent, we sought to establish a role for eIF4B in mammalian cells using small interfering RNA (siRNA). HeLa cells were transfected with eIF4B siRNA, and the effect of eIF4B silencing on protein synthesis was determined. Western blot analysis showed that eIF4B siRNA caused a >90% reduction in eIF4B protein expression compared to control siRNA (Fig. 1A). Translation rates were determined using [^{35}S]methionine/cysteine metabolic labeling. eIF4B siRNA-transfected cells showed an approximately 50% decrease in [^{35}S]methionine/cysteine incorporation compared to control siRNA-transfected cells (Fig. 1B). Next, we wished to determine the step of translation that is impaired as a result of eIF4B silencing using polysome profiling. Cell extracts from control and eIF4B siRNA-transfected cells were fractionated using sucrose gradient centrifugation to separate polysomes from the 40S/60S free ribosomal subunits and 80S ribosomes. Consistent with the reduction in [^{35}S]methionine/cysteine incorporation, a substantial decrease in the amount of polysomes was observed in eIF4B-silenced cells compared to control cells (Fig. 1C). This reduction in polysomes was concomitant with an increase in 80S monomers (Fig. 1C), which is consistent with inhibition of translation initiation (27). Taken together, these results demonstrate that eIF4B is required for optimal translation initiation in cells.

eIF4B silencing reduces translation of mRNAs harboring structured 5′UTRs. Because translation and polysome assembly were only partially inhibited as a consequence of eIF4B silencing, it was conceivable that the translation of just a subset of mRNAs that contain secondary structures in their 5′UTRs was preferentially strongly inhibited. It has been suggested that

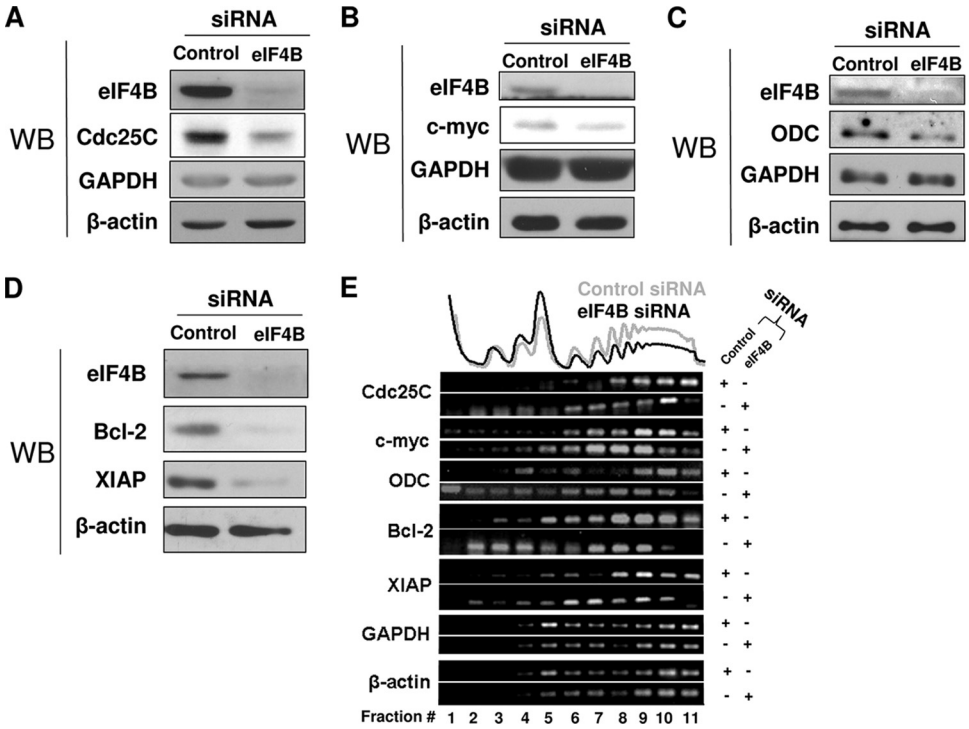

FIG. 2. Translation of mRNAs with structured 5′UTRs is preferentially inhibited in eIF4B-silenced cells. (A to D) Lysates from eIF4B siRNA-silenced HeLa cells were subjected to Western blotting for Cdc25C (A), c-myc (B), ODC (C), and XIAP and Bcl-2 (D) along with eIF4B, GAPDH, and β-actin. (E) Polysomes from eIF4B siRNA-silenced and control siRNA-transfected HeLa cells were fractionated on sucrose density gradients. The effects of eIF4B silencing on the distribution of the indicated mRNAs in gradient fractions are shown.

eIF4B promotes the translation of such mRNAs (11). To test this hypothesis, the protein levels of Cdc25C (ΔG mRNA 5′UTR = −85 kcal/mol [kcal stands for kilocalories]), c-myc (ΔG 5′UTR = −233 kcal/mol), ODC (ΔG 5′UTR = −157 kcal/mol), Bcl-2 (ΔG 5′UTR = −133 kcal/mol), and XIAP (ΔG 5′UTR = −47 kcal/mol) were examined in cell extracts from control and eIF4B-silenced HeLa cells. Cdc25C (40), c-myc (14), and ODC (50) play a role in cell proliferation, and the translation of their mRNAs is regulated, while XIAP and Bcl-2 are antiapoptotic factors, and the translation of their mRNAs is also regulated (17, 23, 33). Transfection of eIF4B siRNA caused a substantial reduction in the expression of Cdc25C, c-myc, ODC, XIAP, and Bcl-2 proteins (Fig. 2A to D). In contrast, the levels of proteins encoded by the relatively unstructured GAPDH and β-actin mRNAs (ΔG 5′UTR = −22 and −16 kcal/mol, respectively) were not changed (Fig. 2A to D).

To determine the effect of eIF4B silencing on translation initiation, we examined the polysomal distribution of Cdc25C, c-myc, ODC, XIAP, and Bcl-2 mRNAs along sucrose density gradients. All of these mRNAs, which harbor relatively structured 5′UTRs, were shifted to lighter polysomal fractions in eIF4B-silenced cells (Fig. 2E). In contrast, there was no significant redistribution of GAPDH or β-actin mRNAs in eIF4B-silenced cells. Taken together, these findings show that eIF4B promotes translation of mRNAs with structured 5′UTRs, and among these mRNAs, it promotes those encoding proteins involved in cell proliferation and survival.

eIF4B silencing impairs cell proliferation and survival. To investigate the roles of eIF4B in cell proliferation and survival, HeLa cells were transfected with eIF4B siRNA, and proliferation was measured up to 5 days after transfection. eIF4B silencing inhibited proliferation as measured by cell counting (Fig. 3A; >70% at day 5 posttransfection). This result was reproduced in a colorimetric MTT analysis (Fig. 3B), which measures mitochondrial activity. Hence, this method was employed for subsequent experiments (see below). eIF4B siRNA caused a dramatic reduction (>90%) in eIF4B protein expression after 3 days as measured by Western blotting, followed by a partial derepression at 6 days (Fig. 3C), probably due to the transient nature of siRNA-mediated silencing. The growth inhibitory effects of eIF4B silencing were reproduced in U2OS osteosarcoma cells and MRC-5 human lung epithelial cells (see Fig. S1 in the supplemental material).

Next, we determined whether the reduced proliferation of eIF4B-silenced cells was due to cell cycle arrest or reduced survival. Cell cycle distribution of control and eIF4B-silenced cells was examined 3 days posttransfection using flow cytometry. eIF4B-silenced cells showed a reduction in the percentage of cells in G_2/M (15% versus 23% in control cells) and accumulation of apoptotic cells (16% versus 3% in control cells; shown as sub-G_1 population) (Fig. 3D). eIF4B silencing did not affect the percentage of cells in the G_1 or S phase. Taken together, these data demonstrate that eIF4B is required for cell cycle progression through G_2/M. In addition, cells unable to pass the intrinsic G_2/M checkpoint become

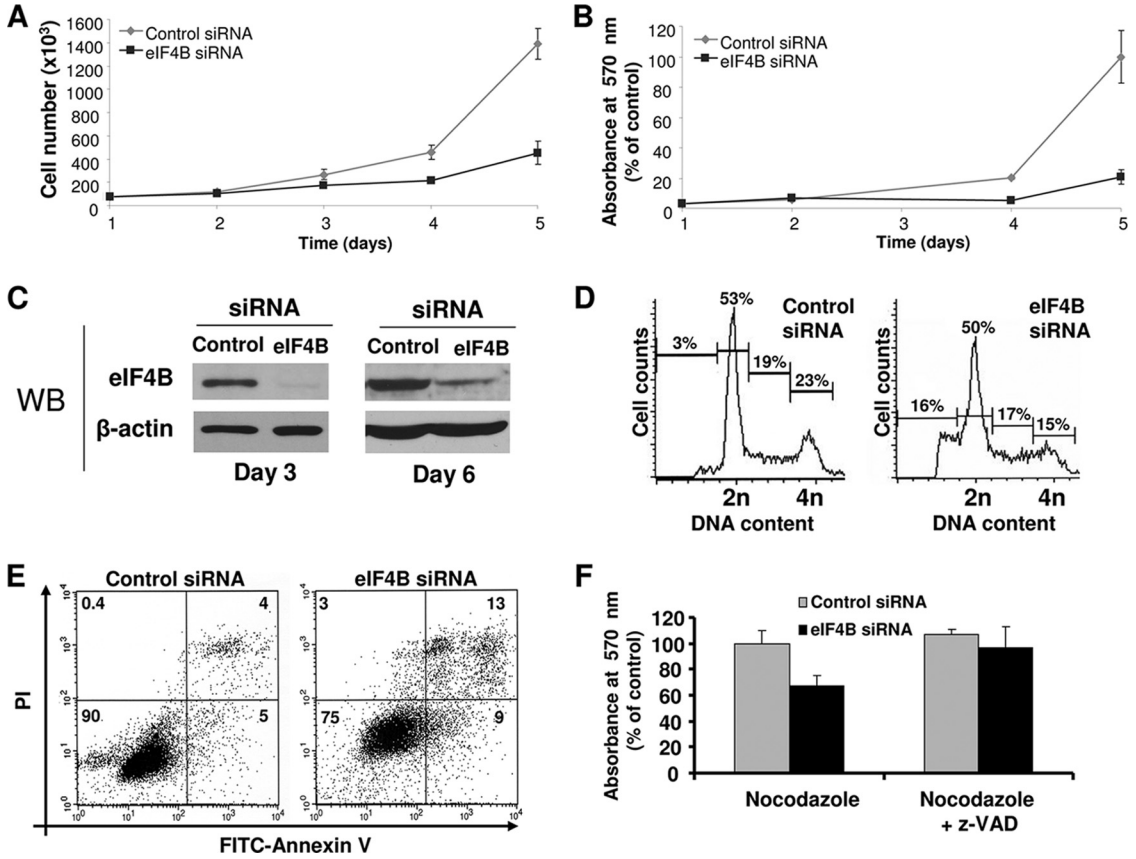

FIG. 3. Effects of eIF4B silencing on cell proliferation and survival. (A) Cell counting using Z1 Coulter Counter (Beckman Coulter). (B) Colorimetric analysis using the MTT assay. Values from control cells on day 5 were set at 100%. (C) Cells from parallel cultures were harvested on the third and sixth day posttransfection, equalized for protein content, and subjected to Western blotting for eIF4B and β-actin. (D) HeLa cells were transfected with control or eIF4B-targeting siRNAs and grown for 3 days before cell cycle analysis by flow cytometry was carried out. (E) Control and eIF4B-silenced cells were doubly stained with FITC-annexin V and propidium iodide (PI), and analyzed by flow cytometry 4 days after siRNA transfection. The number in each quadrant represents the percentage of cells in that quadrant. (F) Control and eIF4B-silenced cells were treated with either nocodazole alone or with nocodazole and zVAD and then incubated for an additional 24 h, when the cells were subjected to the MTT assay. Values from nocodazole-treated control cells were set at 100%.

committed to cell death, as evidenced by the increase in the sub-G_1 population.

To corroborate these data, annexin V and propidium iodide (PI) staining was measured using fluorescence-activated cell sorting (FACS) analysis (Fig. 3E). Differences in the percentages of early apoptotic cells (Fig. 3E, bottom right quadrant; 5% in control versus 9% in eIF4B-silenced cells) and the late apoptotic cells (Fig. 3E, top right quadrant; 4% in control versus 13% in eIF4B-silenced cells) indicate that eIF4B-silenced cells undergo increased apoptosis compared to control siRNA-transfected cells. In addition, eIF4B-silenced cells showed a 15% decrease in the amount of viable cells compared to control (bottom left quadrants in Fig. 3E).

We next examined whether the observed apoptotic effects of eIF4B silencing were caspase dependent. First, we treated eIF4B-silenced and control cells with nocodazole to induce mitotic arrest. As measured by the MTT assay, nocodazole treatment caused a decrease in the number of viable cells in approximately 33% of eIF4B-silenced cells compared to control cells (Fig. 3F). Since nocodazole causes mitotic arrest, the differences in cell number should reflect changes in viability and not the rate of proliferation. Next, we cotreated cells with nocodazole and the pancaspase inhibitor, zVAD. In eIF4B-silenced cells, zVAD treatment reversed the decrease in viable cells, as measured by the MTT assay, which was induced by nocodazole treatment (Fig. 3F), suggesting that this effect was caspase dependent.

To further confirm the involvement of caspases in eIF4B RNAi-induced apoptosis, we examined the status of procaspase 9 and poly(ADP-ribose) polymerase (PARP). Cleavage of these proteins is linked to the activation of apoptotic pathways (12). In cells transfected with eIF4B siRNA, the levels of cleaved PARP increased >8-fold and those of procaspase 9 decreased >5-fold compared to control cells (Fig. 4A).

Our results above (Fig. 2D) show that Bcl-2 expression is suppressed in eIF4B-silenced cells. To assess the role of Bcl-2 in eIF4B RNAi-induced apoptosis, eIF4B-silenced cells were transfected with a plasmid expressing the Bcl-2 protein. This treatment rescued eIF4B-silenced cells from apoptosis as judged by the reduction of cells in the sub-G_1 population from 16% to 6.5% (Fig. 4B). Thus, eIF4B controls apoptosis to a

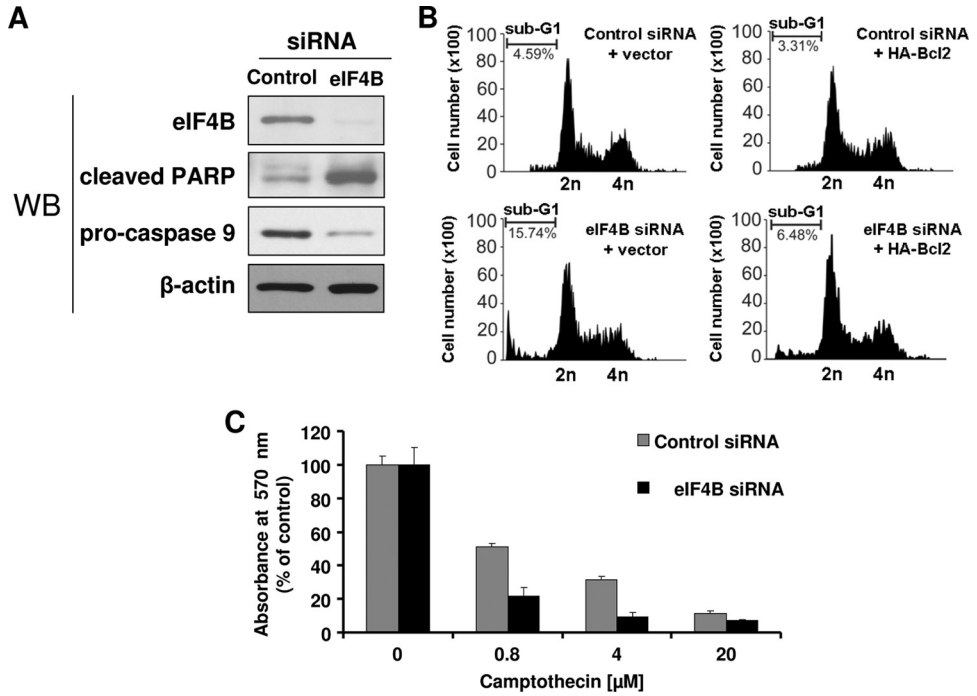

FIG. 4. eIF4B silencing sensitizes cells to camptothecin-induced apoptosis. (A) The cells were transfected with control or eIF4B-targeting siRNAs. Three days posttransfection, cells were lysed and subjected to Western blotting for cleaved PARP, procaspase 9, eIF4B, and β-actin. (B) eIF4B-silenced and control cells were transfected with either an HA-Bcl-2-expressing vector or empty vector. Cell cycle analysis was carried out using flow cytometry. The percentage of sub-G_1 population is shown in each profile. (C) Control or eIF4B-silenced cells were treated with increasing concentrations of the topoisomerase inhibitor, camptothecin. MTT assay was performed 48 h posttreatment. Values of untreated control and eIF4B-silenced cells were adjusted to 100%.

large extent via the translation of Bcl-2 mRNA. However, participation of other prosurvival proteins downstream from Bcl-2 (such as XIAP and others) cannot be ruled out.

Many types of cancer cells are dependent on elevated expression of antiapoptotic proteins for fast proliferation and for chemoresistance and radioresistance (20, 21). Although downregulation of antiapoptotic factors is not sufficient to induce apoptosis, in some studies, it could sensitize cells to cytotoxic agents (5, 8, 17, 22). For example, XIAP silencing sensitizes cancer cells to camptothecin-induced apoptosis (51). A recent clinical study reported that targeting Bcl-2 using an antisense oligonucleotide improved the efficacy of systemic chemotherapy in patients with advanced melanoma (2). Consequently, we wished to study the effect of eIF4B silencing on camptothecin-induced apoptosis. Camptothecin treatment of HeLa cells caused a dose-dependent increase in cell death as determined by MTT assay (Fig. 4C). eIF4B-silenced cells exhibited markedly decreased survival, as treatment with 0.8 or 4 μM camptothecin for 48 h resulted in a >50% decrease in survival of eIF4B-silenced cells compared to control cells (Fig. 4C). At high concentrations of camptothecin (20 μM), both control and eIF4B-silenced cells were equally sensitive (90% cell death). Thus, eIF4B silencing most likely sensitizes cancer cells to weak genotoxic stresses through decreased translation of mRNAs coding for antiapoptotic proteins, such as Bcl-2 and XIAP.

eIF4B silencing inhibits translation independently of caspase activation. Caspase activation mediates apoptosis by a mechanism that involves the cleavage of multiple cellular proteins, including components of the translation machinery (Fig. 5A) (7, 9, 25, 26). eIF4B is cleaved during apoptosis by caspase 3 (7). It is therefore possible that caspase activation, which is induced by eIF4B silencing, leads to inhibition of translation in a positive-feedback loop, whereby caspases cleave eIFs (including residual eIF4B). To address this possibility, cells were transfected with control or eIF4B silencing siRNAs and then treated with zVAD to inhibit caspase activity. Polysome profiling demonstrated that zVAD treatment failed to prevent the reduction in polysome assembly and the concomitant increase in 80S ribosomal subunits observed in eIF4B-silenced cells (Fig. 5B). zVAD did inhibit caspase activity, as it reduced cleavage of PARP and increased the procaspase 9 levels in eIF4B-silenced cells (Fig. 5C). Taken together, these results indicate that the inhibition of translation initiation induced by eIF4B silencing is independent of caspase activity.

DISCUSSION

In this study, we demonstrate that eIF4B preferentially stimulates the translation of mRNAs possessing structured 5′UTRs at the initiation step. This is consistent with the biochemical function of eIF4B to stimulate the helicase activity of the eIF4A component of the eIF4F complex (24, 43). In eukaryotes, translation is largely dependent on eIF4F. eIF4B potentiates ribosome recruitment by stimulating mRNA unwinding activity of the eIF4A helicase subunit of eIF4F and via interaction with ribosome-bound eIF3 pro-

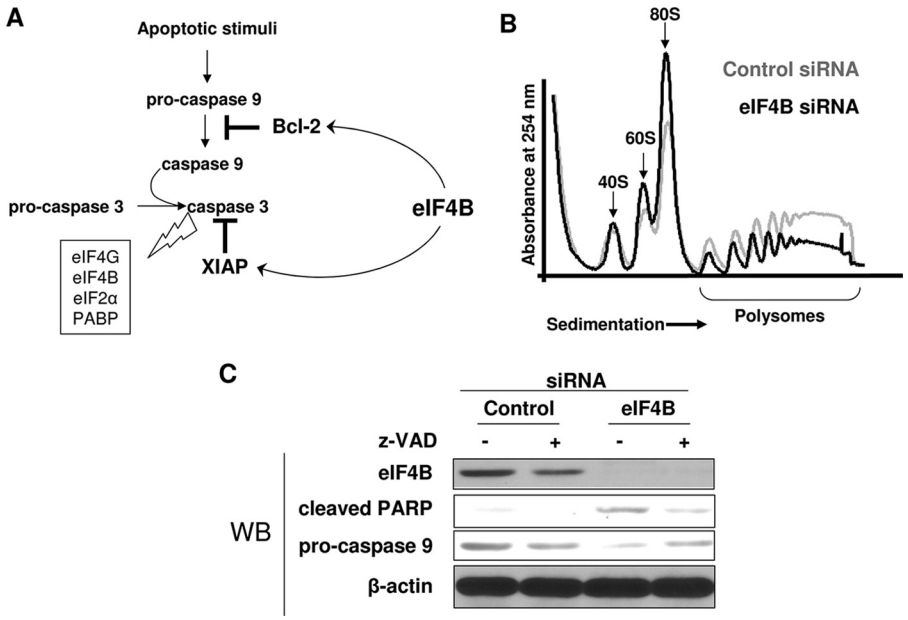

FIG. 5. eIF4B silencing results in translational inhibition in a caspase-independent manner. (A) Schematic diagram depicting the apoptotic pathway leading to caspase 3 activation and cleavage of substrates associated with translation initiation. (B) Polysomal profiles of zVAD-treated control and eIF4B-silenced cells. (C) Protein lysates from control and zVAD-treated cells were subjected to Western blotting for cleaved PARP, procaspase 9, eIF4B, and β-actin.

tein (reviewed in reference 37). Some mRNAs (especially those coding for proteins controlling cell growth, proliferation, and survival) contain highly structured 5′UTRs (39), and their efficient translation is believed to require robust helicase activity. In agreement with this model, our data demonstrate that translation of mRNAs harboring structured 5′UTRs is much more dependent on eIF4B than mRNAs with relatively unstructured 5′UTRs (e.g., β-actin and GAPDH). The recently described helicase-like protein DHX29 (DEAH [Asp-Glu-Ala-His] box polypeptide 29) (40) was shown to have similar discriminatory effects on mRNA translation, depending on 5′UTR complexity, *in vivo* (34). Our results show that upon eIF4B silencing, the expression level of DHX29 protein is not significantly affected (see Fig. S2 in the supplemental material), suggesting that the observed effects are independent of DHX29.

Our results agree with a previous *in vitro* study demonstrating that assembly of the 48S initiation complex on mRNAs containing even moderate secondary structure in the 5′UTR is dependent on eIF4B (11). Interestingly, in the previous study, native eIF4B purified from mammalian cells possessed much higher activity than bacterially expressed recombinant protein, suggesting that posttranslational modifications (e.g., phosphorylation) are important for its activity. The interaction between eIF4B and eIF3 is controlled through phosphorylation of eIF4B Ser422 by S6 kinases (S6Ks) and Rsks, the effectors of PI3K-mTOR (PI3K is phosphatidylinositol 3-kinase, and mTOR is mammalian target of rapamycin) and mitogen-activated protein kinase (MAPK) signaling cascades (47). Involvement of these signaling modules in proliferative and survival responses in cancer cells is well documented (13, 15, 42, 46, 52). Hence, eIF4B phosphorylation regulates the translation of functionally related mRNAs, such as cell cycle regulators and antiapoptotic factors.

eIF4B depletion leads to marked inhibition of the translation of mRNAs coding for the antiapoptotic proteins Bcl-2 and XIAP. This inhibition can explain the strong effect of eIF4B silencing on cell survival and activation of caspase-dependent apoptotic pathways at the G_2/M phase. Bcl-2-mediated rescue of eIF4B-silenced cells from apoptosis suggests that downregulation of Bcl-2 (and possibly downstream regulators) can at least partially explain the enhanced sensitivity of eIF4B-silenced cells to apoptosis. While it is not clear whether cell proliferation and survival are decreased as a direct result of eIF4B silencing or whether they are secondary to a drop in global protein synthesis, it is likely that the negative effects of eIF4B silencing on translation precede those on proliferation and cell survival.

The effects on translation and survival seen in eIF4B-silenced cells are similar to those observed as a consequence of eIF4A inhibition. For instance, the Pdcd4 protein, an inhibitor of eIF4A that exhibits tumor repressor properties, was initially identified as a gene that is upregulated during apoptosis (10, 48). Pdcd4 knockdown in cultured cells decreases apoptosis and increases survival after UV irradiation (6). Inhibition of eIF4A activity by pateamine A (19) or hippuristanol (D. Shahbazian, unpublished observations) leads to the execution of the apoptotic program in various cancer cell lines. Thus, both eIF4A and eIF4B promote translation of antiapoptotic proteins.

Several studies have utilized RNAi against Bcl-2 or XIAP to sensitize different types of cancer cells to cytotoxic treatments (reviewed in reference 4). Our data demonstrate that eIF4B silencing results in a marked decrease of several antiapoptotic

and proproliferative proteins. Thus, small-molecule-mediated inhibition of eIF4B activity in cancer cells could be considered a potential treatment for human malignancies.

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