Biochemical and Biophysical Research Communications 426 (2012) 59–64

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Fbxw5 suppresses nuclear c-Myb activity via DDB1-Cul4-Rbx1 ligase-mediated sumoylation

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ARTICLE INFO

Article history:
Received 2 August 2012
Available online 14 August 2012

Keywords:
c-Myb
Fbxw5
Sumoylation
Ubiquitination

ABSTRACT

The c-*myb* proto-oncogene product (c-Myb) is degraded in response to Wnt-1 signaling. In this process, Fbxw7α, the F-box protein of the SCF complex, binds to c-Myb via its C-terminal WD40 domain, and induces the ubiquitination of c-Myb. Here, we report that Fbxw5, another F-box protein, enhances sumoylation of nuclear c-Myb. Fbxw5 enhanced c-Myb sumoylation via the DDB1-Cul4A-Rbx1 complex. Since the Fbxw5-DDB1-Cul4A-Rbx1 complex was shown to act as a ubiquitin ligase for tumor suppressor TSC2, our results suggest that this complex can function as a dual SUMO/ubiquitin ligase. Fbxw5, which is localized to both nucleus and cytosol, enhanced sumoylation of nuclear c-Myb and induced the localization of c-Myb to nuclear dot-like domains. Co-expression of Fbxw5 suppressed the *trans*-activation of c-*myc* promoter by wild-type c-Myb, but not by v-Myb, which lacks the sumoylation sites. These results suggest that multiple E3 ligases suppress c-Myb activity through sumoylation or ubiquitination, and that v-Myb is no longer subject to these negative regulations.

1. Introduction

The c-*myb* proto-oncogene product (c-Myb) plays an important role in the proliferation of immature hematopoietic cells, colonic epithelial cells, and mammary epithelial cells [1–3], and also in the differentiation of hematopoietic cells [4]. Especially, there is now considerable evidence that c-Myb is associated with human epithelial cancers that arise in colon, breast and brain. c-Myb directly binds to the DNA sequence 5′-AACNG-3′ via its DNA-binding domain in the N-terminus [5,6]. By interacting with the coactivator CBP, c-Myb activates a group of target genes [7]. These target genes include c-*myc* [8], *Bcl-2* [9,10], and *Gbx2* [11], which are involved in cell cycle control, blockage of apoptosis, and growth and differentiation control, respectively. The v-*myb* oncogene (v-Myb) product of avian myeloblastosis virus, which lacks the N- and C-terminal region of c-Myb and has multiple point mutations not found in c-Myb, exhibits oncogenic activity that transforms hematopoietic cells [12]. This is partly due to the deletion of a negative regulatory domain in the C-terminal region of c-Myb [5,13,14], which interacts with the corepressors, BS69 and TIF1β [15,16].

c-Myb activity is regulated by various post-translational modifications. In response to Wnt-1 signal, c-Myb is phosphorylated and degraded [17]. NLK (Nemo-like kinase) binds to c-Myb with HIPK2 (Homeodomain-interacting protein kinase 2) and phosphorylates c-Myb at multiple sites upon Wnt-1 stimulation, and c-Myb is subsequently ubiquitinated and degraded via the proteasome. Fbxw7 (F-box and WD40 domain protein 7, also known as hCDC4, hAGO, or SEL10) is the F-box protein of an SCF complex, which directly binds to c-Myb via its WD40 domain and induces ubiquitination of c-Myb [18]. Thus, Fbxw7 functions as the c-Myb recognition subunit of the SCF E3 ubiquitin ligase complex, which contains Skp1, Cul1, and Rbx1. Since v-Myb lacks the C-terminal region containing multiple NLK phosphorylation sites, v-Myb avoids Wnt-1-induced degradation and exhibits higher activity than c-Myb [19].

c-Myb is also covalently modified by the ubiquitin-like molecule, SUMO (small ubiquitin-like modifier). Sumoylation of nuclear c-Myb at two sites (Lys-499 and Lys-523) by PIASy, an E3 ligase for SUMO-1, increases c-Myb stability and suppresses its *trans*-activating capacity [20,21]. Another SUMO E3 ligase, PIAS-3, promotes SUMO-2 and SUMO-3 modification of c-Myb in response to heat stress or UV stress, which leads to the inhibition of c-Myb activity [22]. TRAF7, which contains WD40 repeats and a RING finger domain, induces the sumoylation of c-Myb in the cytosol, which results in its sequestration in the cytoplasm [23]. Thus, c-Myb activity is regulated by multiple mechanisms via post-translational modification by SUMO and ubiquitin.

Here, we report that another F-box protein, Fbxw5, enhances sumoylation of c-Myb in the nucleus, which suppresses c-Myb activity. Our results indicate that Fbxw5-DDB1-Cul4A-Rbx1 can function as a dual ubiquitin/SUMO E3 ligase.

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http://dx.doi.org/10.1016/j.bbrc.2012.08.032

2. Materials and methods

2.1. Plasmids

The plasmids used to express various forms of c-Myb were described previously [17,23]. The 3xMyc-SUMO-1, 3xMyc-SUMO-2, and 3xMyc-SUMO-3 expression plasmids, which contains three tandem repeats of myc tag at its N-terminus, was generated using a chicken β-actin promoter-containing plasmid or the pcDNA3 vector (Invitrogen). The Myc-ubiquitin, Myc-Nedd8, Cul4A, Cul4B, DDB1, or Skp2 expression plasmids were also constructed using the same vector. The 3xMyc-SUMO-1, 3xMyc-SUMO-2, or 3xMyc-SUMO-3 expression plasmids were constructed using the pcDNA3 vector (Invitrogen). A PCR-based method was employed to construct the Fbxw5 mutant plasmids. The Fbxw5, Fbxw7α, Rbx1, Cul1, and Skp1 expression plasmids were previously described [18].

2.2. In vivo sumoylation assays

For the *in vivo* sumoylation assays, African green monkey kidney CV-1 cells were transfected using Lipofectamin 2000 (Invitrogen) with the plasmids described in the Figure legends. Forty hours after transfection, the cells were scraped into 100 μl of lysis buffer (10 mM Tris–HCl, pH 8.0, 500 mM NaCl, 2% SDS), heated for 10 min at 95 °C, diluted with buffer lacking SDS to reduce the SDS concentration to 0.2%, and then sonicated mildly on ice. After pre-adsorption with protein G-Sepharose, anti-Flag M2 monoclonal antibody was used to precipitate Flag-c-Myb. The eluates were then used for Western blotting with anti-Myc monoclonal antibody (MBL). Ubiquitination and neddylation of c-Myb were similarly examined using pact-Myc-ubiquitin and pact-Myc-Nedd8, respectively. In the case of ubiquitination, the transfected cells were treated with proteasome inhibitor, MG132 (50 μM), for 7 h before lysate preparation.

2.3. Western blotting

For Western blotting, CV-1 cells were transfected using Lipofectamin 2000 with the plasmids indicated in the Figure legend. Forty hours after transfection, cells were lysed in SDS sample buffer with mild sonication and subjected to SDS–PAGE followed by Western blotting using anti-c-Myb antibody and the ECL kit (Amersham Biosciences). The transfection efficiency was determined by measuring β-galactosidase activity in aliquots of cells, and the amounts of lysate used for Western blotting were normalized based on β-galactosidase activity.

2.4. Coimmunoprecipitation assay

For co-immunoprecipitation experiments, human kidney 293T cells (1×10^6 cells per 100-mm dish) were transfected with the plasmids described in the Figure legends using the $CaPO_4$ method. Forty hours after transfection, cells were lysed by mild sonication in 0.5 ml of NET buffer (20 mM Tris–HCl, pH 8.0, 1 mM EDTA, 0.5% NP-40, 10% glycerol protease inhibitor cocktail) containing 150 mM NaCl. The lysates were immunoprecipitated with various antibodies described in the Figure legends, and the immunocomplexes were subjected to SDS–PAGE, followed by Western blotting using the antibody indicated in the Figure legends, and the ECL kit (Amersham Biosciences). An aliquot of lysates was also directly used for Western blotting.

2.5. In vitro sumoylation assay

In vitro sumoylation was performed as described [23]. 293T cells (1×10^6 cells per 100-mm dish) were transfected with HA-DDB1, HA-Cul4A, HA-Cul4B, HA-Cul1, HA-Skp1, HA-Rbx1, and HA-Fbxw5 expression plasmids (8 μg total) using Lipofectamine 2000. Forty hours after transfection, cells were scraped into RIPA buffer and HA-tagged proteins were immunopurified on protein G beads using 12CA5 anti-HA mAb. His-c-Myb was separately expressed in 293T cells, and purified using HIS-select cobalt affinity

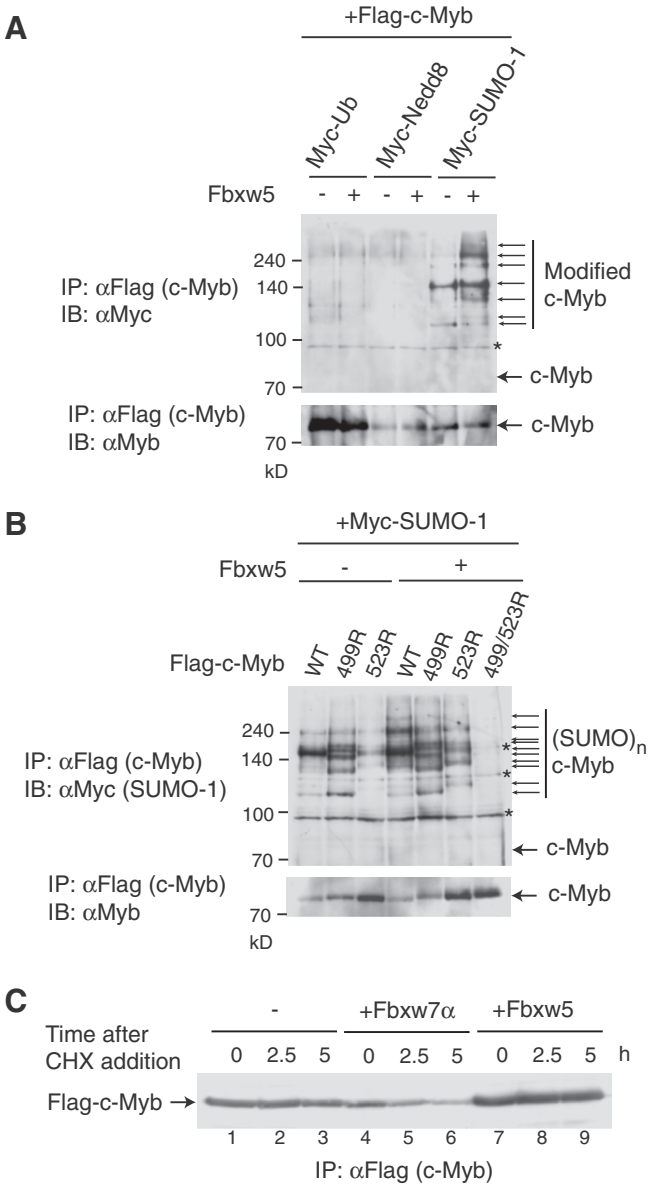

Fig. 1. Fbxw5 induces sumoylation of c-Myb. (A) Fbxw5 induces sumoylation, but not ubiquitination or neddylation of c-Myb. CV-1 cells were transfected with 6 μg of pact-Flag-c-Myb, 2 μg of Myc-ubiquitin, Myc-Nedd8, or pact-3xMyc-SUMO-1 expression plasmids, and 2 μg of the HA-Fbxw5 expression plasmid or the control vector. Lysates from the transfected cells were immunoprecipitated with anti-Flag antibody, followed by Western blotting with anti-Myc (upper) or anti-c-Myb (αCT5) (lower) antibodies. (B) Fbxw5 induces sumoylation of c-Myb at Lys-499 and Lys-523. CV-1 cells were transfected with plasmids expressing wild-type c-Myb or the mutant in which Lys-499 and/or Lys-523 were replaced by Arg, the pact-3xMyc-SUMO-1 plasmid, as well as the Fbxw5 expression plasmid or the control vector, as described above. Sumoylation of c-Myb was examined as described above. (C) Fbxw5 does not induce degradation of c-Myb. CV-1 cells were transfected with 7.5 μg of the Flag-c-Myb expression plasmid, 2 μg of the Fbxw7α or Fbxw5 expression plasmid or the control plasmid, and 0.4 μg of pact-β-gal, which was used as the internal control. The differences in β-galatosidase activity as the transfection internal control was within 10%. After the addition of cycloheximide (CHX) (50 μg/ml) to the cell culture, lysates were prepared at the indicated time, and used for Western blotting with the anti-Flag M2.

gel (Sigma). E1 SAE1/SAE2 (MBL), E2 Ubc9 (MBL), SUMO-1, SUMO-2, and SUMO-3 (MBL), His-c-Myb, and the immunopurified His-tagged proteins were mixed in the reaction buffer (50 mM Tris–HCl, pH 7.5, 5 mM MgCl2, 2 mM ATP) and incubated for 2 h at 37 °C. The reactions were analyzed by SDS–PAGE followed by Western blotting using anti-c-Myb (mAb 1–1) antibody.

2.6. CAT assays

Using the $CaPO_4$ method, CV-1 cells (4×10^5 cells per 100-mm dish) were transfected with the pc-*myc*-CAT reporter containing the human c-*myc* promoter [8], the plasmids used to express various forms of Myb, the plasmid used to express Fbxw5, and the internal control plasmid pact-β-gal. The amount of each plasmid used is indicated in Figure legend. Forty hours after transfection, CAT assays were performed as described [17].

2.7. Subcellular localization

CV-1 cells were transfected with the c-Myb and HA-Fbxw5 expression plasmids, or the c-Myb expression plasmid alone. Immunostaining with anti-c-Myb and anti-HA antibodies was performed as described, as well as the analysis of confocal microscopy images [17].

3. Results

3.1. Fbxw5 stimulates sumoylation of c-Myb

Previously, we examined the interaction between c-Myb and WD40 repeat-containing proteins and found that various WD40 repeat-containing proteins bound to c-Myb, including Fbxw5 [18]. Fbxw5 has been reported to induce ubiquitination of tumor suppressor TSC2 by the DDB1-Cul4-Rbx1 ligase [24]. We also found that Fbxw7α stimulated ubiquitination of c-Myb by the Skp1-Cul1-Rbx1 complex, which leads to the degradation of c-Myb [18]. Thus, we examined whether Fbxw5 regulates c-Myb ubiquitination. However, co-expression of Fbxw5 with c-Myb did not stimulate ubiquitination of c-Myb in CV-1 cells (Fig. 1A). Instead, Fbxw5 enhanced sumoylation of c-Myb, but not neddylation. It has been shown that c-Myb is sumoylated at Lys-499 and Lys-523 by two different SUMO E3 ligases, PIASy and TRAF7 [21,23]. When these two Lys residues were replaced by Arg, Fbxw5 was unable to induce sumoylation of c-Myb (Fig. 1B). Additionally, increasing amounts of Fbxw5 did not affect c-Myb protein levels (Fig. 1C), while Fbxw7α led to a decrease in c-Myb levels, as previously reported [18].

When c-Myb and Fbxw5 were co-expressed in 293T cells, wild-type c-Myb was efficiently co-precipitated with Fbxw5 (Supplementary Figs. S1A and S1B). Additionally, three different C-terminal deletion mutants of c-Myb (CT2, CT3, and CT5) were all co-precipitated with Fbxw5, suggesting that the N-terminal 193-amino acid portion, most of which is the DNA-binding domain, contains the Fbxw5-interacting region. However, ΔDBD and NT2, two c-Myb mutants which lack the DNA-binding domain and the N-terminal 76-amino acid region, respectively, were also co-immunoprecipitated with Fbxw5. These results suggest that c-Myb contains other Fbxw5-interacting regions other than the N-terminus. Fbxw5 also stimulated sumoylation of ΔDBD and NT2, but not ΔNRD (Supplementary Fig. S1C), since it lacks the Lys-499 and Lys-523 sumoylation sites.

In co-immunoprecipitation assays, the ΔF mutant of Fbxw5, which lacks the N-terminal F box, interacted with c-Myb (Supplementary Figs. S2A and S2B). Furthermore, the ΔC and ΔN mutants, which lack the most N-terminal WD repeat (WD1) and the two WD repeats in the C-terminal region (WD2 and WD3), respectively, were also co-immunoprecipitated with c-Myb. Together with our previous data showing that the F box of Fbxw7 binds to c-Myb [18], these results suggest that either WD repeat in the N- or C-terminal region of Fbxw5 is capable of binding to c-Myb. When these three mutants were used for the c-Myb sumoylation assay, they all displayed reduced levels of sumoylation, although the ΔF mutant was able to weakly stimulate c-Myb sumoylation (Supplementary Fig. S2C). Thus, the presence of a WD repeat in Fbxw5 facilitates its interaction with c-Myb, while the F box plays an important role in c-Myb sumoylation.

3.2. Fbxw5 stimulates c-Myb sumoylation by the DDB1-Cul4A-Rbx1 complex

Mammalian cells have three isoforms of SUMO: SUMO-1, SUMO-2, and SUMO-3 [25]. Fbxw5 stimulated c-Myb modification

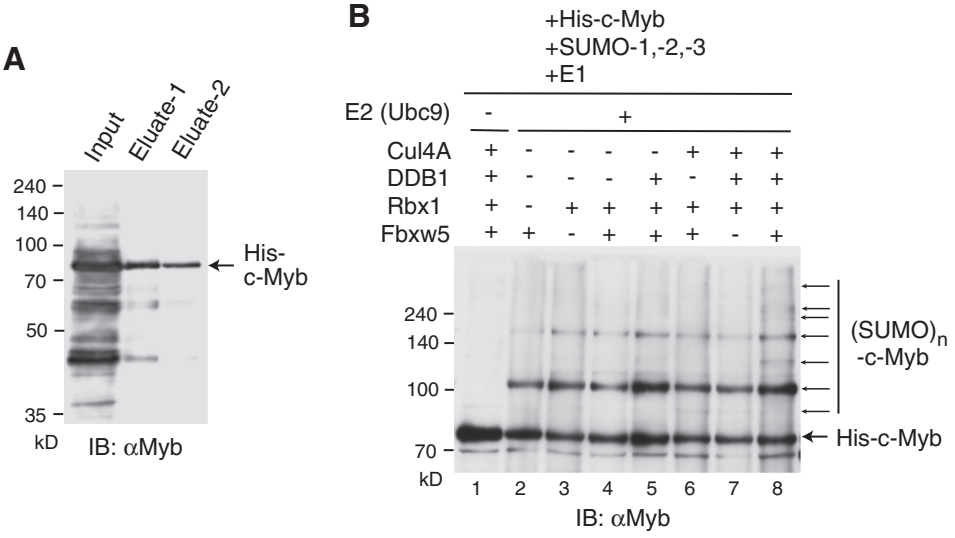

Fig. 2. Fbxw5 enhances sumoylation of c-Myb in vitro with the SCF complex components. (A) Purification of His-c-Myb. His-tagged c-Myb was expressed in 293T cells, mixed with Ni-resin, and eluted with imidazole. The eluates were used for Western blotting with anti-Myb. (B) In vitro sumoylation of c-Myb. The indicated components of the Fbxw5-DDB1-Cul4A-Rbx1 complex were immunopurified from 293T cells as described in the Experimental Procedures. Immuno-purified components and His-c-Myb were incubated with E1 (SAE1/SAE2), E2 (Ubc9), and SUMO-1, SUMO-2, and SUMO-3. The samples were then subjected to Western blotting with the anti-c-Myb (αCT5).

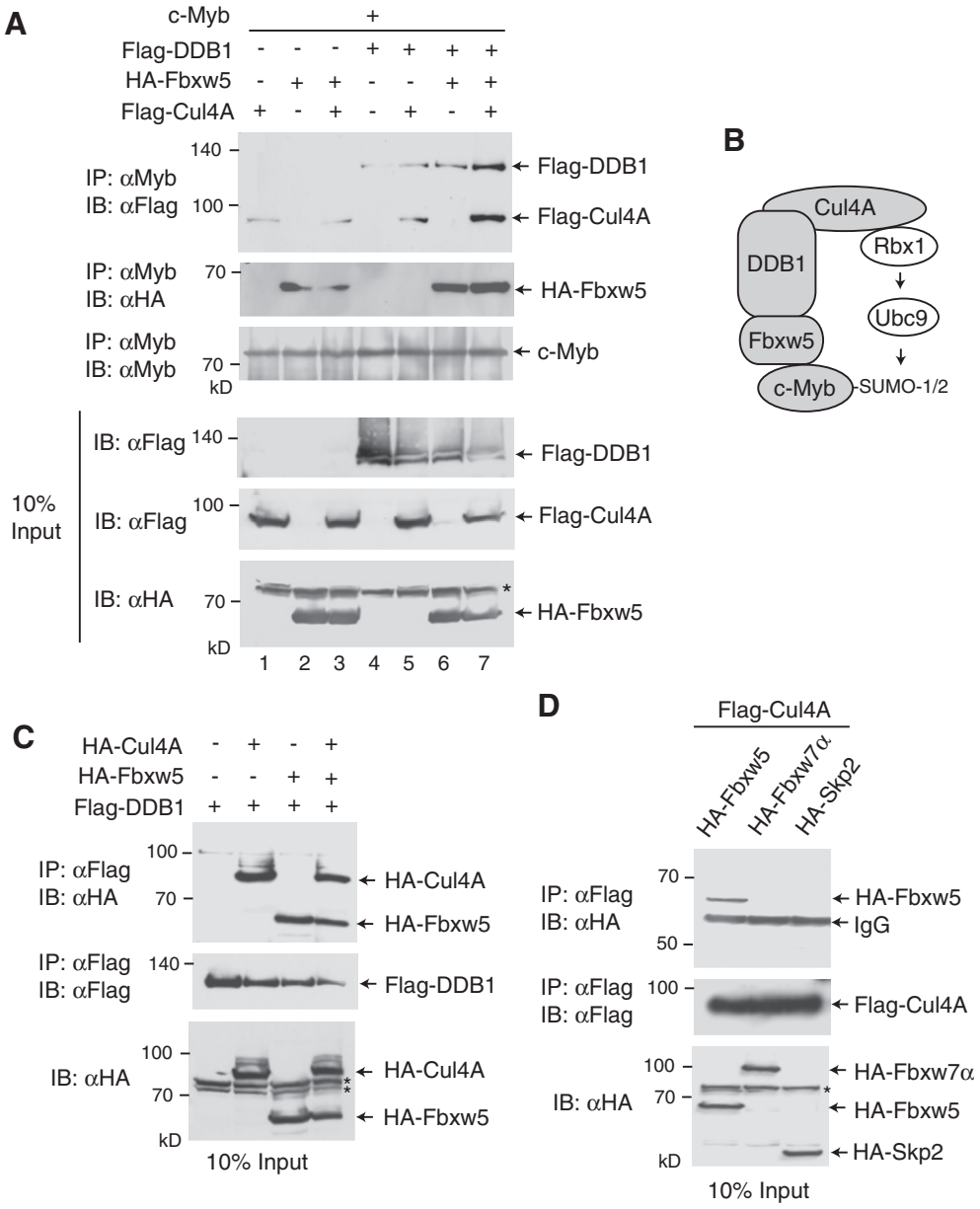

Fig. 3. c-Myb and Fbxw5 interact with the components of the DDB1-Cul4A complex. (A) Interaction of c-Myb with the DDB1-Cul4A complex via Fbxw5. 293T cells were transfected with a 4 μg of the c-Myb expression plasmid and 3 μg each of the Flag-DDB1, HA-Fbxw5, and Flag-Cul4A expression plasmids. Lysates from the transfected cells were immunoprecipitated with anti-c-Myb, and subjected to Western blotting with the antibodies shown (upper). Lysates were also used for direct Western blotting (below). Asterisk indicates a non-specific protein. (B) A schematic of the interaction between c-Myb and the Fbxw5-DDB1-Cul4A-Rbx1 complex. (C) Interaction of Fbxw5 with the Cul4A-DDB1 complex. 293T cells were transfected with 3 μg each of the HA-Cul4A, HA-Fbxw5, or Flag-DDB1 expression plasmids. Cell lysates were immunoprecipitated with anti-Flag M2 antibody, and subjected to Western blotting with the indicated antibody. Lysates were also used for direct Western blotting (below). (D) Interaction of Cul4A and Fbxw5. 293T cells were transfected with the Flag-Cul4A expression plasmid (3 μg) and the HA-Fbxw5, HA-Fbxw7α, or HA-Skp2 expression plasmids (3 μg). Cell lysates were immunoprecipitated with anti-Flag M2, and subjected to Western blotting with the indicated antibody. Lysates were also used for direct Western blotting (below).

by SUMO-1 and SUMO-2, but not SUMO-3 (Supplementary Fig. S3A). When various combinations of the three SUMO forms were used, c-Myb sumoylation was always enhanced by Fbxw5 in the presence of SUMO-1 or SUMO-2 (Supplementary Fig. S3B).

The Fbxw5-DDB1-Cul4A-Rdx1 complex was previously reported to be the ubiquitin ligase for tumor suppressor TSC2 [24]. Furthermore, some ubiquitin ligases, such as Topors, function as a dual ubiquitin/SUMO ligase [26,27]. Therefore, we investigated whether the Fbxw5-DDB1-Cul4A-Rdx1 complex was capable of sumoylating c-Myb in vitro. His-tagged c-Myb was expressed in 293T cells, purified using cobalt affinity gel (Fig. 2A), and used for in vitro sumoylation. We found that sumoylation of c-Myb was enhanced when His-tagged c-Myb was incubated with DDB1, Cul4A, and Rbx1 in the presence of recombinant SUMO1/2/3 and the E2 enzyme Ubc9 (Fig. 2B).

A lot of previous data have indicated that Cul-Rbx complexes, such as Cul4A-Rbx1, form a catalytic core for ubiquitin/sumo ligases. Especially, DDB1 was reported to act as a linker to recruit receptor WD40 protein to the Cull4-Rbx1 complex [24]. Therefore, we examined only for interactions between c-Myb, Fbxw5, DDB1, and Cul4A (the components shown in dark in Fig. 3B), because the Cul4A-Rbx1 interaction has already been well established. When c-Myb was co-expressed with the Fbxw-DDB1-Cul4A complex components, high levels of Fbxw5, Cul4A, and DDB1 were co-immunoprecipitated with c-Myb (Fig. 3A, compare lane 7 with the other lanes). However, when c-Myb was co-expressed with

only one or two of the Fbxw-DDB1-Cul4A complex components, the co-immunoprecipitated level of each protein was reduced. These results suggest that the interaction of c-Myb with Fbxw5 is stabilized by DDB1 and Cul4A (Fig. 3B). In Fig. 3A, the signal intensity of Flag-Cul4A (10% input) in lane 7 is lower than those of Flag-Cul4A in lanes 1, 3, and 5. This may suggest that the formation of the Cul4A-DDB1-Fbxw5 complex reduces the stability of its individual components, although the mechanism responsible for this is currently unknown.

Complex formation between Fbxw5, DDB1, and Cul4A was confirmed by co-immunoprecipitation. Fbxw5 and Cul4A were co-immunoprecipitated with DDB1 (Fig. 3C). Fbxw5 was co-immunoprecipitated with Cul4A, whereas neither Fbxw7α nor Skp2 was co-immunoprecipitated (Fig. 3D), indicating that Cul4A specifically interacts with Fbxw5.

3.3. Fbxw5 suppresses the trans-activation capacity of c-Myb

We then investigated the effect of Fbxw5 on the c-Myb *trans*-activating capacity by analyzing the CAT activity from the c-*myc* promoter, one of the c-Myb target genes. Expression of Fbxw5 decreased the basal c-*myc* promoter activity to 46% of the control level in the absence of c-Myb, possibly due to the ubiquitination or sumoylation of other proteins (Fig. 4A). The c-*myc* promoter activity was enhanced 12-fold by c-Myb, which was reduced to 11% by the expression of Fbxw5. In contrast, the activity of v-Myb was not suppressed by Fbxw5.

3.4. Sumoylation of c-Myb by Fbxw5 induces its localization to nuclear speckles

When wild-type c-Myb was co-expressed with Fbxw5 in CV-1 cells, c-Myb signals appeared as nuclear dot-like structures, although c-Myb was localized uniformly in the nucleoplasm when expressed alone (Fig. 4B). Since many sumoylated proteins localize to PML nuclear bodies [28], we examined whether sumoylated c-Myb by Fbxw5 also co-localized with PML bodies. The signal of the PML nuclear bodies did not overlap with those of sumoylated c-Myb (Fig. 4C). Therefore, sumoylation of c-Myb by Fbxw5 induces its localization to nuclear speckles, but not to PML nuclear bodies.

4. Discussion

We have demonstrated that Fbxw5 directly binds to c-Myb, and induces the sumoylation of c-Myb. While the Fbxw5-Cul4A-DDB1-Rbx1 complex was shown to act as the ubiquitin ligase for tumor suppressor TSC2 [24], our data indicates that this complex can also function as a SUMO ligase. The RING finger-containing protein Topors similarly exhibits dual ubiquitin/SUMO ligase activity, enhancing both the ubiquitination and the SUMO-1 conjugation of p53 [26,27]. While Topors can both ubiquitinate and sumoylate the same substrate, p53, Fbxw5 acts as a ubiquitin ligase for TSC2 and a SUMO ligase for c-Myb. Thus, Fbxw5 functions differently depending on the substrate. Fbxw5 may adopt a different protein conformation when interacting with TSC2 or c-Myb, which may selectively induce binding to ubiquitin or SUMO ligase components.

Up until now, four SUMO E3 ligases for c-Myb have been identified, including Fbxw5 (Supplementary Fig. S4), but the mechanisms by which these E3 ligases regulate c-Myb activity differ. Fbxw5 enhances sumoylation of c-Myb in the nucleus, and inhibits the *trans*-activating capacity of c-Myb by recruiting sumoylated c-Myb to nuclear speckles. On the other hand, TRAF7 stimulates c-Myb sumoylation in the cytoplasm, and inhibits c-Myb activity by sequestering it to the cytoplasm [23]. Thus, the mechanisms by Fbxw5 and TRAF7 negatively regulate c-Myb activity are distinct. Furthermore, despite the fact that both Fbxw5 and PIASy enhance c-Myb sumoylation in the nucleus, sumoylation by Fbxw5 localizes c-Myb to nuclear speckles, while sumoylation by PIASy localizes it to PML nuclear bodies. These data further support the idea that Fbxw5 and PIASy negatively regulate c-Myb by different mechanisms, although the function of the nuclear speckles remains unknown.

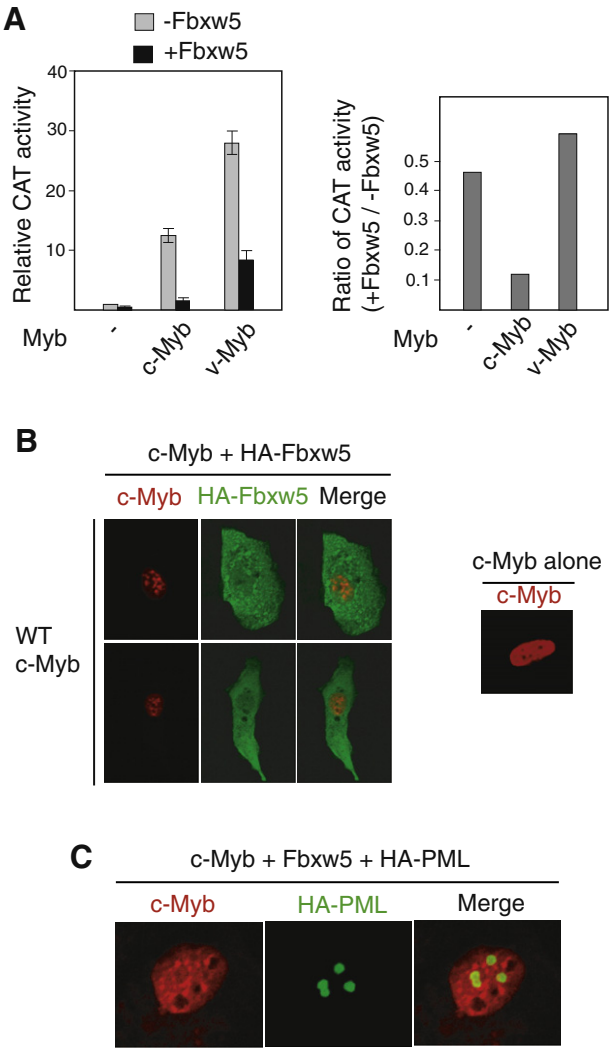

Fig. 4. Fbxw5 suppressed c-Myb *trans*-activating capacity and sequestered c-Myb to nuclear dot-like domains. (A) Suppression of c-Myb *trans*-activating capacity by Fbxw5. (Left) A mixture of 4 μg of the c-*myc* promoter-CAT reporter, 2 μg of the plasmid expressing c-Myb or v-Myb or the control vector, 4 μg of the pact-Fbxw5 or the control vector, and 0.5 μg of the internal control pact-β-gal was transfected into CV-1 cells. Forty hours after transfection, CAT activity was measured, and was normalized to the transfection efficiency (β-gal). The CAT activity relative to that without Myb and Fbxw5 is shown + S.D. ($n = 3$). (Right) The ratio between the CAT activity with and without Fbxw5 is shown. (B) Localization of c-Myb to nuclear dot-like domains in the presence of Fbxw5. (Left) 4 μg of the plasmid expressing wild-type c-Myb, and 3 μg of the pact-HA-Fbxw5 or the control vector was transfected into CV-1 cells. Forty hours after transfection, cells were permeabilized with Triton X-100 and immunostained with anti-c-Myb and anti-HA antibodies, visualized by rhodamine-or FITC-conjugated secondary antibodies, and analyzed by confocal microscopy. c-Myb and HA-Fbxw5 are shown in red and green, respectively. (Right) CV-1 cells were transfected with 4 μg of the c-Myb expression plasmid alone, and its subcellular localization was examined. (C) The Fbxw5-dependent nuclear dot of c-Myb did not colocalize with PML. CV-1 cells were transfected with 4 μg of the c-Myb expression plasmid, 3 μg of the pact-Fbxw5, and 3 μg of the HA-PML expression plasmid. Subcellular localization was analyzed as described above, and c-Myb and HA-PML are shown in red and green, respectively.

Fbxw5 modifies c-Myb using SUMO-1 and SUMO-2, but not SUMO-3. In contrast, TRAF7 and PIASy utilize SUMO-1 to modify c-Myb. SUMO-1 shares 48% identity with SUMO-2 and 46% identity with SUMO-3, while SUMO-2 and -3 share 95% identity, suggesting that SUMO-2 and -3 form a subfamily distinct from SUMO-1 [25]. Furthermore, the conjugation of SUMO-2/SUMO-3 is stimulated by certain types of stress [29]. In response to heat stress and UV light, PIAS-3 stimulates SUMO-2 and SUMO-3 modification of c-Myb, but not SUMO-1 [22]. Therefore, the Fbxw5-dependent modification of c-Myb with SUMO-1 and SUMO-2 differs from the PIAS-3-mediated SUMO-2 and SUMO-3 modification of c-Myb.

We also found that Fbxw5 suppresses the *trans*-activating capacity of c-Myb, but not that of v-Myb. We previously demonstrated that v-Myb is resistant to Fbxw7-mediated ubiquitination and degradation in response to Wnt-1 signal [19]. Thus, v-Myb also avoids negative regulation via sumoylation. While Fbxw5 inhibits the *trans*-activating capacity of c-Myb via sumoylation, it may also enhance the repression of some c-Myb target genes(s). c-Myb has been shown to interact with the coactivator CBP [7], and with corepressors, including N-CoR, mSin3A, and Ski [16], which suggests that some target genes may be repressed by c-Myb. Therefore, Fbxw5-mediated sumoylation of c-Myb may enhance the repression of some c-Myb target gene(s).

Acknowledgments

We thank H. Saitoh for the SUMO-1, SUMO-2, and SUMO-3 plasmids, T. Chiba for the Nedd8, Cul1, DDB1, and Cul4B plasmids, K.I. Nakayama for the Fbxw7α and Fbxw5 plasmids, N. Watanabe for the Skp2 and Rbx1 plasmids, and T. Ohta for the Cul4A and DDB1 plasmids. This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan. No additional external funding received for this study.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.08.032.

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