NIRF is a ubiquitin ligase that is capable of ubiquitinating PCNP, a PEST-containing nuclear protein

Tsutomu Mori, Yuanyuan Li, Hiroaki Hata, Hideo Kochi*

Department of Biochemistry, Fukushima Medical University, School of Medicine, 1 Hikarigaoka, Fukushima, Fukushima 960-1295, Japan

Received 18 September 2003; revised 11 November 2003; accepted 12 December 2003

First published online 29 December 2003

Edited by Ulrike Kutay

Abstract We previously reported the association of a novel Np95/ICBP90-like RING finger protein (NIRF) with a novel PEST-containing nuclear protein (PCNP). NIRF is a nuclear protein with a ubiquitin-like domain, a PHD finger, a YDG/SRA domain, Rb-binding motifs and a RING finger. In this study, we showed that NIRF has auto-ubiquitination activity, the hallmark of a ubiquitin ligase. PCNP was readily ubiquitinated in 293 and COS-7 cells, and NIRF ubiquitinated PCNP in vitro as well as in vivo. Considering that NIRF is implicated in cell cycle regulation, these findings suggest that NIRF and PCNP are a ubiquitin ligase and its substrate, respectively, and may constitute a novel signaling pathway with some relation to cell proliferation.

Key words: PEST protein; Ubiquitin-like domain; RING finger; Ubiquitin ligase; Cell cycle; Proteasome

1. Introduction

Ubiquitin-dependent protein degradation regulates various cellular processes, including cell cycle progression, DNA repair, differentiation and protein transport [1–3]. Ubiquitin ligases confer substrate specificity on the proteins to be degraded and contribute to the efficiency of ubiquitination reactions [4]. PEST sequences, which are rich in proline (P), glutamic acid (E), serine (S) and threonine (T), are found in a number of short-lived proteins, such as transcription factors and cell cycle-associated proteins. The function of those PEST proteins is generally controlled by proteolysis, mostly via ubiquitin-mediated degradation [5].

We previously found a novel PEST-containing nuclear protein (PCNP), and then identified a novel Np95/ICBP90-like RING finger protein (NIRF) as a protein that interacts with PCNP [6]. NIRF possesses a characteristic domain configuration (a ubiquitin-like domain, a PHD finger, a YDG/SRA domain, and a RING finger) shared with Np95/ICBP90, cell proliferation-associated proteins [7,8]. NIRF and Np95/ICBP90 are homologous to each other and belong to a novel family of nuclear proteins [9], which we refer to as the NIRF family. Recent evidence shows that this family is involved in cell cycle regulation [6–10] and genome stability [11].

In this study, we investigated the function of NIRF and the functional relationship between NIRF and PCNP.

2. Materials and methods

2.1. Materials

Ubiquitin-activating enzyme (E1) from rabbit, and ubiquitin-conjugating enzymes (E2s, UbcH5a and UbcH7) were purchased from Boston Biochem (Cambridge, MA, USA), and histidine-tagged ubiquitin from Sigma (St. Louis, MO, USA). MG-132 was obtained from the Peptide Institute (Osaka, Japan). Antibodies used were the following: anti-His monoclonal antibody (HIS-1) and anti-FLAG monoclonal antibody (M2) from Sigma; anti-ubiquitin monoclonal antibody (Ubi-1) from Zymed (San Francisco, CA, USA); anti-myc monoclonal antibody (9E10) from Santa Cruz (Santa Cruz, CA, USA); anti-HA polyclonal antibody from MBL (Nagoya, Japan); affinity-purified anti-PCNP antibody [6]; affinity-purified goat anti-rabbit IgG alkaline phosphatase conjugate (Zymed); and affinity-purified anti-mouse IgG alkaline phosphatase conjugate (Bio-Rad, Hercules, CA, USA).

2.2. Plasmids

The coding sequence of PCNP (GenBank accession number AB037675) was cloned into the vectors pFLAG-CMV-2 (Sigma) and pCAGGS [12]. The coding sequence of NIRF (GenBank accession number AB071698) was cloned into p3×FLAG-CMV-10 (Sigma), pCMV-Myc, pCMV-HA (Clontech, Palo Alto, CA, USA), and pCAGGS. A cDNA for ubiquitin was amplified by polymerase chain reaction from a human brain cDNA library (Clontech) and cloned into p3×FLAG-CMV-10 and pCMV-Myc.

2.3. Cell culture, transfection, and immunoprecipitation

COS-7 and HEK 293 cells were cultured in Dulbecco's modified Eagle's medium (Sigma) supplemented with 10% fetal calf serum (Invitrogen, Carlsbad, CA, USA). Transfection of the plasmids into COS-7 or 293 cells was carried out using the Effectene reagent (Qiagen, Valencia, CA, USA) as recommended by the manufacturer. For transfection, total DNA amount was adjusted to 1 μg per 60 mm dish. Medium was changed once at 24 h after transfection, and cells were further cultured for 12 h with or without MG-132.

For immunoprecipitation, cell extracts were prepared as follows: at 36 h after transfection, cells were harvested and lysed in 1 ml per dish of ice cold lysis buffer (50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 0.5% NP-40, and a protease inhibitor cocktail [Sigma]) for 30 min, followed by centrifugation at 14 000×*g* for 15 min. Protein concentrations of the extracts were estimated by a BCA protein assay kit (Pierce, Rockford, IL, USA), and extracts with an equivalent protein amount were used for immunoprecipitation.

Immunoprecipitation of FLAG-tagged proteins was performed with FLAG-tagged protein immunoprecipitation kit (Sigma) according to the manufacturer's recommendation. Briefly, 30 μl of anti-FLAG M2 affinity gel was added to 1 ml of cell extract, and the sample was

*Corresponding author. Fax: (81)-24-548 8640.
E-mail address: hkochi@fmu.ac.jp (H. Kochi).

Abbreviations: NIRF, Np95/ICBP90-like RING finger protein; PCNP, PEST-containing nuclear protein; Np95, 95-kDa mouse nuclear protein; ICBP90, inverted CCAAT box-binding protein of 90 kDa; E1, ubiquitin-activating enzyme; E2, ubiquitin-conjugating enzyme; E3, ubiquitin–protein ligase; Ubn-PCNP, ubiquitinated form of PCNP

0014-5793/03/$30.00
doi:10.1016/S0014-5793(03)01495-9

shaken for 2 h in ice bath. After washing three times with washing buffer (50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 0.1% NP-40) and once with phosphate-buffered saline, FLAG fusion protein was eluted by the use of 50 μl of 3×FLAG peptide solution (150 ng/μl). The eluted proteins were dissolved in Laemmli's sample buffer [13], and then subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) followed by Western blot analysis.

For immunoprecipitation of PCNP, 1 ml of extract was incubated with 3 μl (15 μg) of affinity-purified anti-PCNP antibody [6]. After the sample was shaken with 30 μl of protein A-Sepharose CL-4B gel (Amersham, Uppsala, Sweden) for 2 h at 0°C, immunoprecipitates bound on the gel were dissolved with SDS–PAGE sample buffer and subjected to SDS–PAGE, followed by Western blotting. The concentrations of the antibodies used for the Western blotting were 0.5 μg/ml (anti-FLAG), 1 μg/ml (anti-ubiquitin), 1 μg/ml (anti-His), 0.1 μg/ml (anti-myc), 2.5 μg/ml (anti-PCNP), 1:2000 dilution (anti-HA), 1:3000 dilution (anti-mouse IgG alkaline phosphatase conjugate), and 1:3000 dilution (anti-rabbit IgG alkaline phosphatase conjugate). The blot was reacted with CDP-Star reagent (Amersham) and the image was captured with a Light Capture System (ATTO, Tokyo, Japan).

2.4. In vitro ubiquitination assay

FLAG-NIRF was purified from extracts of COS-7 cells transfected with p3×FLAG-NIRF by immunoprecipitation. The ubiquitination reaction was carried out for 60 min at 37°C. The complete reaction system consisted of the reaction buffer (25 mM HEPES pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$, 0.05% Triton X-100, 0.5 mM dithiothreitol, 3 mM ATP, 10 μg of histidine-tagged ubiquitin), 200 ng of E1, 200 ng of E2 (UbcH5a or UbcH7), 500 ng of FLAG-NIRF, in a final volume of 50 μl.

For in vitro PCNP ubiquitination assay, 1 μg of the purified recombinant PCNP [6] was included in the reaction mixture. After completion of the ubiquitination reaction, the mixture was incubated with nickel-charged beads (Chelating Sepharose, Amersham) for 20 min at 0°C, followed by washing three times with the ubiquitination reaction buffer containing 0.5 M NaCl. Proteins conjugated with the histidine-tagged ubiquitin were eluted with the buffer containing 0.5 M imidazole, and subjected to SDS–PAGE followed by Western blot analysis with anti-PCNP or anti-His antibody.

3. Results

3.1. NIRF is a novel ubiquitin ligase

NIRF possesses a ubiquitin-like domain and a RING finger (Fig. 1), which are the functional motifs for the interaction with a proteasome [14] and the ubiquitin ligase activity, respectively [15]. The presence of both domains is similar to Parkin, an RBR protein with a ubiquitin ligase activity [16]. Although Parkin does not contain the PHD finger and the YDG/SRA domain, this similarity suggests that NIRF could also be a ubiquitin ligase. To test this, we transfected FLAG-NIRF into 293 cells with or without treatment with MG-132, a proteasome inhibitor [17], and then cell extracts were subjected to immunoprecipitation with anti-FLAG antibody followed by Western blot analysis (Fig. 2A). FLAG-NIRF migrated to an M_r value of 113 kDa nearly equal to that of untagged NIRF expressed from pCAGGS-NIRF (data not shown). This apparent molecular size is markedly larger than a predicted MW of 90 kDa calculated from the amino acid composition, suggesting that NIRF may be subject to certain modifications in vivo. When the blot was immunostained with anti-ubiquitin antibody, the NIRF immunoprecipitated from the MG-132-treated cells exhibited an intense smearing signal resulting from ubiquitin conjugation, while the signal was barely visible without MG-132 (Fig. 2A, right). These results suggest that NIRF could be ubiquitinated in vivo and subsequently subjected to rapid degradation in the absence of proteasome inhibitor. On the other hand, a FLAG-NIRF increase was not apparent in the presence of MG-132 (Fig. 2A, left). This may indicate that the amount of NIRF degraded in the ubiquitin-proteasome pathway is markedly small in this condition as compared with the amount of overexpressed NIRF.

Since many ubiquitin ligases are known to exhibit auto-ubiquitination activity, we next examined whether NIRF is capable of being auto-ubiquitinated in vitro, by employing UbcH5a and UbcH7 as E2 ubiquitin conjugases expected to cooperate with NIRF (Fig. 2B). On a Western blot, the ubiquitinated NIRF was detected by anti-His antibody only in the presence of E1, UbcH5a, and NIRF. Thus, NIRF is auto-ubiquitinated in vitro in cooperation with UbcH5a but not with UbcH7, in spite of conservation of the core domain between both conjugases [18].

3.2. NIRF interacts with PCNP in vivo

We previously found, using a yeast two-hybrid system, a glutathione *S*-transferase (GST) pull-down assay, and a mammalian two-hybrid assay, that both NIRF and PCNP were located in the nucleus and associated with each other in

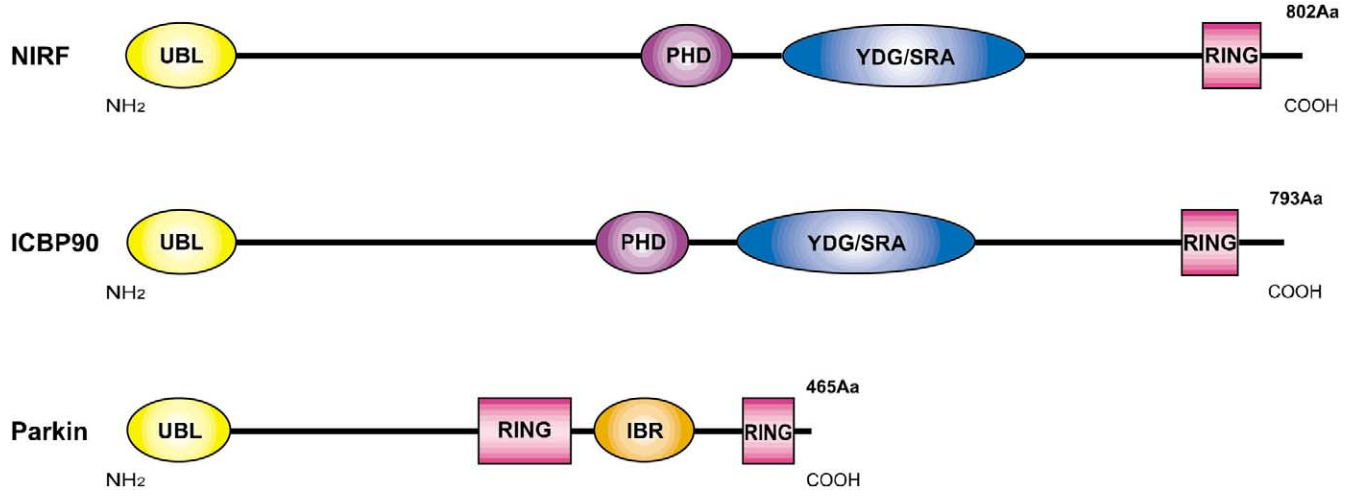

Fig. 1. Schematic representation of the domain composition of NIRF, ICBP90, and Parkin. UBL, ubiquitin-like domain [14]; PHD, plant homeodomain finger [23]; YDG/SRA, SET and RING finger-associated domain containing a conserved motif YDG [24]; RING, RING finger [15]; IBR, in between RING finger motif [16].

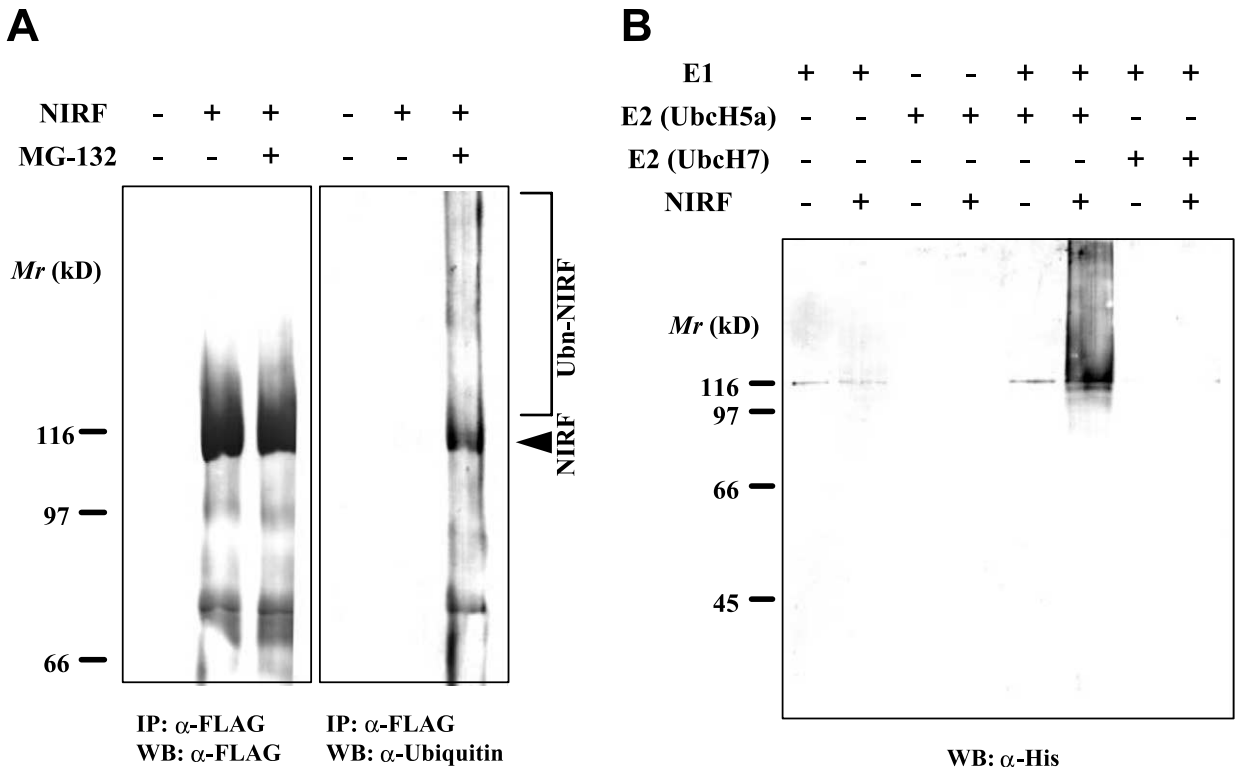

Fig. 2. NIRF is ubiquitinated in vivo, and catalyzes an auto-ubiquitination reaction in vitro. A: 293 cells were transfected with 1 μg of FLAG-NIRF or mock vector. Twenty-four hours later the medium was changed, and the cells were further cultured for 12 h in the presence or absence of MG-132 (5 μM). Then, cell extracts were prepared and subjected to immunoprecipitation (IP) with anti-FLAG M2 affinity gel, followed by Western blot analysis (WB). The blot was immunostained with 0.5 μg/ml of anti-FLAG antibody (left) or 1 μg/ml of anti-ubiquitin antibody (right). B: An in vitro ubiquitination reaction was performed as described in Section 2 with the indicated combinations of the purified FLAG-NIRF, E1, and E2 (UbcH5a or UbcH7). The reaction mixture was subjected to SDS–PAGE, followed by Western blot analysis with 1 μg/ml of anti-His antibody to visualize de novo conjugation of histidine-tagged ubiquitin [25].

vivo as well as in vitro [6]. As shown in Fig. 3, a co-immunoprecipitation assay further validated an in vivo interaction. When extracts from COS-7 cells transfected with plasmids encoding PCNP and/or FLAG-NIRF were subjected to immunoprecipitation by an anti-FLAG antibody followed by Western blot analysis, PCNP was co-immunoprecipitated with FLAG-NIRF (Fig. 3A), indicating at least that PCNP and NIRF participate in the formation of the same supramolecular structure in vivo. And considering the previous results with two-hybrid systems and a GST pull-down assay [6], PCNP and NIRF can directly interact with each other in the complex. This was also ascertained by a reciprocal co-immunoprecipitation experiment, in which the cells were transfected with FLAG-PCNP and/or myc-NIRF (Fig. 3B). Myc-NIRF was co-immunoprecipitated with FLAG-PCNP by anti-FLAG antibody. Moreover, the co-immunoprecipitated myc-NIRF and FLAG-PCNP increased following treatment with MG-132, suggesting that either NIRF or PCNP, or both of them, were subject to ubiquitin-mediated degradation in COS-7 cells. In view of the fact that PEST proteins are often degraded via the ubiquitin-dependent pathway accompanied by ubiquitin ligases [5,19], we then investigated whether PCNP is susceptible to ubiquitination.

3.3. PCNP is readily ubiquitinated in vivo

In vivo ubiquitination of PCNP was examined by transfecting the FLAG-ubiquitin (FLAG-Ub) construct into COS-7 cells followed by immunoprecipitation and Western blot analysis (Fig. 4). In this system, a series of ubiquitinated PCNP species with much higher molecular weights than PCNP itself were easily detected. The ubiquitinated, endogenous PCNPs were still observed without transfection. Since PCNP is an abundant nuclear protein (data not shown), a significant proportion of endogenous PCNP may be ubiquitinated in COS-7 cells. Levels of ubiquitinated PCNP increased markedly in PCNP-transfected cells, and further increased following MG-132 treatment. As was expected from the presence of high-score PEST sequences in PCNP [6], these findings demonstrate that PCNP is readily ubiquitinated in vivo and subsequently degraded by the proteasome.

3.4. NIRF is capable of ubiquitinating PCNP

We next investigated whether the ubiquitin ligase activity of NIRF could contribute to ubiquitination of PCNP. As noted above, PCNP is ubiquitinated in COS-7 cells, but differences in the extent of PCNP ubiquitination were not clear between COS-7 cells with and without NIRF co-transfection (data not shown), possibly due to relatively higher endogenous ubiquitin ligase activity. Therefore, we used 293 cells in order to determine PCNP ubiquitination by NIRF. We transfected PCNP with or without NIRF into 293 cells, and assessed the formation of ubiquitinated PCNP (Fig. 5A). The signal intensity was weak even for NIRF-transfected cells in the

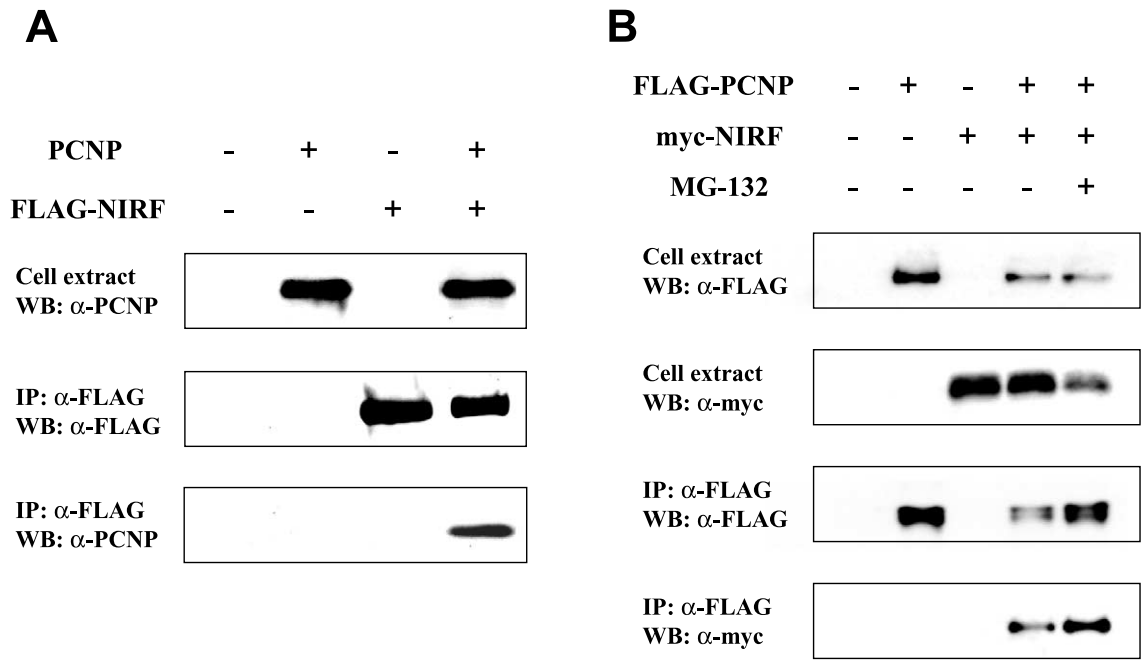

Fig. 3. Assessment of the in vivo association of NIRF with PCNP. A: COS-7 cells were co-transfected with 0.5 μg each of the plasmids encoding PCNP (pCAGGS-PCNP) and FLAG-NIRF (p3×FLAG-NIRF) or the corresponding empty vectors (pCAGGS and p3×FLAG-CMV-10) and cultured for 36 h. Cell extracts were prepared and immunoprecipitated with anti-FLAG M2 affinity gel followed by Western blot analysis with 0.5 μg/ml of anti-FLAG monoclonal antibody (middle panel) or 2.5 μg/ml of the affinity-purified anti-PCNP polyclonal antibody [6] (lower panel). The cell extracts were also subjected to SDS–PAGE and immunostained with the anti-PCNP antibody (upper panel). B: COS-7 cells were co-transfected with 0.5 μg each of the plasmids encoding FLAG-PCNP (pFLAG-PCNP) and myc-NIRF (pCMV-Myc-NIRF) or the corresponding empty vectors (pFLAG-CMV-2 and pCMV-Myc). Twenty-four hours later the medium was changed, and cells were further cultured for 12 h in the presence or absence of MG-132 (5 μM). Cell extracts were prepared and subjected to immunoprecipitation with anti-FLAG M2 affinity gel followed by Western blot analysis with 0.5 μg/ml of anti-FLAG antibody or 0.1 μg/ml of anti-myc antibody (lower two panels). The extracts were also subjected to SDS–PAGE and immunostained with these antibodies (upper two panels).

absence of MG-132 (lanes 2 and 3), probably owing to rapid destruction of ubiquitinated PCNP in 293 cells. By the addition of MG-132, however, the signal intensity increased, and it became clear that NIRF enhanced ubiquitination of PCNP (lanes 4 and 5). In Fig. 5B, we analyzed the mono-ubiquitinated form of PCNP. The amounts of mono-ubiquitinated PCNP increased in the NIRF-transfected cells (Fig. 5B, lower panel), and were proportional to the extents of PCNP ubiquitination (see Fig. 5A). Thus, overexpressed NIRF was capable of ubiquitinating PCNP in vivo.

Again NIRF was co-immunoprecipitated with PCNP (Fig. 5B, upper panel), indicating that ubiquitination of PCNP occurred in association with NIRF. While the amount of NIRF co-immunoprecipitated with PCNP was less without MG-132 than with MG-132, this must be due to rapid degradation of ubiquitinated PCNP in the absence of proteasome inhibitor. In fact, the amounts of NIRF in the NIRF-transfected cell extracts were practically equal with and without MG-132 (data not shown).

The capability of NIRF to be a ubiquitin ligase for PCNP ubiquitination was also examined in vitro. Purified PCNP was incubated in the ubiquitination mixture containing histidine-tagged ubiquitin, and the ubiquitinated PCNPs were visualized on Western blot with either anti-PCNP or anti-His antibody (Fig. 6). As shown in the figure, PCNP was ubiquitinated only in the presence of NIRF. Thus, NIRF as a ubiquitin ligase is capable of ubiquitinating PCNP in vitro as well as in vivo.

4. Discussion

4.1. NIRF family of proteins as possible ubiquitin ligases

NIRF and ICBP90 are moderately analogous to each other in amino acid sequence (53% identical and 67% similar), and counterparts of both have been found in mice, i.e. mouse NIRF (GenBank accession number AB116653) and Np95 [7]. These four proteins share a characteristic domain configuration, and constitute a novel family of nuclear proteins (Fig. 1) [9]. As noted above, NIRF is also structurally related to Parkin, an RBR protein with ubiquitin ligase activity [16]. This similarity between NIRF and Parkin is seen in the domain composition, and both NIRF and Parkin exhibit ubiquitin ligase activity, whereas they are not significantly similar in their amino acid sequences. In this connection, ubiquitin ligase activity can be a general functional property shared among RING finger proteins irrespective of divergences in their entire structure [4,15]. Thus, all the members of the NIRF family could be ubiquitin ligases with multi-domain structures.

4.2. Multi-functionality of the NIRF family

To date, the NIRF family is considered to be cell proliferation-associated, and involved in tumorigenesis in various ways. As to human NIRF, mRNA expression is high in the proliferating phase but significantly low in the G0/G1 phase in normal cells while it is consistently high in tumor cells. Moreover, the human NIRF gene maps to 9p24.1, just within a

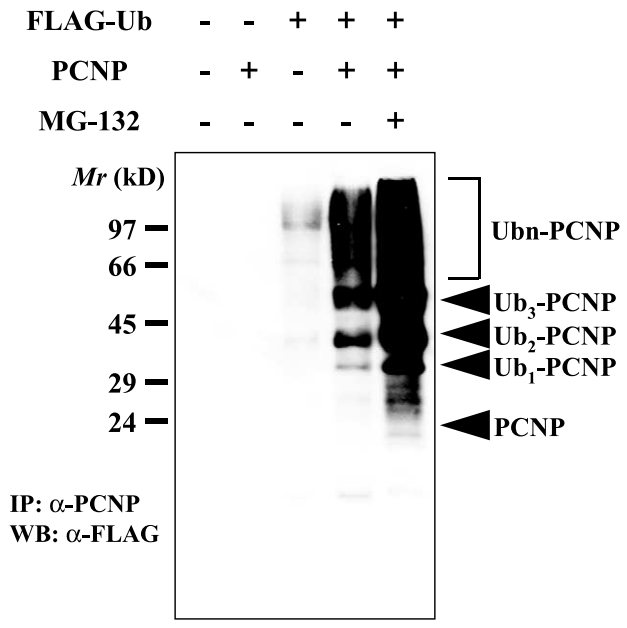

Fig. 4. PCNP is readily ubiquitinated in vivo. COS-7 cells were co-transfected with 0.5 μg each of the plasmids encoding FLAG-ubiquitin (p3×FLAG-ubiquitin) and PCNP (pCAGGS-PCNP) or the corresponding empty vectors (p3×FLAG-CMV-10 and pCAGGS). Twenty-four hours later the medium was changed, and the cells were further cultured for 12 h in the presence or absence of MG-132 (5 μM). Cell extracts were prepared and subjected to immunoprecipitation with 15 μg/ml of the affinity-purified anti-PCNP polyclonal antibody [6] followed by Western blot analysis with 0.5 μg/ml of anti-FLAG antibody.

small amplicon in some tumor cells [6], suggesting that the NIRF gene could behave as an oncogene. In addition, the finding that 9p24.1 is lost in various types of tumors at the top of frequency (48%) has led to the prediction that an essential tumor suppressor exists in this region [20]. NIRF is involved in cell cycle control as a negative regulator (to be published elsewhere). On the contrary, Np95/ICBP90 are positive regulators of cell proliferation [8–10]. Np95 is proposed to be an oncogene candidate [10] and contributes to genome stability in response to various genotoxic insults [11]. Taken together, the NIRF family members may be ubiquitin ligases contributing not only to cell cycle regulation but also to tumorigenesis.

4.3. Ubiquitination of PCNP by NIRF

The PEST hypothesis predicts that the proteins with 'PEST sequences' tend to be degraded rapidly, often via the ubiquitin-proteasome pathway [5]. In fact, many PEST proteins have been defined to be short-lived. Also they often act as regulatory proteins [21,22]. We originally identified NIRF as a partner interacting with PCNP in yeast two-hybrid screening [6]. In this study, PCNP was ubiquitinated by NIRF both in vivo and in vitro. This may imply that NIRF and PCNP may be involved in the same signaling pathway concerned with cell cycle regulation and/or genome stability.

Both cell cycle progression and genome stability are controlled by an intricate network of signaling molecules including ubiquitin ligases, transcription factors, and protein kinases. NIRF could play a role in this network by ubiquitinating certain substrates including PCNP, leading to control of a signaling pathway for cellular homeostasis. The

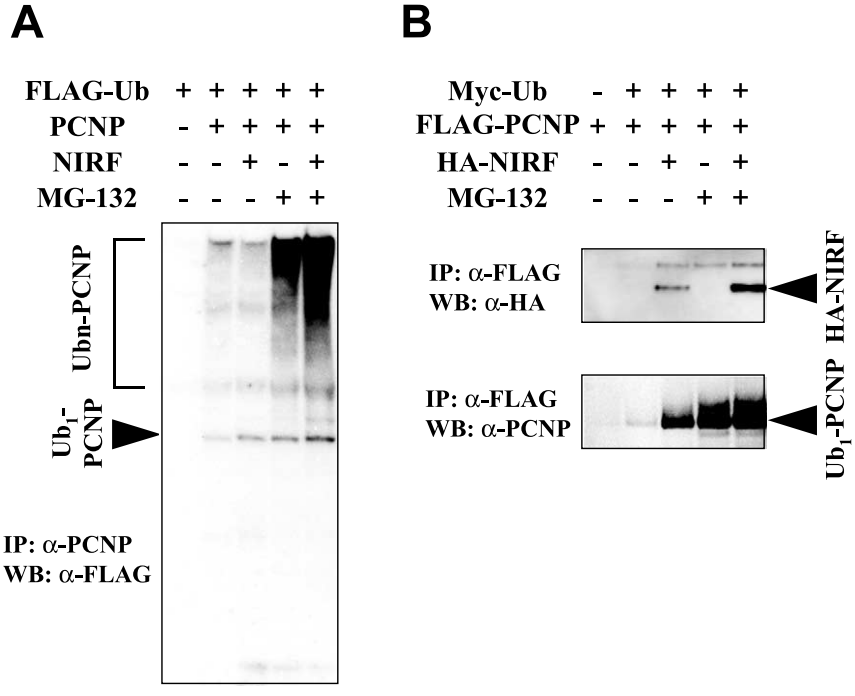

Fig. 5. NIRF can ubiquitinate PCNP in vivo. A: 293 cells were co-transfected with 0.33 μg each of the plasmids encoding FLAG-ubiquitin (p3×FLAG-ubiquitin), PCNP (pCAGGS-PCNP) and NIRF (pCAGGS-NIRF) or the corresponding empty vectors (p3×FLAG-CMV-10 and pCAGGS). Treatment of the cells with MG-132 and detection of ubiquitinated PCNP were done as described in the legend to Fig. 4. B: 293 cells were co-transfected with 0.33 μg each of the plasmids encoding myc-ubiquitin (pCMV-Myc-ubiquitin), FLAG-PCNP (pFLAG-PCNP) and HA-NIRF (pCMV-HA-NIRF) or the corresponding empty vectors (pCMV-Myc, pFLAG-CMV-2 and pCMV-HA). Treatment of the cells with MG-132 was done as in A. Cell extracts were immunoprecipitated with anti-FLAG M2 affinity gel followed by Western blot analysis with the 1:2000 diluted anti-HA antibody (upper panel) or 2.5 μg/ml of the affinity-purified anti-PCNP antibody (lower panel).

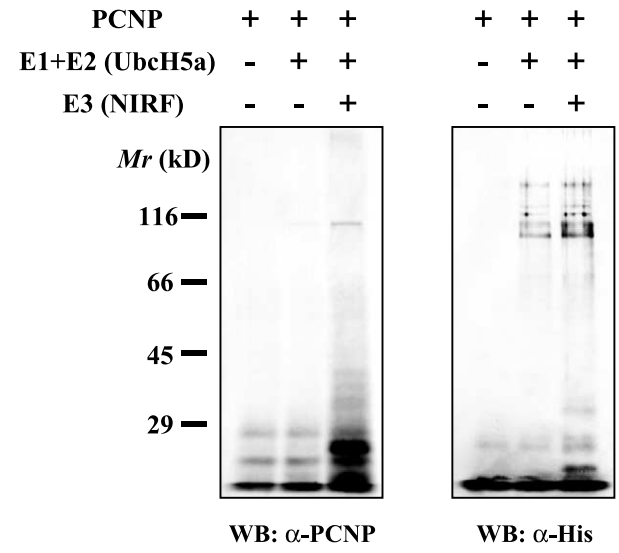

Fig. 6. In vitro ubiquitination of PCNP by NIRF. The in vitro ubiquitination reaction was performed as described in Section 2 with the indicated combinations of PCNP, E1 and E2 (UbcH5a), FLAG-NIRF. After completion of the ubiquitination reaction, the proteins conjugated with the histidine-tagged ubiquitin were purified with nickel-charged Sepharose beads, and then subjected to SDS–PAGE followed by Western blot analysis. The blots were probed with 2.5 μg/ml of the affinity-purified anti-PCNP antibody (left) or 1 μg/ml of the anti-His antibody (right).

NIRF family of proteins could be subject to phosphorylation [7], which may complicate their properties even further. Inquiry into the network surrounding NIRF and PCNP will be necessary.

References

[1] Pickart, C.M. (2001) Mol. Cell 8, 499–504.
[2] Weissman, A.M. (2001) Nat. Rev. Mol. Cell Biol. 2, 169–178.
[3] Doherty, F.J., Dawson, S. and Mayer, R.J. (2002) Essays Biochem. 38, 51–63.
[4] Pickart, C.M. (2001) Annu. Rev. Biochem. 70, 503–533.
[5] Rechsteiner, M. and Rogers, S.W. (1996) Trends Biochem. Sci. 21, 267–271.
[6] Mori, T., Li, Y., Hata, H., Ono, K. and Kochi, H. (2002) Biochem. Biophys. Res. Commun. 296, 530–536.
[7] Fujimori, A., Matsuda, Y., Takemoto, Y., Hashimoto, Y., Kubo, E., Araki, R., Fukumura, R., Mita, K., Tatsumi, K. and Muto, M. (1998) Mamm. Genome 9, 1032–1035.
[8] Hopfner, R., Mousli, M., Jeltsch, J.M., Voulgaris, A., Lutz, Y., Marin, C., Bellocq, J.P., Oudet, P. and Bronner, C. (2000) Cancer Res. 60, 121–128.
[9] Mousli, M., Hopfner, R., Abbady, A.Q., Monte, D., Jeanblanc, M., Oudet, P., Louis, B. and Bronner, C. (2003) Br. J. Cancer 89, 120–127.
[10] Bonapace, I.M., Latella, L., Papait, R., Nicassio, F., Sacco, A., Muto, M., Crescenzi, M. and Di Fiore, P.P. (2002) J. Cell Biol. 157, 909–914.
[11] Muto, M., Kanari, Y., Kubo, E., Takabe, T., Kurihara, T., Fujimori, A. and Tatsumi, K. (2002) J. Biol. Chem. 277, 34549–34555.
[12] Niwa, H., Yamamura, K. and Miyazaki, J. (1991) Gene 108, 193–199.
[13] Laemmli, U.K. (1970) Nature 227, 680–685.
[14] Upadhya, S.C. and Hegde, A.N. (2003) Trends Biochem. Sci. 28, 280–283.
[15] Jackson, P.K., Eldridge, A.G., Freed, E., Furstenthal, L., Hsu, J.Y., Kaiser, B.K. and Reimann, J.D. (2000) Trends Cell Biol. 10, 429–439.
[16] Marin, I. and Ferrus, A. (2002) Mol. Biol. Evol. 19, 2039–2050.
[17] Saito, Y., Tsubuki, S., Ito, H. and Kawashima, S. (1990) Neurosci. Lett. 120, 1–4.
[18] Schwarz, S.E., Rosa, J.L. and Scheffner, M. (1998) J. Biol. Chem. 273, 12148–12154.
[19] Takahashi, R., Imai, Y., Hattori, N. and Mizuno, Y. (2003) Ann. NY Acad. Sci. 991, 101–106.
[20] Knuutila, S., Aalto, Y., Autio, K., Bjorkqvist, A.M., El-Rifai, W., Hemmer, S., Huhta, T., Kettunen, E., Kiuru-Kuhlefelt, S., Larramendy, M.L., Lushnikova, T., Monni, O., Pere, H., Tapper, J., Tarkkanen, M., Varis, A., Wasenius, V.M., Wolf, M. and Zhu, Y. (1999) Am. J. Pathol. 155, 683–694.
[21] Chevaillier, P. (1993) Int. J. Biochem. 25, 479–482.
[22] Sekhar, K.R. and Freeman, M.L. (1998) Recept. Signal Transduct. Res. 18, 113–132.
[23] Aasland, R., Gibson, T.J. and Stewart, A.F. (1995) Trends Biochem. Sci. 20, 56–59.
[24] Baumbusch, L.O., Thorstensen, T., Krauss, V., Fischer, A., Naumann, K., Assalkhou, R., Schulz, I., Reuter, G. and Aalen, R.B. (2001) Nucleic Acids Res. 29, 4319–4333.
[25] Leng, R.P., Lin, Y., Ma, W., Wu, H., Lemmers, B., Chung, S., Parant, J.M., Lozano, G., Hakem, R. and Benchimol, S. (2003) Cell 112, 779–791.