

Overexpression of Clast4 Reduces Cell Proliferation

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The eIF4E protein is the key regulator of translation initiation. The interaction of eIF4E with eIF4G triggers the translation of mRNA, and several proteins interrupt this association to modulate translation. Human 4E-T is one of the eIF4E-binding partners that represses the translation of bound mRNAs, and it is involved in the transport of eIF4E to processing bodies (P-bodies). Although Clast4, the mouse homolog of human 4E-T, might play critical roles in the regulation of translation, its properties are not well known. In this report, we deciphered the properties of Clast4 by determining its phosphorylation state, binding to eIF4E, and effects of overexpression on cell proliferation. Clast4 was phosphorylated by protein kinase A (PKA) *in vivo* on several residues of its amino terminus. Nevertheless, the PKA phosphorylation of Clast4 appeared to have no effect on either its eIF4E-binding ability or localization. Clast4 interacted with eIF4E1 and CPEB. The conserved eIF4E-binding sequence in Clast4, YXXXXL ϕ , was important for binding eIF4E1A but not eIF4E1B. Similar to that of another well-known eIF4E regulator, the eIF4E binding protein (4E-BP), the overexpression of Clast4 decreased cell proliferation. These results suggest that Clast4 acts as a global translation regulator in cells.

Key words : 4E-T, Clast4, CPEB, eIF4E1, translation initiation

Introduction

In eukaryotes, the translation of mRNA is tightly regulated to orchestrate many cellular mechanisms such as cell proliferation, differentiation, neural cell plasticity, embryo development, and oocyte maturation [12, 19]. Therefore, misregulation of these steps is related with cell malfunction, such as the creation of cancer cell [1]. Translation starts with the formation of eukaryotic initiation factor 4F (eIF4F) complex, which recruits ribosomes and other initiation factors [6]. The eIF4F complex consists of eIF4E, eIF4G, and eIF4A. The eIF4E interacts with the 5' cap structure, m⁷GpppN, which is present on all nuclear-encoded mRNAs. The eIF4G, a scaffold protein, binds the eIF4E and recruits an eIF4A, ATP-dependent RNA helicase, to resolve the mRNA secondary structure [6]. Because eIF4E is the first protein incorporated into the translation initiation complex in mRNA among eIF4F complex factors, it is a major target for regulation. The eIF4G subsequently binds eIF4E for the basal

translation initiation complex, and this interaction is regulated by factors such as the 4E-BP. Hypophosphorylated 4E-BP competes with eIF4G to bind with eIF4E through its YXXXXL ϕ (ϕ is the hydrophobic residue) motif, which is also found in the eIF4G protein [11]. By hyperphosphorylation through the mTOR signaling pathway, 4E-BP dissociates from eIF4E, resulting in the docking of eIF4G to eIF4E to initiate translation [7]. Mutation or unregulated hyperphosphorylation of 4E-BP induces an increase of proliferation due to weakening or loss of interaction with eIF4E in cancer cells [17]. Translation initiation is regulated not only by proteins on the 5' untranslated region (UTR) but also by the factors on the 3'UTR. Cytoplasmic polyadenylation element-binding protein (CPEB) is loaded on the cytoplasmic polyadenylation element (CPE) of 3'UTR [12]. CPEB is implicated in the repression of translation by interacting poly(A)-specific ribonuclease (PARN) to shorten the poly(A) tail or by binding one of the proteins such as Maskin, Neuroguidin, and eIF4E transporter (4E-T) to obstruct the association of eIF4E with eIF4G [2, 9]. Following a translation initiation signal (e.g., growth factors or nutrients), CPEB is phosphorylated, and it facilitates poly(A) tail elongation on the 3'UTR. The poly(A)-binding protein (PABP) is placed on the poly(A) tail to generate a closed-loop structure by the interaction with eIF4G on the 5'UTR. This structure stabilizes the initiation complex and enhances the

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initiation of translation [12].

Clast4 is a mouse homolog of human 4E-T [20], a protein responsible for the transport of eIF4E from cytoplasm to nucleus or P-body [4]. It is believed that mRNA decay and/or storage take place in the P-body, because they contain some proteins related to RNA degradation such as PAT1, Dcp1 and Xrn1 [14]. Human 4E-T is also a P-body component protein, and the depletion of 4E-T induces a remarkable decrease of P-body assembly in HeLa cells [5]. Given that Clast4 also possesses the eIF4E-binding motif, YXXXXL ϕ , it appears to be a translation regulator interrupting the interaction between eIF4E and eIF4G. This is consistent with the fact that the *Drosophila* ortholog of Clast4, Cup, interacts with eIF4E and 3'UTR-bound factors to repress translation during ovary development and growth [15]. It has been reported that *Xenopus* 4E-T can associate with CPEB similar to how Maskin does in the *Xenopus* oocyte, and *Xenopus* 4E-T also binds eIF4E1B, a germinal-specific isoform of eIF4E, in an YXXXXL ϕ -independent manner, unlike eIF4E1A, which is a canonical form of eIF4E1 [13]. However, the regulation mechanism of the mouse Clast4 protein is still unclear. Therefore, in this report we deciphered the properties of Clast4 by examining its phosphorylation, binding properties, and overexpression effect on cell proliferation.

Materials & Methods

Materials

The anti-4E-T and anti-phospho (Ser/Thr) PKA substrate antibodies were purchased from Bethyl (Montgomery, AL) and Cell Signaling (Danvers, CO), respectively. FLAG epitope antibody and other chemicals were purchased from Sigma Aldrich (St. Louis, MO). Oligonucleotide synthesis and DNA sequencing was performed by Cosmo Genetech (Seoul, Korea). The eIF4E1B gene was isolated from mouse ovaries by RT-PCR with a forward primer of T A G C C C G G G T A C C A T G A A-CAAAGTTGAGGTTGGAGGGC and a reverse primer of TTAACGCGCCGCCACCACAACTTGTGTTGCTAAG.

Construction of Clast4 mutants

The Clast4 cDNA was obtained from Open Biosystems (MMM1013-7512592, Clone ID: 5359290) and cloned into the pcDNA 3.1 topo (Invitrogen Corp., Carlsbad, CA) or pCMV Tag2 (Clontech, Mountain View, CA) vectors. To determine the localization of Clast4, the Clast4-GFP fusion gene was

generated by inserting the GFP gene into the Clast4-pcDNA 3.1 construct. The truncated forms of Clast4 (Fig. 1D) were made by cutting with restriction enzymes or by PCR reaction. To generate the non-phosphorylated form of Clast4, indicated serine or threonine sites of Clast4 (Ser212, Thr256, Ser258, Ser352) were mutated using the QuikChange Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA) with specific primers.

Immunoprecipitation and *in vitro* kinase assay

HEK293T cells were transfected with JetPEI (Polyplus-transfection Inc., NY) at 60% confluence, and harvested with lysis buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 0.1% NP40, 1 mM EDTA, 1 μ g/ml leupeptin, 1 mM aprotinin, 1 mM PMSF, 1 mM sodium orthovanadate, 1 mM NaF) 36 h after transfection. Harvested cell lysate was incubated with anti-FLAG antibody at 4°C and then precipitated using protein G sepharose (GE Healthcare Bio-Sciences, Piscataway, NJ). The immunoprecipitates were washed with lysis buffer and kinase buffer (25 mM Tris-HCl pH 7.5, 5 mM β -glycophosphate, 0.2 mM dithiothreitol, 1 mM sodium orthovanadate, 1 mM magnesium chloride) three times, consecutively. The immunoprecipitates were incubated with kinase buffer containing 250 μ M ATP and 10 μ Ci/ml [γ -³²P] ATP (3000 Ci/mmol, PerkinElmer) for 30 min at 37°C with or without 5 units of PKA catalytic subunit (Promega, Madison, Wisconsin), Plk1, Aurora A kinase (SignalChem, Richmond, CA), and Cdk1, respectively. The samples were resolved on the 8% SDS-PAGE gel and transferred to PVDF membranes. Radiolabeled Clast4 was detected with the X-ray film (Kodak, Rochester, NY) or by a phosphor image analyzer (Typhoon 9400, GE Healthcare Bio-Sciences).

In vivo phosphorylation

For the *in vivo* phosphorylation test, Clast4-transfected cells were cultured in the DMEM with 0.2% FBS for 16 hr. To activate *in vivo* PKA, 10 μ M Forskolin and 0.1 mM IBMX were treated for 30 min with or without 10 μ M H89. The immunoprecipitated Clast4 was detected with anti-phospho (Ser/Thr) PKA substrate antibody following SDS-PAGE and transferred to a PVDF membrane.

Co-immunoprecipitation

HA-tagged eIF4E1B-transfected cells were divided into 60 mm dishes, and FLAG-tagged Clast4 fragments were sub-

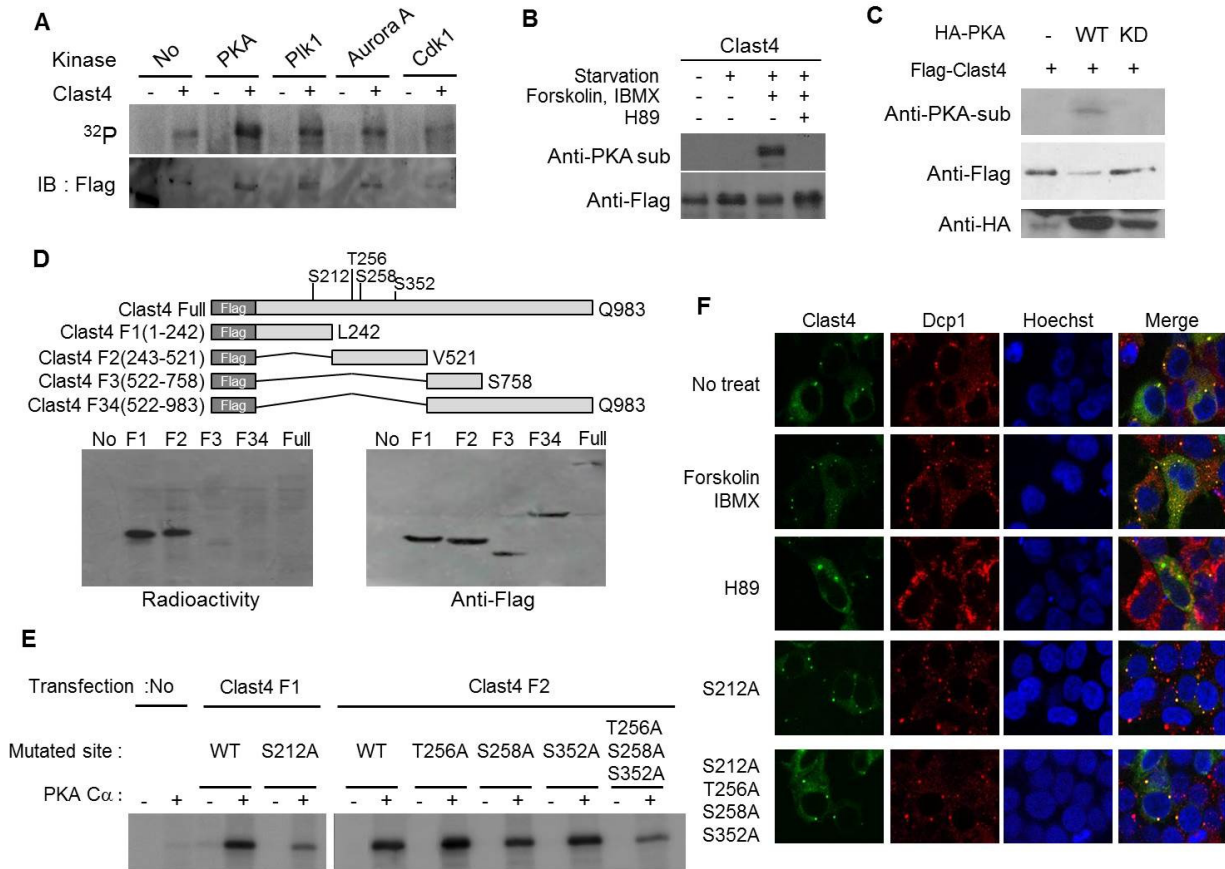


Fig. 1. PKA phosphorylates Clast4 *in vivo*. (A) Immunoprecipitated FLAG-Clast4 was incubated with PKA, Plk1, Aurora A kinase, and Cdk1/cyclin B complex with 5 μ Ci [γ - 32 P] ATP. The phosphorylation state and the expression of Clast4 were checked by autoradiography (upper panel) and FLAG-specific antibody (lower panel) after SDS-PAGE. (B) Clast4-transfected HEK293T cells were treated with forskolin and IBMX. Immunoprecipitated Clast4 was detected with anti-phospho (Ser/Thr) PKA substrate antibody (upper panel) and FLAG-specific antibody (lower panel). The phosphorylation of Clast4 was completely diminished by the addition of 10 μ M of H89. (C) Clast4-transfected cells were co-transfected with either wild-type (WT) or kinase-dead mutant (KD) of PKA catalytic subunit. Western blot analysis for immunoprecipitates using anti-phospho (Ser/Thr) PKA substrate antibodies indicated that Clast4 is phosphorylated by WT PKA overexpression (upper blot). The expression of Clast4 and PKA constructs was confirmed using anti-FLAG or HA antibodies, respectively (lower two blots). (D) The amino terminus of Clast4 is the major PKA phosphorylation site. A schematic diagram of the FLAG-tagged full-length or truncated form of Clast4 is shown (upper image). The putative PKA phosphorylation sites (S212, RRRNDSYT; T256, S258, RTRRRRTASVK; S352, GSRSSSLG) were indicated. The *in vitro* kinase assay with PKA catalytic subunits in [γ - 32 P] ATP showed that F1 and F2 fragments were phosphorylated by PKA (left panel). The immunoprecipitated proteins were monitored using FLAG antibody (right panel). (E) The putative PKA-phosphorylated serine or threonine amino acids were changed to alanine. The phosphorylation states of mutated F1 or F2 constructs were checked by autoradiography after an *in vitro* kinase assay. (F) The localization of EGFP-tagged wild-type or phosphorylation sites mutated Clast4 were checked after transfection into HEK293T cells. The localization of Clast4 to P-body was increased by PKA inhibitor treatment but not by the mutation of phosphorylation sites. Dcp1 and Hoechst were used as P-body and nucleus indicators, respectively.

sequently transfected. After immunoprecipitation with FLAG antibody, the western blotting was performed with HA antibody.

Immunocytochemistry

HEK293T cells were cultured on the poly-L-lysine-coated slide glass and transfected with GFP-tagged Clast4. After se-

rum starvation for 16 hr, forskolin and IBMX were treated with or without H89. The cells were fixed in 4% formaldehyde in PBS for 1 hr and were permeabilized in blocking buffer (1% BSA, 0.3% Triton-X-100 in PBS). The Dcp1 antibody in blocking buffer was added for 2 hr followed by the treatment of CY3-conjugated anti-rabbit (PA43004, GE Healthcare Bio-Sciences) antibody. The samples were ob-

served under a confocal laser-scanning microscope (LSM 510 META, Carl Zeiss, Germany).

Proliferation test

The GFP, Clast4, or 4E-BP-transfected cells (2,000/well) were plated in 96-well culture plates, and 20 μ l of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, 0.5 mg/ml in PBS) was added at the indicated time point to check the number of viable cells. After incubation for 4 h at 37°C, generated formazan was dissolved in MTT assay solution (4 mM HCL, 0.1% NP40 in isopropanol). The absorbance of the colored solution was quantified by measuring at 590 nm with a reference filter of 620 nm using a microplate reader (MQX 200R, BioTek Instruments, Winooski, VT, USA).

Results and Discussion

Clast4 is phosphorylated by PKA

It has been reported that Clast4 is phosphorylated by an unknown kinase during oocyte maturation [20]. To identify the kinase(s) that phosphorylates Clast4 during cell cycle progression, we performed *in vitro* kinase assays with several of the kinases involved in cell cycle regulation. Among the kinases tested, PKA could phosphorylate Clast4 much more strongly than the other kinases (i.e., polo-like kinase 1 (plk1), Aurora A kinase, and cyclin-dependent kinase 1 (Cdk1)) (Fig. 1A). Because human 4E-T is shuttled to the P-body, and PKA is related to the P-body formation in yeast [18], we focused on the role of PKA in Clast4 phosphorylation. To confirm the phosphorylation of Clast4 *in vivo*, PKA was activated by the treatment of forskolin, an adenylyl cyclase activator, and IBMX, a phosphodiesterase inhibitor. After the immunoprecipitation of Clast4, the phosphorylation state of Clast4 was detected with anti-phospho (Ser/Thr) PKA substrate antibody that could detect the phosphorylated conserved site (RXXpS/T). Clast4 was phosphorylated *in vivo* by the activation of PKA, and this effect was completely removed by treatment of H89, a PKA inhibitor (Fig. 1B). In addition, the phosphorylation state of Clast4 in the cell was increased by the overexpression of PKA catalytic subunit, not by that of the kinase-dead form (Fig. 1C). These results show that Clast4 is *per se* a PKA substrate in the cell.

Using web-based programs (<http://scansite.mit.edu/> and <http://www.phosphosite.org/homeAction.do>), the S212,

S256, S258, and S352 sites were found to be potential PKA phosphorylation sites. To delineate PKA phosphorylation sites in Clast4, several truncated forms of Clast4 were generated. F1 and F2 (but not F3 or F4) fragments were phosphorylated by PKA catalytic subunit in [γ -³²P] ATP-containing reaction buffer (Fig. 1D), indicating that the amino-terminal half of Clast4 contains the major PKA phosphorylation sites. To define the phosphorylated residues in the F1 and F2 fragments, S212 of F1 and T256, S258, and S352 of F2 residues were mutated to alanine, a non-phosphorylated amino acid residue, and *in vitro* phosphorylation was conducted (Fig. 1E). The degree of [γ -³²P] incorporation into the F1 fragment was reduced by the mutation of S212A. In the F2 fragment, the signal intensity was not significantly reduced in the single amino acid mutants, but it was dramatically decreased by the mutation of all putative phosphorylation sites. These results strongly suggest that multiple sites of Clast4 can be phosphorylated by PKA.

The depletion of human 4E-T induces a significant reduction in the size of the P-body [5], and the oxidative stress promotes multisite human 4E-T phosphorylation by c-Jun N-terminal kinase (JNK) to facilitate the formation of larger P-body [3]. In addition, PKA catalytic subunit Tpk associates with the translation initiation factors Pab1 and Rps3 in exponentially growing cells and possibly controls cap-dependent translation by regulating Rpg1 and eIF4G₁ proteins in yeast. Glucose starvation promotes the loss of interaction between Tpk and initiation factors followed by their accumulation into P-bodies [18]. Therefore, we checked the effect of PKA phosphorylation on the P-body localization of Clast4 by the transfection of GFP-fused wild-type or phosphorylation site mutant Clast4. After transfection into HEK293T cells, Clast4 was found in both the P-body and cytoplasm (Fig. 1F). Although treatment of PKA inhibitor (H89) led to an increase of P-body size, mutation of the PKA site on Clast4 had no effect on either its localization or the size of the P-body (Fig. 1F). It would be very interesting to investigate the relationship between Clast4 phosphorylation by other kinases and P-body formation.

Clast4 interacts with eIF4E1 and CPEB

It has been proposed that human 4E-T plays a similar role with Maskin, another eIF4E-binding protein, modulating the initiation of translation by linking eIF4E on the Cap structure and CPEB on the 3'UTR of mRNA. However, to our knowledge, no report has described the interaction of Clast4 with

CPEB and eIF4E in the mammalian system, which led us to investigate the binding properties of Clast4 with these proposed partners. Co-immunoprecipitation results show that Clast4 associates with CPEB, eIF4E1A, and eIF4E1B (Fig. 2A). Because eIF4E1A can bind with the protein that contains the eIF4E-binding motif, YXXXXL ϕ , the corresponding tyrosine residue of Clast4 was changed to alanine, and the binding specificity between Clast4 and either eIF4E1A or eIF4E1B was monitored. The binding affinity between Clast4 and eIF4E1A was dramatically reduced by this mutation, but it was not for eIF4E1B (Fig. 2B). These results suggest that Clast4 associates with both eIF4E1s in a different manner of eIF4E-binding motif dependency. By co-immunoprecipitation with several truncated forms of Clast4, the amino terminus of Clast4 was identified as a binding part to eIF4E1B (Fig. 2C). Given that the Clast4 can bind with

eIF4E1s and CPEB, Clast4 could be involved in mRNA translation regulation. However, the way in which the binding of Clast4 with other factors is regulated and the kinds of mRNA that are modulated by the action of Clast4 are currently unclear.

The binding of 4E-BP with eIF4E relies on phosphorylation by the mTOR signal pathway, and the hyper-phosphorylated human 4E-T has a weak affinity for eIF4E in mitosis [16]. Clast4 is phosphorylated by an unknown kinase in the MI and MII phases during mouse oocyte maturation [20]. Therefore, the effect of Clast4 phosphorylation on the binding properties with eIF4E was monitored. The co-immunoprecipitation result demonstrated that the mutant of the putative PKA phosphorylation sites of Clast4 does not have an effect on the binding capacity between Clast4 and eIF4E (Fig. 2D). Although this result is somewhat dis-

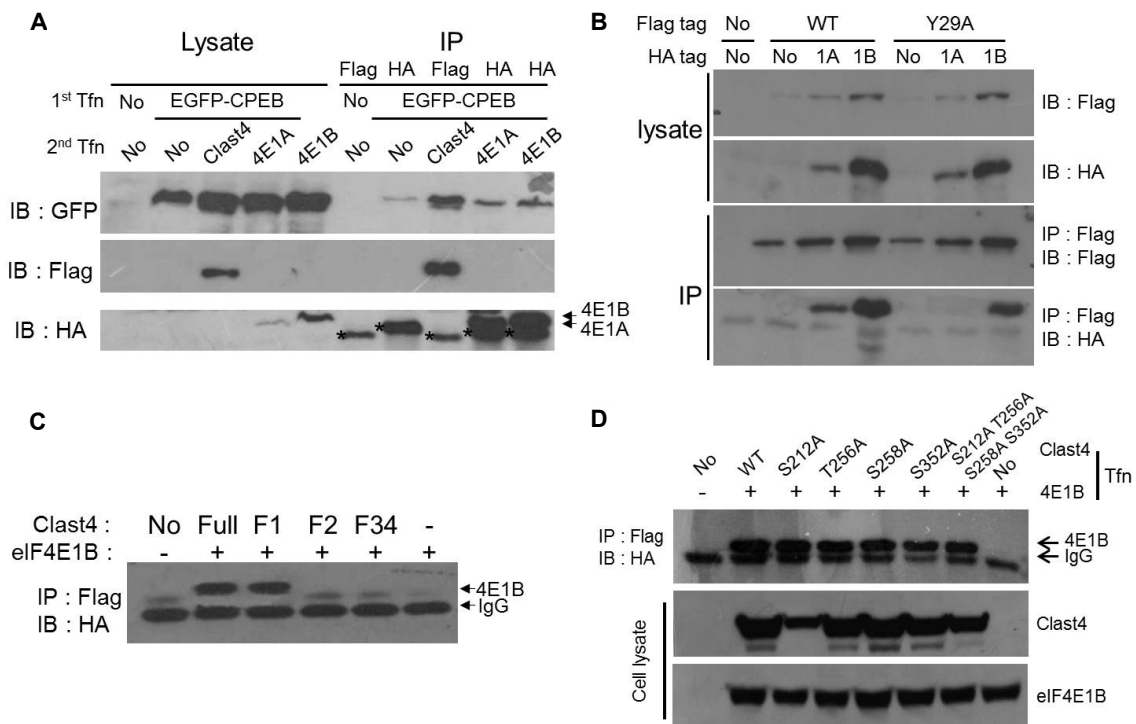


Fig. 2. Clast4 interacts with eIF4E1s and CPEB (A) After co-transfection of EGFP-CPEB and one of FLAG-Clast4, HA-eIF4E1A, or HA-eIF4E1B, the interaction between two proteins was checked by co-immunoprecipitation. Clast4 bound CPEB directly, while eIF4E1A and eIF4E1B were not able to bind directly with CPEB. Asterisks indicate the IgG light chain. (B) After either the wild-type or Y29A mutant form of Clast4 was transfected, either eIF4E1A or eIF4E1B was consecutively transfected. The association with Clast4 and eIF4E was monitored by co-immunoprecipitation. While the wild-type of Clast4 was shown to bind with eIF4E1A and eIF4E1B, the binding affinity of Clast4 with eIF4E1A (but not with eIF4E1B) was abolished by the mutation of tyrosine 29 to alanine. (C) eIF4E1B associated with the amino-terminus of Clast4. After the co-transfection of HA-eIF4E1B and one of the truncated forms of FLAG-Clast4, the co-immunoprecipitates were detected using HA antibody. (D) None of the putative PKA phosphorylation sites was involved in its ability to bind eIF4E. One of the FLAG-tagged Clast4 phosphorylation site mutants and HA-tagged eIF4E1B were transfected into HEK293T cells, and the co-immunoprecipitation of Clast4 and eIF4E1B was monitored.

appointing, we think that ruling out one candidate kinase that might be able to regulate Clast4 is also informative for further studies. As several kinases such as Aurora A kinase, Plk1, Greatwall kinase, and MAP kinase are activated during the MI - MII transition in oocyte maturation, these kinases could be involved in Clast4 phosphorylation. In fact, several kinases—including PKA, Plk1, Aurora A kinase, and cdc2/cyclin B—can phosphorylate Clast4 at least *in vitro* (Fig. 1). To elucidate the role of Clast4 in translation regulation, the kinases responsible for Clast4 phosphorylation must be revealed.

Overexpression of Clast4 delays cell proliferation

Because 4E-BP binds eIF4E to hinder translation initiation, and the expression level of 4E-BP correlates with oncogenic activation [17], we checked whether the overexpression of Clast4 influences cell proliferation. The overexpression of 4E-BP decreased cell proliferation by up to 30% compared to GFP overexpression. Interestingly, Clast4 overexpression also reduced cell proliferation as much as 4E-BP did (Fig. 3). This result demonstrated that Clast4 might act as an mRNA translation regulator similar to 4E-BP. Although 4E-BP has received attention as an important regulator of translation initiation, Clast4 seems to be a new candidate

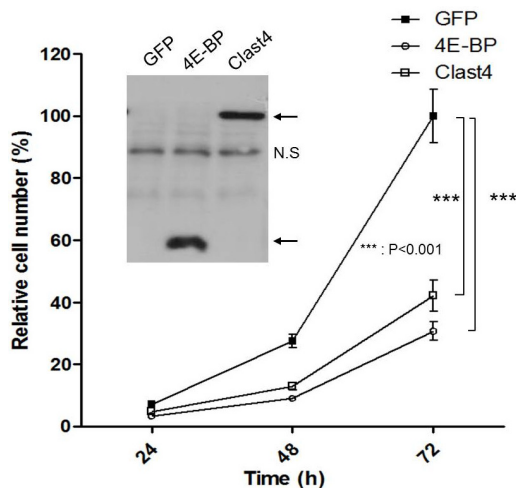


Fig. 3. Overexpression of Clast4 decreases the proliferation rate of the cell. (A) The GFP, 4E-BP, or Clast4 construct was introduced into 4×10^3 HEK293T cells, and the cell numbers were counted at the indicated times. Statistically significant differences of cell proliferation in Clast4 or 4E-BP expression cells compared to GFP expression cells are represented by asterisks (***) $p < 0.001$. Results represent three separate experiments. The inset panel indicates the expression of transfected proteins (NS: nonspecific band).

for the performance of a similar function. Recently, another result showed that treatment of human 4E-T siRNA in HeLa cells increases global protein synthesis by about 55%, which reinforces this idea [10]. Since the *Drosophila* homolog, CUP, promotes deadenylation and inhibits the decapping of mRNA targets [8], it is possible that Clast4 inhibits translation using a different mechanism from that of 4E-BP. It would be very interesting to determine the new function of Clast4 during inhibition of translation.

In this study, we found that Clast4 is phosphorylated by PKA *in vivo* on several residues of its amino terminus and Clast4 can associate with CPEB and eIF4E1. The overexpression of Clast4 decreases cell proliferation. Collectively, all of these results suggest that Clast4 acts as a novel global translation regulator in the cell.

Acknowledgements

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References

- Audic, Y. and Hartley, R. S. 2004. Post-transcriptional regulation in cancer. *Biol Cell* **96**, 479-498.
- Cao, Q. and Richter, J. D. 2002. Dissolution of the maskin-eIF4E complex by cytoplasmic polyadenylation and poly(A)-binding protein controls cyclin B1 mRNA translation and oocyte maturation. *EMBO J* **21**, 3852-3862.
- Cargnello, M., Tcherkezian, J., Dorn, J. F., Huttlin, E. L., Maddox, P. S., Gygi, S. P. and Roux, P. P. 2012. Phosphorylation of the eukaryotic translation initiation factor 4E-transporter (4E-T) by c-Jun N-terminal kinase promotes stress-dependent P-body assembly. *Mol Cell Biol* **32**, 4572-4584.
- Dostie, J., Ferraiuolo, M., Pause, A., Adam, S. A. and Sonenberg, N. 2000. A novel shuttling protein, 4E-T, mediates the nuclear import of the mRNA 5' cap-binding protein, eIF4E. *EMBO J* **19**, 3142-3156.
- Ferraiuolo, M. A., Basak, S., Dostie, J., Murray, E. L., Schoenberg, D. R. and Sonenberg, N. 2005. A role for the eIF4E-binding protein 4E-T in P-body formation and mRNA decay. *J cell Biol* **170**, 913-924.
- 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.
- Heesom, K. J. and Denton, R. M. 1999. Dissociation of the eukaryotic initiation factor-4E/4E-BP1 complex involves phosphorylation of 4E-BP1 by an mTOR-associated kinase.

- FEBS Lett* **457**, 489-493.
8. Igreja, C. and Izaurralde, E. 2011. CUP promotes deadenylation and inhibits decapping of mRNA targets. *Genes Dev* **25**, 1955-1967.
 9. Jung, M. Y., Lorenz, L. and Richter, J. D. 2006. Translational control by neuroguidin, a eukaryotic initiation factor 4E and CPEB binding protein. *Mol Cell Biol* **26**, 4277-4287.
 10. Kamenska, A., Lu, W. T., Kubacka, D., Broomhead, H., Minshall, N., Bushell, M. and Standart, N. 2014. Human 4E-T represses translation of bound mRNAs and enhances microRNA-mediated silencing. *Nucleic Acids Res* **42**, 3298-3313.
 11. 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 gamma and the translational repressors 4E-binding proteins. *Mol Cell Biol* **15**, 4990-4997.
 12. Mendez, R. and Richter, J. D. 2001. Translational control by CPEB: a means to the end. *Nat Rev Mol Cell Biol* **2**, 521-529.
 13. Minshall, N., Reiter, M. H., Weil, D. and Standart, N. 2007. CPEB interacts with an ovary-specific eIF4E and 4E-T in early Xenopus oocytes. *J Biol Chem* **282**, 37389-37401.
 14. Mir, M. A., Duran, W. A., Hjelle, B. L., Ye, C. and Pangniban, A. T. 2008. Storage of cellular 5' mRNA caps in P bodies for viral cap-snatching. *Proc Natl Acad Sci USA* **105**, 19294-19299.
 15. Piccioni, F., Zappavigna, V. and Verrotti, A. C. 2005. A cup full of functions. *RNA Biol* **2**, 125-128.
 16. Pyronnet, S., Dostie, J. and Sonenberg, N. 2001. Suppression of cap-dependent translation in mitosis. *Genes Dev* **15**, 2083-2093.
 17. She, Q. B., Halilovic, E., Ye, Q., Zhen, W., Shirasawa, S., Sasazuki, T., Solit, D. B. and Rosen, N. 2010. 4E-BP1 is a key effector of the oncogenic activation of the AKT and ERK signaling pathways that integrates their function in tumors. *Cancer Cell* **18**, 39-51.
 18. Tudisca, V., Simpson, C., Castelli, L., Lui, J., Hoyle, N., Moreno, S., Ashe, M. and Portela, P. 2012. PKA isoforms coordinate mRNA fate during nutrient starvation. *J Cell Sci* **125**, 5221-5232.
 19. Ule, J. and Darnell, R. B. 2006. RNA binding proteins and the regulation of neuronal synaptic plasticity. *Curr Opin Neurobiol* **16**, 102-110.
 20. Villaescusa, J. C., Allard, P., Carminati, E., Kontogiannea, M., Talarico, D., Blasi, F., Farookhi, R. and Verrotti, A. C. 2006. Clast4, the murine homologue of human eIF4E-Transporter, is highly expressed in developing oocytes and post-translationally modified at meiotic maturation. *Gene* **367**, 101-109.

초록 : Clast4의 과발현에 의한 세포 증식의 감소

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eIF4E는 번역개시과정에서 중심조절자 역할을 한다. eIF4E와 eIF4G의 결합이 mRNA의 번역을 촉발하기 때문에, 여러 단백질들이 이 결합을 저해함으로써 번역과정을 조절한다. 인간 4E-T는 eIF4E 결합단백질 중의 하나로, 결합한 mRNA의 번역을 저해할 뿐 아니라, eIF4E를 processing body (P-body)로 이동시키는 기능을 가지고 있다. Clast4는 인간의 4E-T와 상동성을 가지는 생쥐 단백질로 번역 조절에 중요한 기능을 할 것으로 추측되지만, 그 특징은 아직 잘 알려져 있지 않다. 본 연구에서는 Clast4의 인산화된 상태와 eIF4E와의 결합력, Clast4 과발현시 세포증식의 변화에 대한 특징을 관찰하였다. Clast4는 PKA에 의해 *in vivo*에서 아미노말단의 몇몇 잔기가 인산화되는 것으로 확인되었다. 그러나 PKA에 의해 인산화된 Clast4는 eIF4E와의 결합력이나 Clast4의 세포 내 위치에는 큰 변화가 없었다. Clast4는 eIF4E1과 CPEB와 결합하며, Clast4의 보존된 eIF4E 결합 서열인 YXXXXLφ가 eIF4E1A와의 결합에서는 중요하지만 eIF4E1B와의 결합에서는 큰 영향이 없는 것으로 관찰되었다. 잘 알려져 있는 eIF4E 조절자인 4E-BP의 경우와 유사하게 Clast4를 과발현하였을 때 세포의 증식이 감소되었다. 이러한 결과는 Clast4가 세포 내에서 전반적인 번역 조절에 관여하고 있다는 것을 시사한다.