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α4 Phosphoprotein Interacts With EDD E3 Ubiquitin Ligase and Poly(A)-Binding Protein

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ABSTRACT

Mammalian α4 phosphoprotein, the homolog of yeast Tap42, is a component of the mammalian target-of-rapamycin (mTOR) pathway that regulates ribogenesis, the initiation of translation, and cell-cycle progression. α4 is known to interact with the catalytic subunit of protein phosphatase 2A (PP2Ac) and to regulate PP2A activity. Using α4 as bait in yeast two-hybrid screening of a human K562 erythroleukemia cDNA library, EDD (*E*3 isolated by *d*ifferential *d*isplay) E3 ubiquitin ligase was identified as a new protein partner of α4. *EDD* is the mammalian ortholog of *Drosophila hyperplastic discs* gene (*hyd*) that controls cell proliferation during development. The EDD protein contains a PABC domain that is present in poly(A)-binding protein (PABP), suggesting that PABP may also interact with α4. PABP recruits translation factors to the poly(A)-tails of mRNAs. In the present study, immunoprecipitation/immunoblotting (IP/IB) analyses showed a physical interaction between α4 and EDD in rat Nb2 T-lymphoma and human MCF-7 breast cancer cell lines. α4 also interacted with PABP in Nb2, MCF-7 and the human Jurkat T-leukemic and K562 myeloma cell lines. COS-1 cells, transfected with Flag-tagged-pSG5-EDD, gave a (Flag)-EDD–α4 immunocomplex. Furthermore, deletion mutants of α4 were constructed to determine the binding site for EDD. IP/IB analysis showed that EDD bound to the C-terminal region of α4, independent of the α4-PP2Ac binding site. Therefore, in addition to PP2Ac, α4 interacts with EDD and PABP, suggesting its involvement in multiple steps in the mTOR pathway that leads to translation initiation and cell-cycle progression. J. Cell. Biochem. 110: 1123–1129, 2010.

KEY WORDS: α4 PHOSPHOPROTEIN; EDD E3 UBIQUITIN LIGASE; POLY(A)-BINDING PROTEIN

The α4 phosphoprotein, homolog of yeast Tap42, is an essential component of the mTOR pathway that responds to nutrients and growth factors to control ribosome biosynthesis and the initiation and elongation phases of translation in mammalian cells [Schmelzle and Hall, 2000; Gingras et al., 2001; Raught et al., 2001]. mTOR has intrinsic serine/threonine kinase activity [Brown et al., 1995] and phosphorylates two well-known effectors, the translational repressor eukaryotic initiation factor 4E (eIF4E)-binding protein (4E-BP1) and ribosomal protein S6 kinase (p70S6K). The mTOR-inactivated 4E-BP1 releases eIF4E, which becomes available to form the eIF4F complex (eIF4E, 4G, 4A), thereby increasing translation of 5′-cap mRNAs. The mTOR-activated p70S6K phosphorylates ribosomal protein S6 [Clemens, 2001; Bjornsti and Houghton, 2004], which may potentially modulate mRNA biogenesis [Tee and Blenis, 2005]. mTOR, acting through an effector which has yet to be identified, also increases the translation of 5′-terminal oligopyrimidine-tract (5′-TOP) mRNAs which encode components of the translational machinery, thereby upregulating ribosome biogenesis [Findlay et al., 2005].

The α4 cDNA, first cloned from a murine bone marrow cDNA library, was recognized to encode a phosphoprotein component related to immunoglobulin receptor-mediated signal transduction [Inui et al., 1995], but was found to be widely expressed [Inui et al., 1995; Everett and Brautigan, 2002]. We cloned and identified the rat α4 as a prolactin-downregulated cDNA in the prolactin-dependent rat Nb2 T-lymphoma cell line, using differential display [Boudreau

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et al., 2002]. Mammalian α4 associates with the catalytic subunits of protein phosphatase 2A (PP2Ac), PP4, and PP6 [Inui et al., 1995; Chen et al., 1998; Boudreau et al., 2002]. α4 is a regulator of PP2A activity. α4 has been shown to inhibit PP2A, PP4, and PP6 in transfected Jurkat T-leukemic cells [Nanahoshi et al., 1999], whereas transient overexpression of α4 activates PP2A in transfected COS-1 cells [Nien et al., 2007]. In human Jurkat T-leukemic and rat Nb2 T-lymphoma cells, mTOR indirectly controls phosphorylation of p70S6K and 4E-BP1 by restraining the activity of PP2A [Peterson et al., 1999; Bishop et al., 2006].

In addition to PP2Ac, there has been evidence that α4 has other protein partners. When Nb2 cells were pulsed with [^{32}P]-orthophosphate in the presence of prolactin and a phorbol ester, several unidentified [^{32}P]-phosphoproteins were immunoprecipitated by anti-α4 antibodies [Boudreau et al., 2002]. We have sought to identify these proteins associated with α4 since their identification may provide more insight into the action of α4.

In this study, yeast two-hybrid analysis identified EDD ubiquitin ligase as a protein partner of α4. EDD has a domain that is also found in poly(A)-binding protein (PABP). Using co-immunoprecipitation (co-IP) analysis, this study has confirmed interaction of α4 with EDD and PABP in Nb2, COS-1, human hematopoietic, and breast cancer cell lines.

MATERIALS AND METHODS

ANTIBODIES

Our anti-α4 polyclonal antibody has been described previously [Boudreau et al., 2002]. Polyclonal anti-EDD antibodies were kindly provided by Dr. Michelle Henderson (previously at the Garvan Institute Sydney, Australia) or were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA) or Abcam, Inc. (Cambridge, MA). Other antibodies, used at the indicated concentrations, were purchased from the following sources: mouse anti-PP2Ac (1:2,500), BD Transduction Laboratories (Mississauga, Ontario, Canada); mouse anti-Xpress (1:5,000), Invitrogen (Burlington, Ontario, Canada); mouse anti-Flag M2 (10 μg/ml), Sigma–Aldrich Canada Ltd (Oakville, Ontario, Canada); rabbit anti-PABP (1:500), Santa Cruz Biotechnology, Inc.; secondary antibodies donkey anti-rabbit IgG-HRP conjugate (1:5,000), Amersham Pharmacia Biotechnology (Baie d'Urfe, Quebec, Canada), and goat anti-mouse IgG-HRP (1:1,250), Santa Cruz Biotechnology, Inc. Normal IgG was purified from preimmune rabbit serum using protein A-sepharose.

YEAST TWO-HYBRID ANALYSIS

Our full-length rat α4 cDNA was used as bait in yeast two-hybrid screening of a human K562 erythroleukemia Matchmaker cDNA library following the manufacturer's protocol (Clontech Laboratories, Inc., Palo Alto, CA). Candidate yeast colonies were assayed for β-galactosidase activity. Yeast mating and Leu^{+}-selection were performed to confirm positive clones.

CELL CULTURES

Suspension cultures of prolactin-dependent rat Nb2 T-lymphoma cells were maintained in Fischer's medium containing 10% fetal bovine serum (FBS; contains prolactin) and 10% horse serum (prolactin-free) as previously described [Too et al., 1987]. In some experiments, Nb2 cells were growth-arrested in medium containing 10% horse serum for 48 h, and then stimulated to enter the cell cycle by prolactin treatment (10 ng/ml). Human Jurkat T-leukemic and K562 myeloma cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS. MCF-7 cells were maintained in DMEM containing 10% (v/v) heat-inactivated FBS and supplemented with 1× MEM non-essential amino acids, 1 mM sodium pyruvate, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. In some experiments, MCF-7 cells were made quiescent in medium containing 1% charcoal-stripped FBS (steroid-free) for 24 h, and then cultured in 10% complete FBS. SV40-transformed African green monkey COS-1 kidney cells were maintained in 5% FBS-DMEM. All cell culture reagents were from Invitrogen or Sigma–Aldrich Canada Ltd.

DNA TRANSFECTIONS

Cells were transiently transfected as previously described [Boudreau et al., 2002]. Briefly, 2×10^5 cells/well in six-well dishes were cultured in 5% FBS–DMEM for 24 h and then washed with serum-free DMEM. To each well was added a LipofectAMINE–DNA mixture containing 5 μL LipofectAMINE^{TM} reagent (Invitrogen) and 1 μg Flag-tagged pSG5-EDD, pDNA3-α4 mutant (D4 or D5) or the vector alone, in a total volume of 1 ml DMEM. After 5 h at 37°C, the LipofectAMINE–DNA mixture was replaced with 5% FBS-DMEM. After 24–48 h, cell lysates were prepared for further analysis.

DELETION MUTANTS OF α4

The deletion mutants of α4, D4 and D5, were generated by PCR using the full-length pcDNA3-α4 [Boudreau et al., 2002] as template. PCR products were electrophoresed and excised from 1% agarose gels and cleaned using UltraClean 15 DNA Purification Kit (Medicorp, Inc., Montreal, Quebec, Canada). D4 and D5 DNAs were subcloned into pcDNA3 or pcDNA4/HisMax TOPO (Invitrogen), respectively, and DNA sequenced. The D4 mutant protein lacked the PP2Ac-binding site and contained the C-terminal epitope that was used to raise polyclonal anti-α4 antibodies [Boudreau et al., 2002]. The D5 mutant protein contained a N-terminal Xpress-tag and the C-terminal epitope.

IMMUNOPRECIPITATION AND IMMUNOBLOTTING (IP/IB)

Total cell lysates, 50×10^6 cells/treatment, were prepared for IP in RIPA buffer containing protease inhibitors [Dodd et al., 2000]. Immunocomplexes were used for sodium dodecylsulfate–polyacrylamide gel electrophoresis, followed by IB. Immunoreactive signals were detected with Super Signal ULTRA Kit (Pierce Chemical Co., Rockford, IL) or Immobilon Kit (Millipore Corp., Billerica, MA).

RESULTS

YEAST TWO-HYBRID SCREENING IDENTIFIES EDD AS A PARTNER OF α4

Using rat α4 as bait, yeast two-hybrid analysis identified PP2Ac and PP4c as partners of α4 (Table I). This validated our screening process since human α4 is known to interact with PP2Ac, PP4c, and PP6c

TABLE I. Identities of Yeast Two-Hybrid Clones Interacting With Rat α4 Bait

Clone	Protein	Nucleotide identity
16.16A	Human PP2Ac	535/555 (96%)
13.20.2	Human PP4c	671/673 (99%)
	Rat PP4c	606/670 (90%)
	Mouse PP4c	585/644 (90%)
10.1B.23	Human ubiquitin-protein ligase EDD	668/683 (97%)

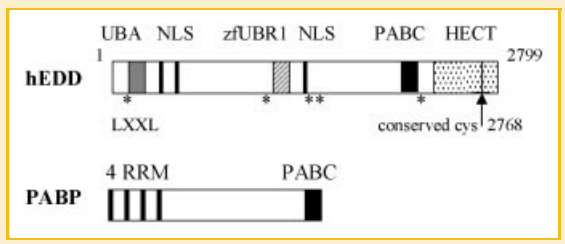

Fig. 1. EDD has a PABC domain that is also present in PABP. Structure of EDD: *UBA domain* binds mono-/multi-ubiquitin chains. *zfUBR1 domain* is RING-like zinc finger. *PABC domain* is also found in the C-terminus of PABP. *HECT domain* has E3 ubiquitin ligase activity. *LXXL motif* (*) is a potential steroid receptor binding site. NLS (▮), there are three putative nuclear localization signals. Structure of PABP: RRM, there are four RNA recognition motifs. PABC domain is the PABP C-terminal domain.

[Chen et al., 1998]. We also identified EDD E3 ubiquitin protein ligase as a new protein partner of α4 (Table I).

EDD is a progestin-induced gene in T47D breast cancer cells [Callaghan et al., 1998; Henderson et al., 2002] and is the mammalian ortholog of *Drosophila hyperplastic discs* gene (*hyd*) that controls cell proliferation during development [Mansfield et al., 1994]. The EDD/HYD/Rat100 proteins are large (~300 kDa), highly conserved, and predominantly nuclear [Henderson et al., 2002]. Nuclear localization of EDD has significance since α4 is a predominantly nuclear protein [Boudreau et al., 2002].

The EDD protein has a UBA domain, a RING-like zinc finger domain, a poly(A)-binding protein (PABP)-like domain, and a HECT domain [Henderson et al., 2002; Saunders et al., 2004] (Fig. 1). HECT and RING are the signature domains of the two main classes of E3 ubiquitin ligases. HECT proteins have intrinsic E3 ubiquitin ligase activities and target substrates for ubiquitin-mediated proteolysis. RING proteins may display intrinsic E3 ligase activity or are part of E3 ligase complexes [Callaghan et al., 1998; Beckmann et al., 2005]. EDD is the only known E3 ligase with both RING and HECT domains [Henderson et al., 2002]. The PABP-like domain in EDD is homologous to the C-terminus of PABP (PABC), which recruits translation factors to the poly(A)-tails of mRNAs. Since EDD and PABP each contains a PABC domain (Fig. 1), we have postulated that α4 may also interact with PABP.

α4 INTERACTS WITH EDD AND/OR PABP IN HEMATOPOIETIC CANCER CELLS

Since the interaction between α4 and EDD was identified through screening of a K562 cDNA library, formation of the α4–EDD complex was further investigated in hematopoietic cancer cell lines, such as rat Nb2 T-lymphoma, human K562 myeloma, and Jurkat T-leukemic cells. IP/IB analysis showed interaction of α4 with EDD in Nb2 cells (Fig. 2). The anti-EDD antibody from Santa Cruz Biotechnology, Inc. (anti-EDD-SC) was suitable for IB only, and it detected a faint 300-kDa immunoreactive band in the α4–immunocomplex prepared from 50×10^6 cells (Fig. 2; lane 4). A faint 300-kDa band was also detected in the total cell lysate (Fig. 2; lanes 1 and 5), using 50 μg protein/lane. We routinely co-IP with 20×10^6 Nb2 cells or load 20 μg protein/lane for strong immunoreactive signals [Boudreau et al., 2002; Dauphinee et al., 2005]. The absence of the α4–EDD immunocomplex in human Jurkat and K562 cells (Fig. 2; lanes 2 and 3) was surprising since the α4–EDD interaction was identified from screening a K562 cDNA library. The weak 300-kDa bands, or their absence, may be due to low levels of the EDD protein in these cells and/or low affinity of the anti-EDD antibodies from Santa Cruz Biotechnology, Inc. Therefore, anti-EDD antibodies from other sources and a Flag-tagged EDD construct were subsequently used to demonstrate a clearer interaction between α4 and EDD. Figure 2 also demonstrated that anti-PP2Ac antibodies did not immunoprecipitate EDD (Fig. 2; lanes 6–8), showing that EDD interacted specifically with α4 only, and not with the α4 partner PP2Ac.

IP/IB analysis confirmed the interaction of α4 with PABP in Nb2, Jurkat, and K562 cells (Fig. 2; lanes 2–4). PABP also

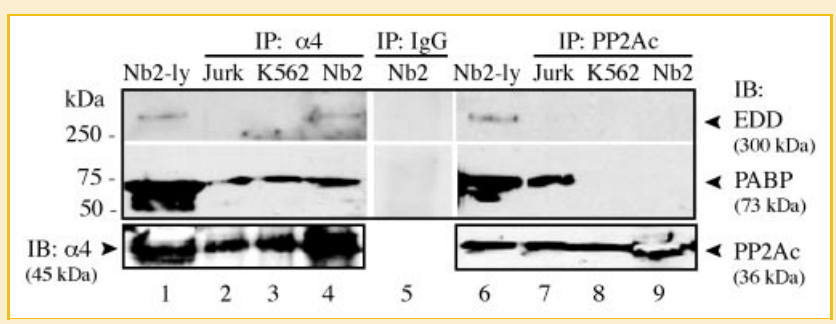

Fig. 2. α4 interacts with EDD and/or PABP in hematopoietic cancer cells. Lysates of Jurkat, K562, or Nb2 cells (50×10^6 each) were used for IP using anti-α4 or anti-PP2Ac antibodies or normal IgG, and followed by IB as indicated. Nb2-ly: total Nb2 lysate (50 μg protein/lane). Anti-EDD-SC (from Santa Cruz) was used for IB.

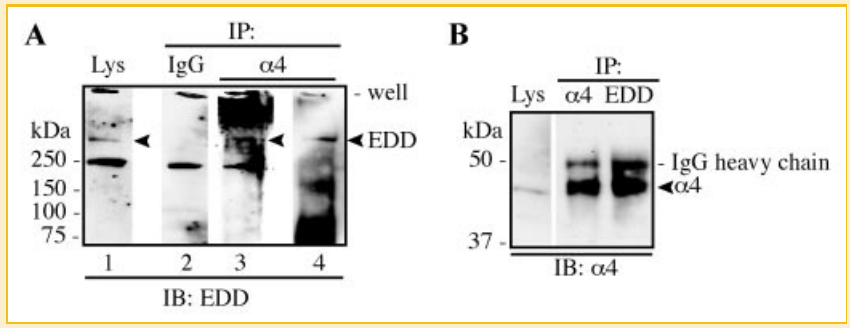

Fig. 3. IP of α4 or EDD in Nb2 cells. Nb2 lysates were used for IP of α4 or EDD as indicated, and normal IgG served as a control. IP of EDD was performed using anti-EDD-GI (from Garvan Institute). A: IB using anti-EDD-GI (lanes 1–3) was performed and detected a weak 300-kDa EDD and a dark 250-kDa non-specific band. Lane 3 was stripped and re-probed using anti-EDD-SC (lane 4). Arrowheads indicate the 300-kDa EDD. B: IP of α4 or EDD, followed by IB for α4 (45 kDa). Lys: Nb2 cell lysate.

co-immunoprecipitated strongly with the α4 partner, PP2Ac, but only in Jurkat cells (Fig. 2; lane 6), which may suggest cell specificity or formation of a transient PABP–PP2Ac complex only under conducive conditions.

Using anti-EDD antibodies from Garvan Institute (anti-EDD-GI), which were suitable for both IP and IB analyses, we detected a 300-kDa immunoreactive band in Nb2 cell lysates (Fig. 3A; lane 1) and in the Nb2 α4-immunocomplex (Fig. 3A; lane 2). When lane 2 was stripped and reprobed with anti-EDD_{SC}, a cleaner 300-kDa immunoreactive band was obtained (Fig. 3A; lane 3). The anti-EDD-GI antibodies also immunoprecipitated α4 (Fig. 3B), confirming the α4–EDD interaction.

α4 INTERACTS WITH EDD AND PABP IN TRANSFECTED COS-1 CELLS

The two anti-EDD antibodies detected the 300-kDa, EDD-specific immunoreactive band weakly, as well as non-specific bands (Figs. 2 and 3). Therefore, in a further attempt to obtain better immunoblots, COS-1 cells were transfected with a Flag-tagged-pSG5-EDD construct or vector alone (Con). IP of Flag or α4, followed by IB using anti-EDD_{SC} antibodies, showed the 300-kDa EDD (Fig. 4A). IB, using anti-α4 antibodies, detected α4 in the Flag-tagged complex (Fig. 4B). Expression of the Flag-tagged EDD was weak, and this could be due to poor transfection efficiency of the construct into COS-1 cells. However, when new anti-EDD antibodies (Abcam, Inc.) became available, co-IP analysis with these antibodies clearly demonstrated formation of an α4–EDD–PABP complex in COS-1 cells (Fig. 4C).

α4 INTERACTS WITH EDD IN MCF-7 BREAST CANCER CELLS

We have noted that, in IP/IB analyses, the 45-kDa α4 immunoreactive band was often partially hidden under the dense 50-kDa IgG heavy chain. Although our full-length rat α4 cDNA predicts a 39-kDa protein, the α4 protein in Nb2 cells is 45-kDa due to post-translational glycosylation [Boudreau et al., 2002]. Therefore, we sought to identify other cell lines to use for this study. Western

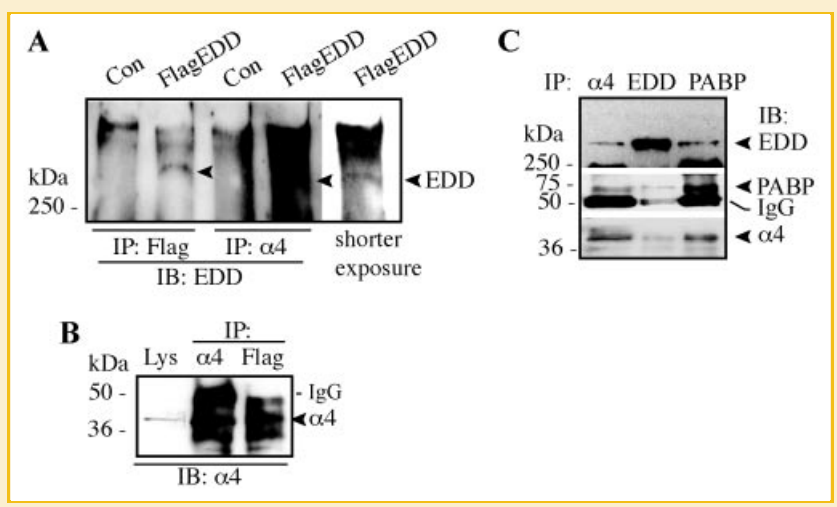

Fig. 4. α4 interacts with EDD and PABP in COS cells. In (A) and (B), COS-1 cells were transfected with Flag-tagged EDD or vector control. IP was performed using anti-Flag or anti-α4 antibodies, and followed by IB as indicated. A: IB was performed using anti-EDD-GI. Due to the dark intensity, lane 4 was re-exposed for a shorter time (lane 5). Arrowheads indicate the 300-kDa EDD. B: IB was performed using anti-α4 antibodies. Lys: COS cell lysate shows the position of the endogenous α4. C: Total COS-1 lysates were used for IP and the same blot was cut for IB with different antibodies as indicated. The anti-EDD antibodies were from Abcam, Inc.

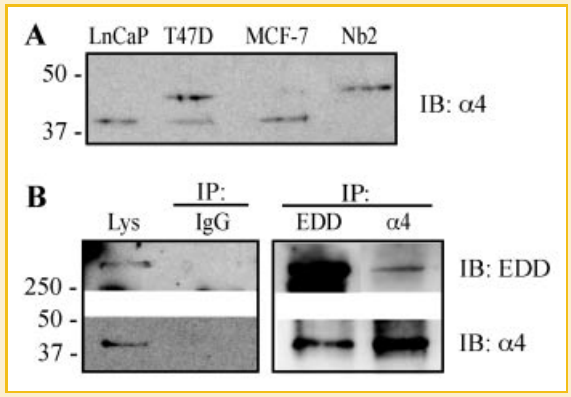

Fig. 5. α4 interacts with EDD in MCF-7 cells. A: IB for α4 in human cancer cell lines. B: MCF-7 cell lysates were used for IP/IB as indicated. IP with IgG served as control. The anti-EDD antibodies were from Abcam, Inc.

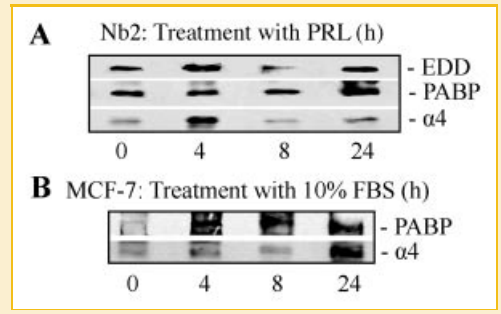

Fig. 6. α4 interaction with PABP and EDD is cell-cycle independent. Nb2 and MCF-7 cells were made quiescent as described in the Materials and Methods Section. Quiescent (A) Nb2 cells and (B) MCF-7 cells were treated with prolactin (10 ng/ml) or 10% FBS for the indicated times. IP of α4 was performed, followed by IB for α4, EDD, and PABP. EDD antibodies were from Abcam, Inc.

analysis showed that α4 was 39-kDa in size in some cell lines, such as human MCF-7 breast cancer and LNCaP prostate cancer cells, whereas T47D breast cancer cells expressed both the 39- and 45-kDa α4 (Fig. 5A). Similar to Nb2 cells, the higher molecular weight α4 in these human cancer cell lines could be due to glycosylation. However, other possibilities, such as phosphorylation and splice isoforms, could not be excluded at this stage. More importantly, co-IP experiments using MCF-7 or LNCaP cells would allow a better separation of 39-kDa α4 from the IgG heavy chain. We next confirmed that α4 also interacted with EDD in MCF-7 cells (Fig. 5B).

α4 INTERACTION WITH PABP AND EDD IS NOT DEPENDENT ON THE CELL CYCLE

Since α4 binds to PABP, we investigated whether this interaction was affected by re-entry of cells into the cell cycle. Therefore, prolactin-dependent Nb2 cells were made quiescent in medium containing 10% horse serum (prolactin-free) before treatment with prolactin. MCF-7 cells were made quiescent with 1% charcoal-stripped FBS (steroid-free), and then given 10% complete FBS. In each case, IP of α4 was performed, followed by IB. As seen in Figure 6, α4 formed a complex with PABP and/or EDD in quiescent and actively growing cells. The co-IP analysis was not absolutely quantitative but we can conclude that α4 is capable of binding PABP and EDD, regardless of the cell cycle.

GENERATION AND DETECTION OF α4 MUTANTS

Deletion mutants of α4, D4 and D5, were generated to determine the α4 binding sites for EDD (Fig. 7). When the D4 or D5 constructs were transfected into MCF-7 cells breast cancer cells, Western analysis showed the D4 and D5 proteins to be approximately 33 and 23 kDa, respectively. The D4 mutant, lacking the PP2A-binding site, was detectable using polyclonal anti-α4 antibodies. The D5 mutant was detected using either anti-Xpress or anti-α4 antibodies (Fig. 7).

EDD BINDS TO THE C-TERMINAL REGION OF α4

The D4 and D5 constructs were transfected into MCF-7 cells for Western and IP/IB analyses. In total lysates, both the 39-kDa endogenous α4 and the 33-kDa D4 protein were detected in cells transfected with the D4 construct (Fig. 8A). Untransfected, control cell lysates only had the endogenous α4. Co-IP analysis detected the D4 protein in the EDD immunocomplex, the D4 protein co-migrated alongside the D4 protein in total cell lysates (Fig. 8A).

Similarly, the 23-kDa D5 protein was detected only in D5-transfected MCF-7 cells and not in untransfected controls, using either anti-α4 or anti-Xpress antibodies (Fig. 8B). Co-IP analysis showed interaction of EDD with the D5 protein (Fig. 8B). Therefore, EDD binds to the C-terminal region of α4, independent of the α4-PP2Ac binding site (see Fig. 7).

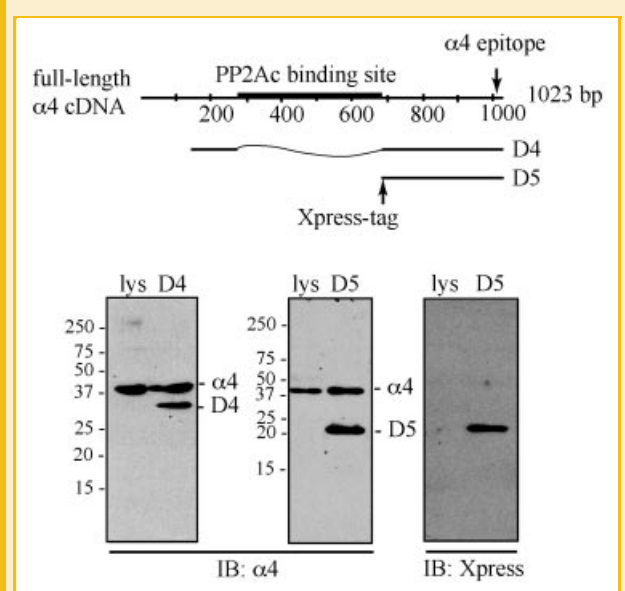

Fig. 7. Deletion mutants of α4. MCF-7 cells were transfected with the D4 or D5 construct. After 48 h, cell lysates were prepared for Western analysis. D4, detected using anti-α4 antibodies, was ~33 kDa. D5, detected using either anti-α4 or anti-Xpress antibodies, was ~23 kDa.

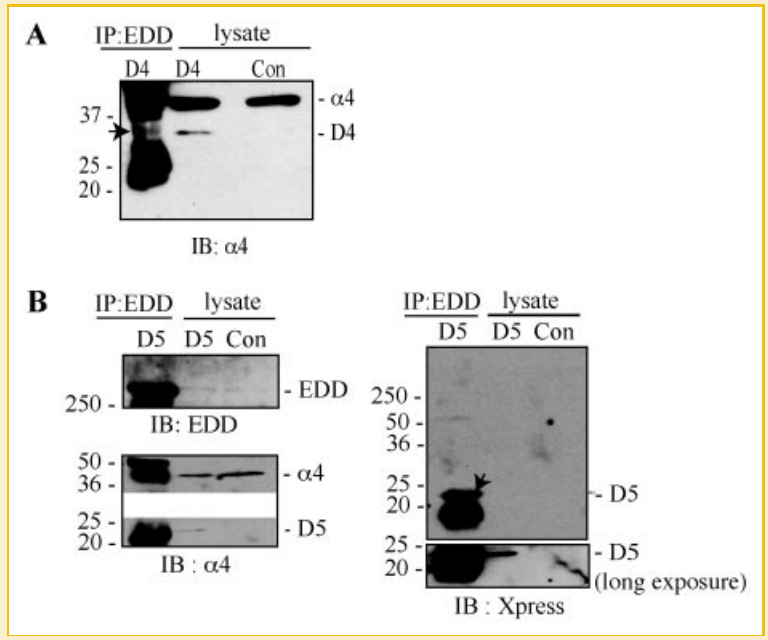

Fig. 8. EDD interacts with D4 and D5. MCF-7 cells were transfected with the D4 or D5 construct, whereas control cells (Con) were not. Total lysates of transfected and controls cells were used for Western analysis. A: IP of EDD was followed by IB for α4 antibodies. Arrow: D4 protein in the EDD immunocomplex. B: IP of EDD was followed by IB, using anti-α4 (left panel) or anti-Xpress (right panel) antibodies. In the latter, the D5 protein in cell lysates was detected after a longer film exposure. Arrow: D5 protein in the EDD immunocomplex. Anti-EDD antibodies for IP and IB were from Abcam, Inc.

DISCUSSION

We have identified EDD E3 ubiquitin ligase and PABP as two new protein partners of the α4 phosphoprotein. Our findings suggest a new role(s) for α4 in the initiation of translation, in addition to its regulation of PP2Ac activity in the mTOR pathway.

EDD is a critical regulator of protein turnover during the initiation of translation. EDD binds to PABP-interacting protein 2 (Paip2) and targets Paip2 for degradation [Yoshida et al., 2006]. In the absence of Paip2, the free PAPB binds to mRNA to initiate protein translation [Yoshida et al., 2006]. PABP is a highly conserved protein that serves as a scaffold to organize the mRNA–protein complex [Mangus et al., 2003]. The N-terminal RRM motifs of human PABP (Fig. 1) bind to the poly(A)-tail of mRNAs and to eIF4G at the 5′-cap of mRNAs to enable mRNA circularization. The C-terminal PABC domain recruits mRNA-processing factors to promote mRNA maturation [Kozlov et al., 2004].

The α4 phosphoprotein has also been shown to interact with a microtubule-associated protein (MAP) called MID1 [Liu et al., 2001; Trockenbacher et al., 2001]. MID1 is a RING-finger E3 ubiquitin ligase that normally targets PP2Ac for degradation through its binding to α4. Mutation of *MID1* causes impairment of ubiquitin-ligase activity, resulting in a marked accumulation of PP2Ac and hypophosphorylation of other MAPs.

The possibility that α4 undergoes ubiquitination by EDD is under further investigation. Mono-ubiquitination may increase α4 stability, whereas polyubiquitination may target it for proteolytic degradation. In any event, changes in α4 stability may be expected to affect its interaction with its protein partners and/or their activities. For example, altered levels of α4 may either increase or decrease PP2Ac activity to hypo/phosphorylate p70S6K and 4E-BP1 [Nanahoshi et al., 1999; Nien et al., 2007]. The effect of α4 turnover on PABP and the significance of the α4–EDD–PABP complex are not known and will be investigated.

In summary, α4 has multiple partners. Its interaction with PP2Ac, EDD, and PABP suggests that α4 is involved in multiple steps in the mTOR pathway leading to the initiation of translation.

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