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The intronic region of *Fbxl12* functions as an alternative promoter regulated by UV irradiation

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ABSTRACT

The ubiquitin ligases, SCF complexes, consist of Cul1, Skp1, Rbx1 and the substrate recognition components F-box proteins. Previous studies have reported that one of these F-box proteins, Fbl12, which is produced by *Fbxl12* gene, regulates both cell cycle and differentiation. In this paper, we show that the intronic region of *Fbxl12* gene acts as an alternative promoter and induces expression of a short form of Fbl12 that lacks F-box domain (Fbl12ΔF). We also found that UV irradiation increases Fbl12ΔF mRNA in cells. Finally, Fbl12ΔF may promote the subcellular localization of Fbl12 from nucleus to cytoplasm through their binding. Our data provide the possibility that Fbl12ΔF induced by alternative promoter controls the SCF^{Fbl12} activity in response to UV stimulation.

1. Introduction

The SCF ubiquitin ligase plays important roles in a wide variety of cellular functions [1,2]. The SCF complex contains major four proteins, which are Cul1, Rbx1, Skp1, and F-box protein. Cul1 acts as a scaffold protein that bridge between Rbx1 and Skp1. Rbx1 is a small RING-finger protein associated with the carboxyl terminus of Cul1 and recruits a specific ubiquitin-conjugating enzyme (UBC) to this complex. Meanwhile, Skp1 binds to the amino terminus of Cul1, and also recruits F-box protein, which is a substrate recognition protein, and retains it in this complex. Although the Cul1, Rbx1, and Skp1 are invariable component in the SCF ubiquitin ligase, the F-box proteins are variable and contribute to substrate specificity. So far, more than fifty of human F-box proteins have been identified and fell into three major classes, depending on the types of substrate recognition domains [3]. One of F-box proteins, Fbl12, has a leucine-rich repeat and has structural similarity to another F-box protein, Skp2 [4]. It has been shown that SCF^{Fbl12} enhances ubiquitination of Ca^{2+}/CaM-dependent kinase I (CaMKI) and Ku80, resulting in the regulation of cell cycle and DNA repair [5,6]. In addition, mutation of Fbl12 is associated with renal carcinoma [7], suggesting that dysfunction of SCF^{Fbl12} activity may cause cancer occurrence as well as SCF^{Skp2} [8]. Moreover, Fbl12 transcripts have been shown to be upregulated in response to a treatment with transforming-growth factor-β (TGF-β), leading to the differentiation of osteoblast cells through p57 ubiquitination [4]. Taken together, these studies provide evidences that Fbl12 possesses multiple functions, especially control of proliferation, differentiation and stress responses.

The alternative promoters produce a variety of mRNA from single gene locus, leading to the proteomic diversity and complexity in mammalian cells. It is thought that modulation of promoter activity is influenced by diverse elements, such as tissue, developmental stage, and environmental stresses. Recent comprehensive analyses have demonstrated that a large number of genomic loci contain putative alternative promoters [9,10]. For instance, p21B, which is a variant of p21 transcribed by the usage of alternative promoter, is expressed by a treatment with antitumor compounds, followed by an induction of apoptosis but not cell cycle arrest [11]. It has also shown that the functional deficit of alternative promoter perturbs transcripts, resulting in an occurrence of several human disorders. Intriguingly, *in silico* analyses have revealed that the average cancer-related genes are mediated by two promoters, whereas the average among others are less than two [12]. Therefore, the regulation of alternative promoter activity gives rise to an increase of transcripts from limited number of genes, as well as alternative RNA splicing and RNA editing, and links to the systematic protein production in living organisms.

In this study, we report that the intronic region of *Fbxl12* has a promoter activity regulated by UV irradiation. This alternative promoter initiates transcription of a short form of Fbl12

Abbreviations: SCF, SKP1-CUL1-F-box protein; TNF-α, Tumor necrosis factor-α; TGF-β, Transforming growth factor-β

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transcripts, which lacks F-box region (Fbl12ΔF). In addition, expression of Fbl12ΔF redistributes Fbl12 from nucleus to cytoplasm. Thus, our data suggest that the intronic region of *Fbxl12* gene carries a novel element to regulate UV-dependent Fbl12 functions.

2. Materials and methods

2.1. Materials and antibodies

The antibodies used in this study were listed as follows: anti-Flag (M2, SIGMA) and anti-Myc (9E10, Santa Cruz) antibodies for immunoblot analyses, and anti-GFP (Cat.#598, MBL) and Myc (9E10, Santa Cruz) antibodies for immunocytochemistry. The reagents used in this study were as follows: tunicamycin (SIGMA), Hoechst 33342 (Life Technologies), MG132 (Peptide Institute), TNF-α (R&D systems), TGF-β (Peprotech), H_2O_2 (WAKO), Actinomycin D (SIGMA).

2.2. Cell culture, transfection and stimulation

HEK293, HEK293T, and HeLa cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (WAKO) containing 5-10% fetal bovine serum, penicillin (100 units) and streptomycin (100 mg; P/S). Cells were transfected with plasmids using Lipofectamine 2000 (Life Technologies) or polyethyleneimine (Polyscience) according to the manufacturer's instructions.

2.3. Plasmid construction

The second intron of *Fbxl12* were amplified from mouse genome and subcloned into BglII sites of pGL3. The genomic sequence encoding the each intronic region were amplified from pGL3-Fbl12ΔF and subcloned into the MluI and BglII sites of pGL3. pcDNA3-Flag-Fbl12ΔF construct were described previously [4]. The Fbxl12 cDNA was subcloned into the EcoRI and XhoI sites of pCS4-EGFP and pCS4-Myc. The Fbl12ΔF cDNA was subcloned into the EcoRI and XhoI sites of pCS4-Myc.

2.4. RT-PCR

Total RNAs were isolated using Isogen II (NIPPON GENE) from either HEK293 cells or C57BL/6 J adult mouse tissues. The brain, heart, skeletal muscle, liver, spleen, thymus were dissected from 8-month-old mice, and kidney, spinal cord from 4.5-month-old mice. The cDNA were synthesized by SuperScript III Reverse Transcriptase (Life Technologies) or ReverTra Ace (TOYOBO) according to the manufacturer's instructions.

2.5. RT-qPCR

Total RNAs were isolated from HEK293 cells with of without UV stimulation using Isogen II (NIPPON GENE). The cDNA were synthesized by SuperScript III Reverse Transcriptase (Life Technologies) or ReverTra Ace (TOYOBO) according to the manufacturer's instructions. Quantitative RT-PCR was performed with THUNDERBIRD SYBR qPCR Mix (TOYOBO) and appropriated primers, and analyzed using Thermal Cycler Dice Real Time System (TAKARA).

2.6. Co-immunoprecipitation analysis

HEK293T cells were lysed in extraction buffer (0.5% NP-40, 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM, EDTA, 1 mM DTT) and centrifuged at 14,000 rpm for 5 minutes. The cell lysates were mixed with anti-Flag agarose beads (SIGMA) for 3 hours at 4 °C. The immunoprecipitants were washed and separated by SDS-PAGE, transferred to PVDF membrane, probed with either anti-Flag or anti-Myc antibodies, and detected with HRP-conjugated secondary antibodies and chemiluminescence reagent (Amersham ECL Plus Western Blotting Detection Reagents, GE Healthcare).

2.7. Immunocytochemistry and time-laps imaging

HeLa cells plated on 15 mm coverslips and grown in 12-well plates were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 10 minutes at room temperature. The coverslips were washed in PBS, blocked with 5% bovine serum albumin (BSA) in PBS with 0.4% Triton X-100, then incubated with the indicated primary antibodies for 1 hour at room temperature or overnight at 4 °C. Following PBS wash, samples were incubated with secondary antibodies (Alexa Fluor 594 anti-mouse IgG [1:500], Alexa Fluor 488 anti-rabbit IgG [1:500]) for 30 minutes at room temperature in blocking solution. Cells were imaged using a fluorescence microscope (BIOREVO BZ-9000, Keyence). The images were quantified using GraphPad Prism (GraphPad software) and Image J software. Time-laps imaging was performed using fluorescence microscope (BIOREVO BZ-9000, Keyence) and Incubation System for Microscopes (TOKAI HIT).

2.8. Luciferase assay

HEK293 cells were transfected with the reporter plasmid, which contains the firefly luciferase gene, and a renilla luciferase expression plasmid as an internal control, then treated with indicated stimulation. Cell lysates were subsequently assayed for both firefly and renilla luciferase activities with Dual-Luciferase Reporter Assay System (PROMEGA), and the former activity was normalized on the basis of the latter. Luciferase activity was measured using luminometer Flash'n Glow (BERTHOLD).

2.9. Cell proliferation assay

HEK293 cells were transfected with either control or Fbl12ΔF plasmids. The transfected cells were plated on 96 well plates and incubated for two days. Cellular proliferation was analyzed using microplate reader Model 680 (BIO-RAD) and Cell Counting kit-8 (DOJINDO) according to the manufacturer's instructions.

3. Results

3.1. The mRNA encoding the third exon of Fbxl12 is expressed in cells

The F-box domain of Fbl12 is essential for the interaction with Skp1, which is an adaptor protein that connects Fbl12 to Cul1. Previous study has suggested that Fbl12ΔF, which lacks F-box domain, functions as a dominant negative mutant that suppresses SCF^{Fbl12} ubiquitin ligase activity [4]. While investigating the SCF^{Fbl12} functions, we noticed that the third exon of *Fbxl12* gene begins with an ATG, which is well conserved among mammals evolutionarily. Interestingly, this third exon is precisely consistent with the Fbl12ΔF coding region (Fig. 1A). We also found that the mRNA encoding the third exon of *Fbxl12* containing 5'- and 3'-untranslated region (UTR) is already registered in the database (http://www.ensembl.org/index.html) (Fig. 1B). Therefore, we hypothesized that mRNA encoding Fbl12ΔF is expressed in cells as an independent transcript. To ask this hypothesis, we developed the PCR primers that are capable of amplifying only Fbl12ΔF mRNA through recognition of the 5'- UTR flanking coding region. Because the Fbl12ΔF mRNA in database (Transcription ID; ENSMUST00000140702) is started from -142 base pair (bp) of the translation initiation codon, we designed four primers

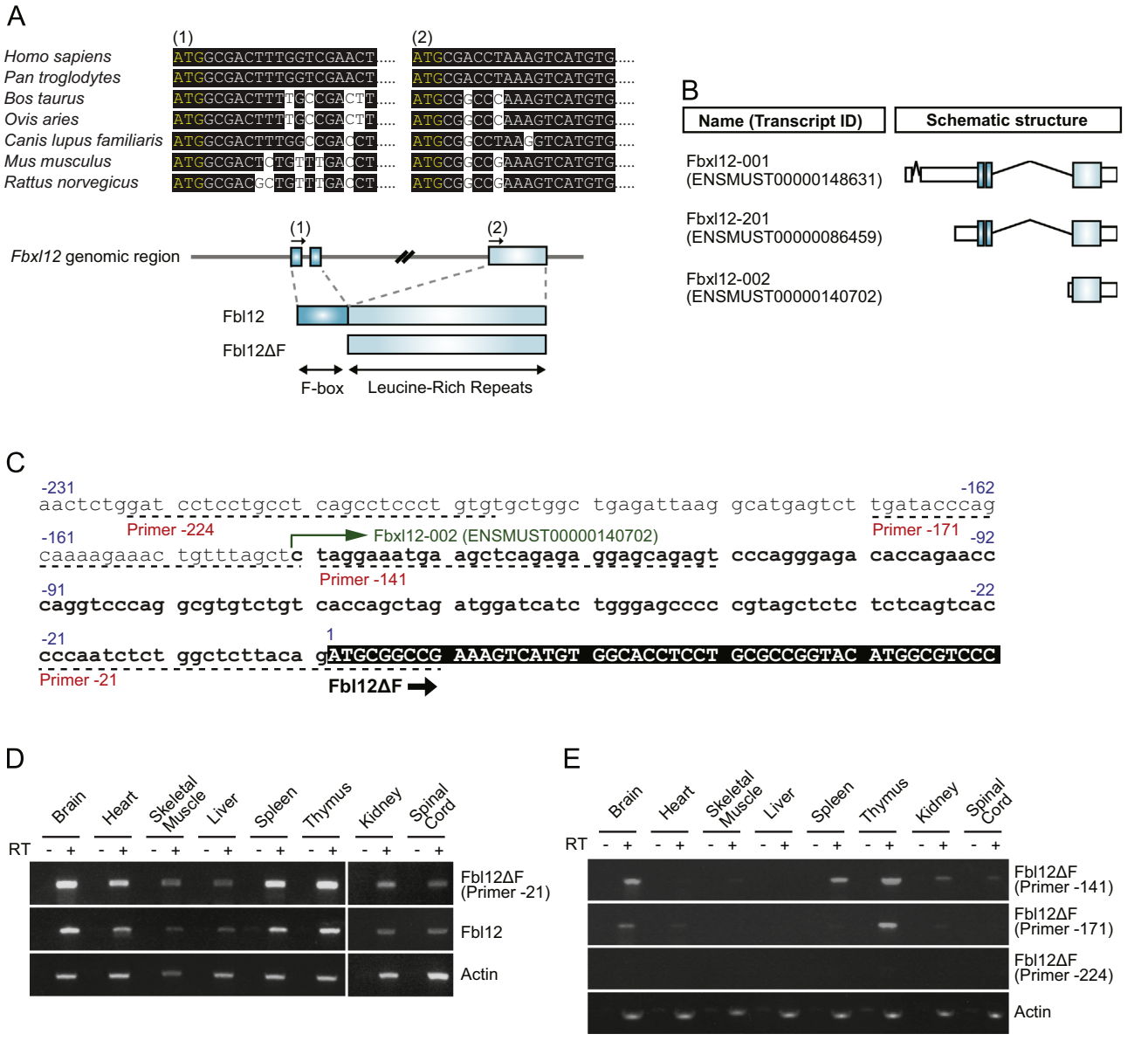

Fig. 1. The mRNA encoding the third exon of *Fbxl12* is expressed in cells. (A) Sequence alignment of first and third exons of *Fbxl12* from *Homo sapiens, Pan troglodytes, Bos taurus, Ovis aries, Canis lupus familiaris, Mus musculus, Rattus norvegicus.* Conserved genome sequences are highlighted in black and initiation codons are shown in yellow (upper panel). Schematic structure of Fbl12 protein. The third exon of *Fbxl12* encodes a leucine-rich repeat region of Fbl12 (lower panel). (**B**) Schematic structure of Fbl12 transcripts from Ensembl database. (**C**) Sequence of second intron flanking Fbl12ΔF initiation codon. The transcript sequence registered in Ensembl database (ENSMUST00000140702) is in italics. The sequence of Fbl12ΔF is highlighted in black. (**D** and **E**) RT-PCR analyses of either Fbl12ΔF or Fbl12 mRNA in mice tissues, including brain, heart, skeletal muscle, liver, spleen, thymus, kidney and spinal cord.

corresponding to either the neighborhood of the registered Fbl12ΔF mRNA or the outside of that (Fig. 1C). To first confirm whether Fbl12ΔF mRNA is produced independently *in vivo*, we conducted RT-PCR analyses using the primers that recognized the outside of third exon. The Fbl12ΔF mRNA was detected as well as full length Fbl12 mRNA in each mouse tissues (Fig. 1D), suggesting that Fbl12ΔF mRNA is transcribed ubiquitously *in vivo*. To next examine the transcription-starting site, we used different sets of primers. The starting sites in Fbl12ΔF mRNA in heart, skeletal muscle and liver may be proximal region of initiation codon; on the other hand, those in brain and thymus are distal region. Moreover, the amount of PCR products was significantly decreased when the primers recognized at -224 bp of the initiation codon were used (Fig. 1E). These data suggest that the transcription-starting sites differ among tissues and are located within this region.

3.2. The intronic region of Fbxl12 functions as an alternative promoter regulated by UV

Because Fbl12ΔF mRNA exists as an independent transcript, we made the assumption that the intronic region between second and third exons of *Fbxl12* possesses a promoter activity. We first confirm whether Fbl12ΔF mRNA is expressed in HEK293 cells. To examine this, we purified total RNA from HEK 293 cells and conducted RT-PCR using the primer that recognized the 5'-UTR region. The Fbl12ΔF mRNA was slightly detected in HEK293 cells (Fig. 2A), demonstrating that Fbl12ΔF mRNA exists as an independent transcript. To next investigate the promoter activity, we introduced luciferase reporter constructs harboring either intronic region of mouse *Fbxl12* (pFbl12ΔF) or regular promoter of that (pFbl12) into HEK293 cells (Fig. 2B). Contrary to the pFbl12 activity responsible for the full length Fbl12 expression, the activity of pFbl12ΔF was comparable to a mock control under basal

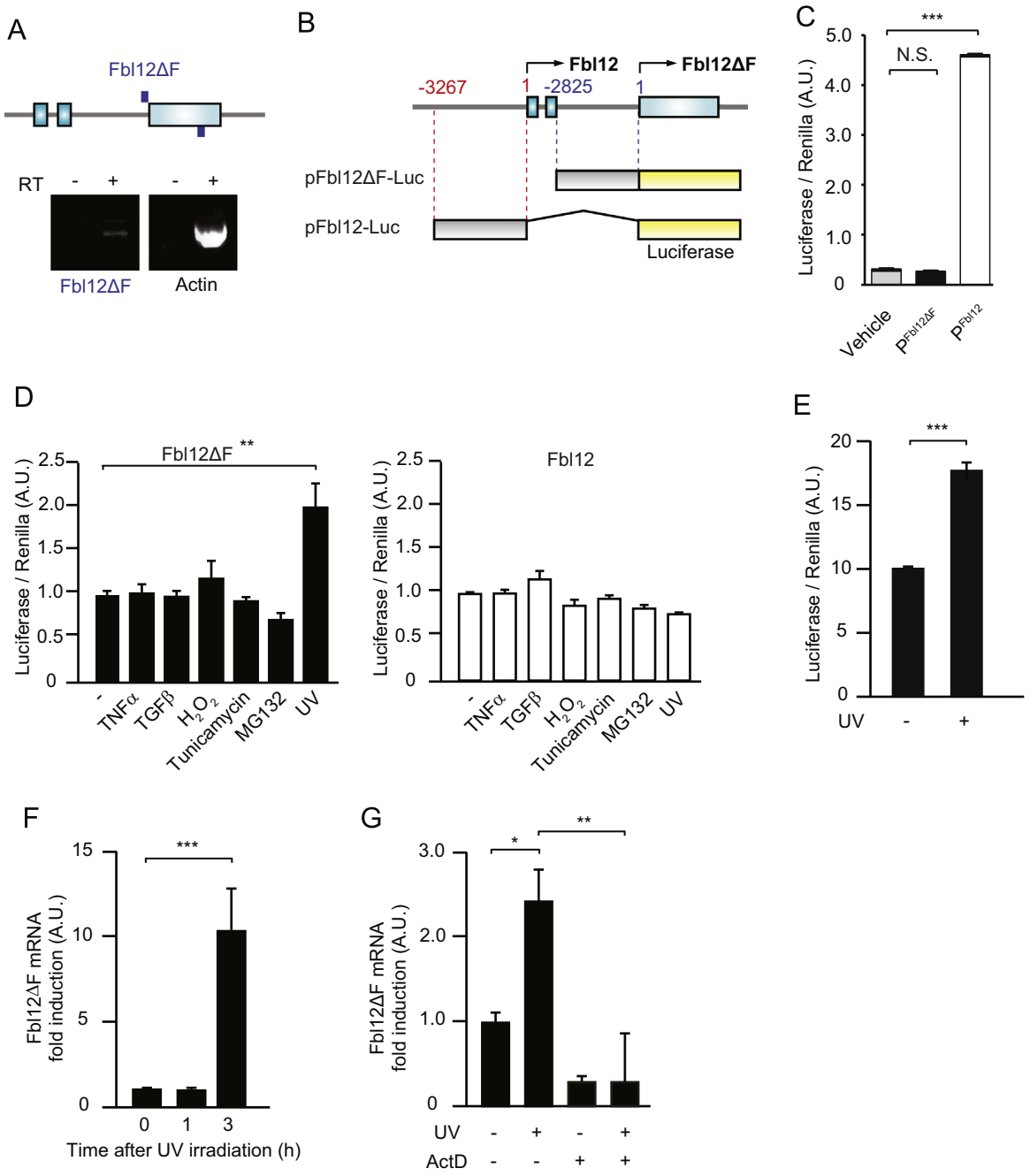

Fig. 2. The intronic region functions as an alternative promoter regulated by UV. (**A**) Diagrams of primer binding sites. The small squares indicate the annealing sites of Fbl12ΔF primer (upper panel). RT-PCR analysis of either Fbl12ΔF mRNA in HEK293 cells (lower panel). **(B)** Schematic structure of the reporter constructs. **(C)** HEK293 cells were transfected with reporter plasmids, pFbl12ΔF-Luc and pFbl12-Luc. Both promoter activities were analyzed by luciferase assay ($n=3$, mean ± SEM, N.S., not significant, ***$p < 0.001$ by one-way ANOVA). **(D)** HEK293 cells were transfected with either pFbl12ΔF-Luc or pFbl12-Luc, then stimulated with wither 5 ng/ml TNF-α, 1 ng/ml TGF-β, 1 mM H_2O_2, 5 μg/ml Tunicamycin, 10 μM MG132, or 12 μW/cm^2 UV for 8 hours. The promoter activity was analyzed by luciferase assay ($n=3$, mean ± SEM, **$p < 0.01$ by one-way ANOVA). **(E)** NIH3T3 cells were transfected with either pFbl12ΔF-Luc, then stimulated with 12 μW/cm^2 UV for 6 hours. The promoter activity was analyzed by luciferase assay ($n=3$, mean ± SEM, ***$p < 0.001$ by student's t-test). **(F)** HEK293 cells were stimulated with 4 μW/cm^2 UV and incubated for 3 hours. The Fbl12ΔF mRNAs were then subjected to quantitative RT-PCR analysis. Results were normalized to actin expression ($n=3$, mean ± SEM, *** p < 0.001 by one-way ANOVA). **(G)** HEK293 cells were stimulated with 4 μW/cm^2 UV and incubated for 3 hours in the presence or absence of 5 μM Actinomycin D. The Fbl12ΔF mRNAs were then subjected to quantitative RT-PCR analysis. Results were normalized to 5.8 S rRNA expression ($n=3$, mean ± SEM, * $p < 0.05$, ** $p < 0.01$ by one-way ANOVA).

condition (Fig. 2C). This suggest that the promoter activity is not high at steady state in cells. We therefore pursued whether physiological stimuli are involved in an increase of pFbl12ΔF activity. In consequence, we found that UV irradiation activated the Fbl12ΔF transcription approximately two-fold than control, but not other external stimuli such as TNF-α, TGF-β, MG132, Tunicamycin and H_2O_2 (Fig. 2D). To next examine a specificity of cell type, we used another cell line, mouse fibroblasts. We transfected NIH3T3 cells with reporter constructs harboring the intronic region of *Fbxl12*, then analyzed promoter activity by luciferase assay. UV irradiation significantly enhances promoter activity in NIH3T3 cells (Fig. 2E), suggesting that UV responsive phenomena may be general. To further confirm this endogenously, we performed qRT-PCR. Consistent with luciferase assay, UV irradiation enhanced a synthesis of Fbl12ΔF mRNA after UV irradiation for three hours (Fig. 2F). Because it seems not to be rapid reaction, mRNA

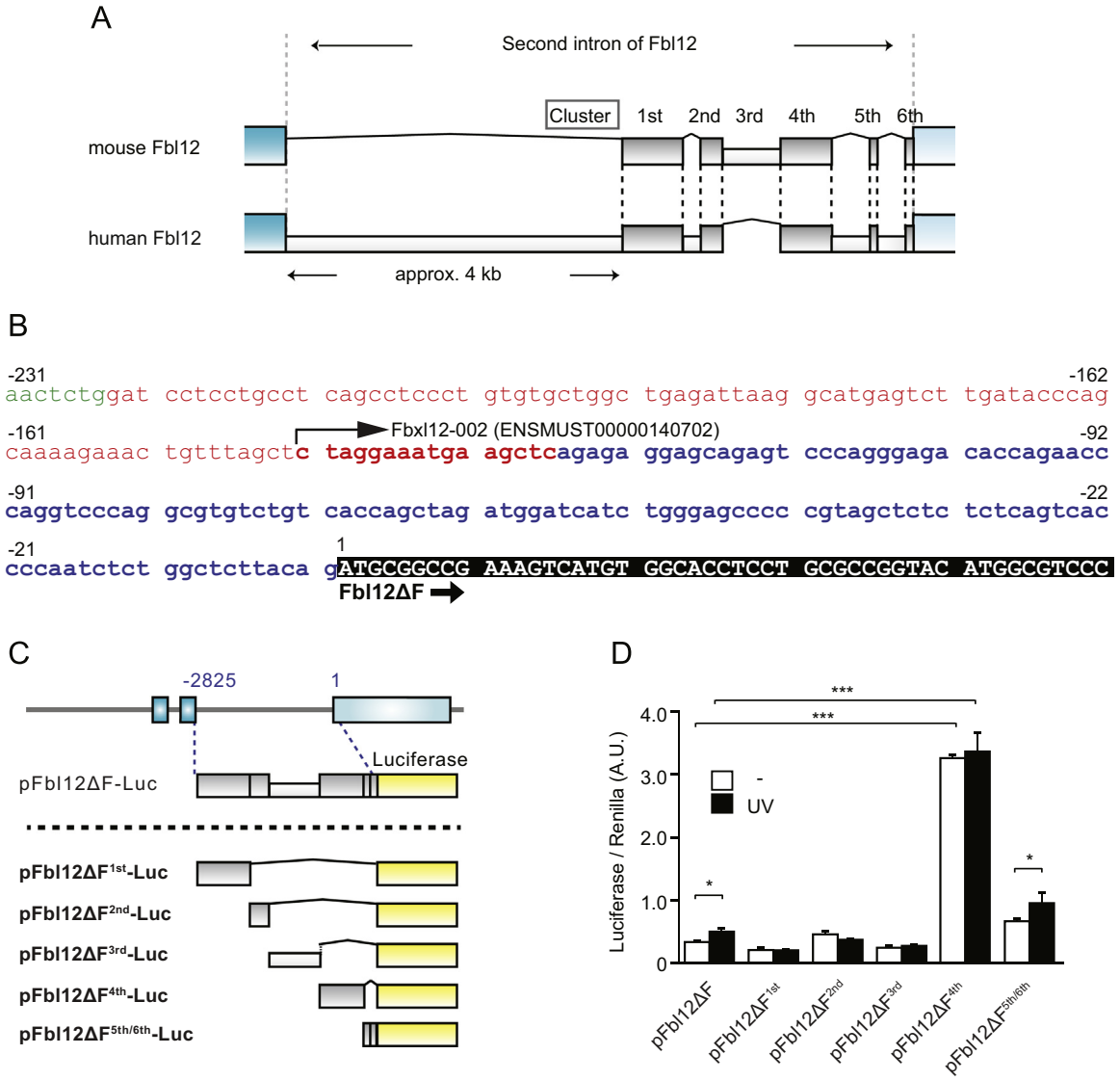

Fig. 3. The 3' regions of second intron are responsible for the promoter activity. (A) Scheme of second intronic region of *Fbxl12* gene. There are at least five clustering regions conserved from mouse to human. **(B)** Sequence of second intron flanking mouse Fbl12ΔF initiation codon. The fourth, fifth and sixth cluster regions are shown in green, red and blue, respectively. The sequence of Fbl12ΔF is highlighted in black. **(C)** Diagrams of the reporter constructs. The luciferase gene was fused to the deletion constructs of mouse *Fbxl12* intron. **(D)** HEK293 cells were transfected with reporter plasmids, then stimulated with 8 μW/cm^2 UV for 6 hours. Fbl12ΔF promoter activity was analyzed by luciferase assay ($n=3$, mean ± SEM, $*p<0.05$, $***p<0.001$ by one-way ANOVA).

synthesis regulated by UV could be indirect. One possibility is that UV-induced target gene acts as a mediator that triggers Fbl12ΔF mRNA synthesis. Finally, to investigate whether this effect is dependent on not an inhibition of mRNA degradation but an enhancement of transcription, we used transcription inhibitor; actinomycin D. Treatment with actinomycin D clearly blocked UV-induced gene expression, suggesting that UV irradiation promotes Fbl12ΔF transcription, but not suppression of mRNA degradation (Fig. 2G). Taken together, our data suggest that Fbl12ΔF expression is enhanced by a usage of novel promoter at second intronic region in response to UV.

3.3. The 3' regions of second intron are responsible for the promoter activity

To dissect the region responsible for UV stimulation, we made a series of deletion constructs of second intronic region. There are some sequence similarity between human and mouse as the gene cluster, though the intronic region in human genome is longer than mouse genome (Fig. 3A). In the process of this study, we found that both fifth and sixth clusters in Fig. 3A encode the region of putative 5'-UTR (Fig. 3B). We then subdivided intronic region into these clusters based on this alignment and developed reporter plasmids (Fig. 3C). To investigate the promoter activities, we introduced these constructs into HEK293 cells, followed by stimulation with UV. Consistent with our data in Fig. 2, the promoter activity of pFbl12ΔF was increased in response to UV irradiation. We found that the basal luciferase activity was considerably dependent on the fourth cluster region (pFbl12ΔF^{4th}). Intriguingly, the 3'-region including pFbl12ΔF^{5th} and pFbl12ΔF^{6th} appears to be slightly links to reporter activity regulated by UV (Fig. 3D). These data imply that pFbl12ΔF^{4th}-dependent promoter activity is hampered by another region under basal condition, and UV-recognition element may lie in pFbl12ΔF^{5th} and pFbl12ΔF^{6th}.

3.4. Fbl12ΔF affects subcellular localization of Fbl12

It is well known that UV irradiation is a typical cellular stress that causes cell cycle arrest. Because Fbl12ΔF expression is induced by UV, we next examined whether overexpression of

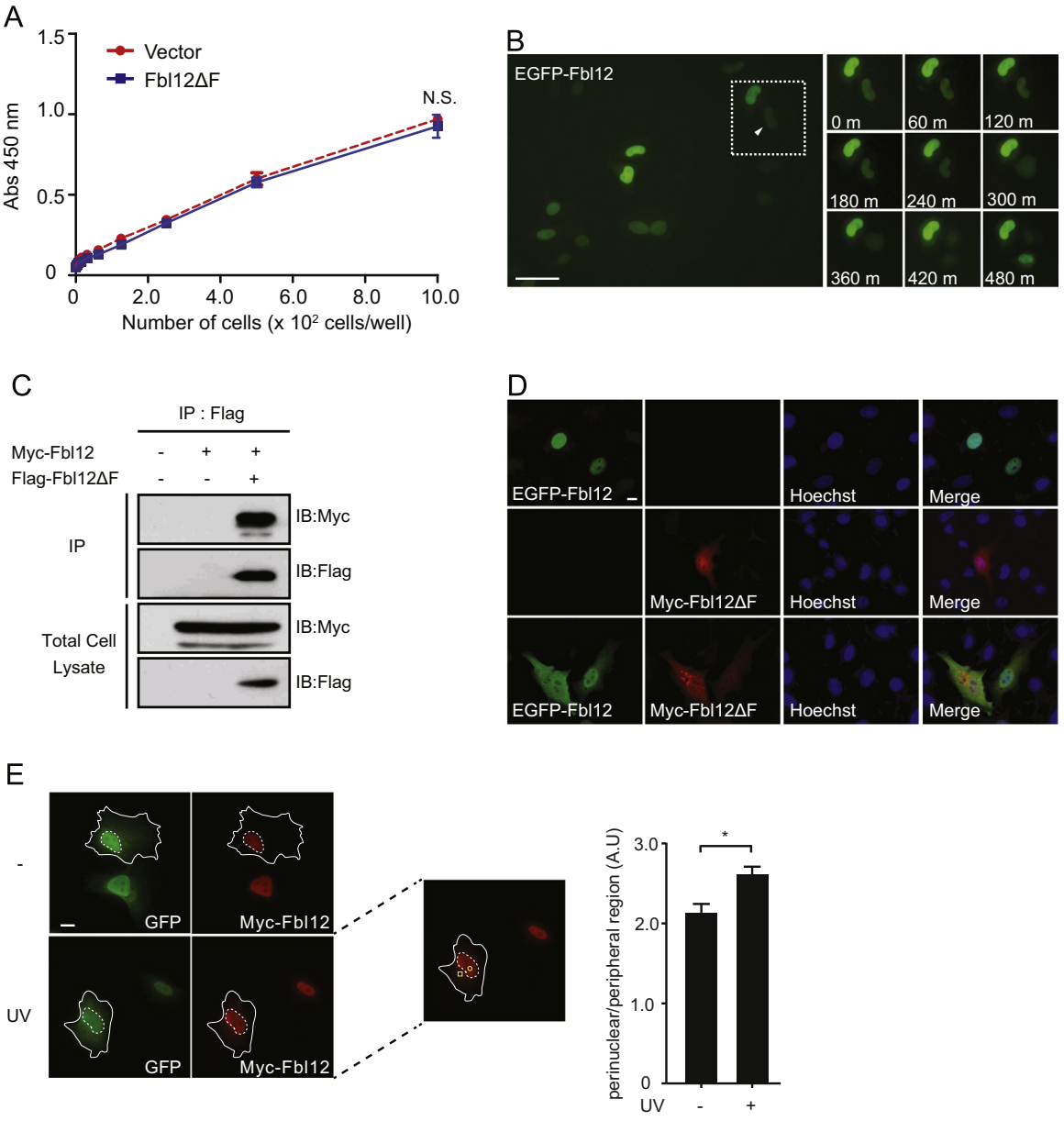

Fig. 4. Fbl12ΔF affects subcellular localization of Fbl12. (**A**) HEK293 cells were transfected with either control or Fbl12ΔF plasmids. cellular proliferation was analyzed using cell counting kit-8. **(B)** Subellular localization of EGFP-Fbl12 in HeLa cells. the arrowhead shows the cells undergoing mitosis. EGFP-Fbl12 Is exclusively localized in nucleus except the period of cell division. the scale bar represents 50 μm. **(C)** HEK293T cells were transfected with either Myc-Fbl12 or flag-Fbl12ΔF and were subjected to immunoprecipitation using anti-flag antibodies. the immunoprecipitants and the total cell lysates were immunoblotted with either anti-Myc or anti-flag-antibodies. **(D)** HeLa cells transfected with either EGFP-Fbl12 or Myc-Fbl12ΔF and were subjected to Immunocytochemistry using anti-GFP or anti-Myc antibodies. the scale bar represents 10 μm. **(E)** HeLa cells transfected with both EGFP and Myc-Fbl12ΔF. the cells were subjected to Immunocytochemistry in the presence or absence of UV stimulation for 3 hours. the scale bar represents 10 μm (left panels). ratio of cytoplasmic to nuclear Fbl12 fluorescence intensity ($n=60$, mean ± SEM, $*p<0.05$ by student's t-test). the solid line indicates the edge of cell, the dash line indicate the nucleus. the small yellow circle indicates nuclear region and the square indicates cytoplasmic region.

Fbl12ΔF is implicated in cellular proliferation. To examine this, we transfected HEK293 cells with Fbl12ΔF plasmids and measure the proliferation rate. Accordingly, overexpression of Fbl12ΔF has little effect on the proliferation (Fig. 4A), implying that Fbl12ΔF expressed by UV is not implicated in cell cycle arrest. Given that Fbl12ΔF does not regulate cellular proliferation, Fbl12ΔF may be associated with another cellular function such as subcellular localization of full length Fbl12. To examine this, we performed time-lapse imaging to verify the spatiotemporal localization of Fbl12. We found that EGFP-Fbl12 is exclusively localized in nucleus except cell division (Fig. 4B and Suppl.Movie 1). As some F-box proteins form homo-dimer through their binding [13], we investigated the possibility that Fbl12ΔF binds to Fbl12, followed by the changes of Fbl12 subcellular localization. To examine this, we first check the interaction between them. We obtained the data showing that Fbl12 interacts with Fbl12ΔF effectively (Fig. 4C). We next asked whether Fbl12ΔF alters Fbl12 subcellular localization. Because there is no specific antibody against both Fbl12 and Fbl12ΔF, we transfected HeLa cells with EGFP-Fbl12 and Myc-Fbl12ΔF. Usually, Fbl12 localizes in the nucleus under basal condition and Fbl12ΔF exists in both nucleus and cytoplasm. However, when Fbl12 was co-expressed with Fbl12ΔF, Fbl12 was observed in both nucleus and cytoplasm (Fig. 4D). We further asked if UV irradiation regulates the subcellular localization of Fbl12. To do this, we introduced both EGFP and Myc-Fbl12 into HeLa cells, upon stimulation with UV, and observed the changes of subcellular localization. After stimulation of UV, the localization of Myc-Fbl12 in cytoplasm is slightly increased (Fig. 4E). This data

suggests that UV stimulation alters the localization of Fbl12 from nucleus to cytoplasm via probably newly synthesis of Fbl12ΔF protein.

Supplementary material related to this article can be found online at 1016/j.bbrep.2015.07.010.

4. Discussion

In this study, we found that the second intron of *Fbxl12* gene acts as an alternative promoter regulated by UV irradiation. We also narrowed down the region responsible for a transcription of Fbl12ΔF. Both expression of Fbl12ΔF and UV irradiation appear to promote translocation of Fbl12 from nucleus to cytoplasm. Our data suggest that subcellular localization of Fbl12 is regulated by UV irradiation through Fbl12ΔF synthesis. Therefore, we think that Fbl12ΔF could be a novel mediator that controls SCF^{Fbl12} properties.

Previous studies have reported that F-box family proteins are crucial for the control of both complexity and diversity in cells. It is also known that defect of F-box family protein cause abnormal cellular homeostasis, leading to several diseases including cancer [14]. Intriguingly, a wide variety of transcripts encoding F-box family proteins exist in cells (http://www.ensembl.org/index.html) (Suppl.Table 1). It is plausible that these variants modulate properties of SCF ubiquitin ligases. For instance, Skp2 variant, which lacks C-terminal region, is normally localized in cytoplasm and inhibits ubiquitination of the substrates such as cyclin D1 [15]. Furthermore, splicing variants of Fbx4, exhibit different subcellular localization and inhibit cyclin D1 degradation [16], supporting our idea that various F-box proteins could alter SCF ubiquitin ligase properties such as localization and activity by themselves. The bottom line seems to be that these transcripts are produced using not only alternative splicing but also alternative promoter. So far, it has not been reported that variants of F-box proteins are expressed by an alternative promoter. However, our *in silico* analyses suggest that several F-box proteins, including Skp2, are transcribed using putative alternative promoter (see Suppl.Table 1). Probably, these processing machineries contribute to the diversity of F-box family proteins.

The molecular mechanism that underlies UV-induced Fbl12ΔF expression has not been fully elucidated. Because Fbl12ΔF mRNA synthesis is not rapid reaction, the gene expression of Fbl12ΔF could occur after a transcription of other targets, which regulate Fbl12ΔF expression. On the other hand, we found several transcription binding sites in the intronic region. It is known that some mediators such as p53 are associated with UV-induced gene expression. In fact, putative p53 binding sites exist in this region. Thus, p53 could be a potential mediator that regulates UV-induced Fbl12ΔF expression directly despite a slow response to mRNA synthesis.

Although the physiological consequences of Fbl12ΔF expression remain to be elucidated, it is likely that Fbl12ΔF modulates the function of SCF^{Fbl12}. It is known that UV irradiation significantly influences the expression of p21, a member of CDK inhibitor [17]. As Fbl12 can also bind to p21 (our unpublished data), UV-induced Fbl12ΔF may regulate p21 abundance *in vivo*. In fact, Fbl12ΔF binds to p21 and slightly increased the level of p21 (Tsuruta et al. in preparation). Therefore, induction of Fbl12ΔF by UV-irradiation may influence the level of p21 expression, although the expression of Fbl12ΔF *per se* did not influence the cell proliferation. Another possibility is that Fbl12ΔF modulates DNA repair through SCF^{Fbl12}-induced Ku80 ubiquitination. Previous studies have suggested that SCF^{Fbl12} is recruited to double-strand break via interaction with Ku80, leading to ubiquitination and elimination of Ku80 from double-strand break [6]. This process is crucial for the control of DNA repair. Thus, it is likely that expression of Fbl12ΔF causes negative feedback after DNA damage, though we have not tested whether another DNA damage such as γ-irradiation increases transcriptional activity.

Recently, it has been demonstrated that the cis-regulatory elements located in 5'- and 3'-UTRs modulates RNA stability. For instance, one of ELAV family proteins, HuR, associates AU-rich elements (AREs) in 3'-UTR of target mRNA, leading to stabilization [18]. As another example, the iron regulatory proteins (IRPs) recognize the iron response element, which is encoded by 3'-UTR of transferrin receptor 1 (TfR1), and prevent TfR1 mRNA from decay when intracellular iron level become low [19]. Since we found that $pFbl12\Delta F^{5th/6th}$, which is consisitent with 5'-UTR, could be involved in UV response, it is not rule out the possibility that $pFbl12\Delta F^{5th/6th}$ may recruit regulatory protein to suppress RNA decay in response to UV stimulation. In fact, the promoter activity of the intronic region was comparable to the mock control without UV (Fig. 2C). This may suggest that some factors normally associates with intronic region and mediates mRNA fate.

In conclusion, we found that the second intronic region of *Fbxl12* functions as an alternative promoter and enhances transcription of Fbl12ΔF in response to UV, leading to the regulation of Fbl12 localization. Our data suggest that UV-induced Fbl12ΔF is a novel mediator, which controls SCF^{Fbl12} activity, and may provide a new regulatory mechanism of DNA damage response.

Acknowledgments

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at 10.1016/j.bbrep.2015.07.010.

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