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Degradation of c-Fos by the 26S Proteasome Is Accelerated by c-Jun and Multiple Protein Kinases

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c-Fos is associated with c-Jun to increase the transcription of a number of target genes and is a nuclear proto-oncoprotein with a very short half-life. This instability of c-Fos may be important in regulation of the normal cell cycle. Here we report a mechanism for degradation of c-Fos. Coexpression of c-Fos and c-Jun in HeLa cells caused marked increase in the instability of c-Fos, whereas v-Fos, the retroviral counterpart of c-Fos, was stable irrespective of the coexpression of c-Jun. Interestingly, deletion of the C-terminal PEST region of c-Fos, which is altered in v-Fos by a frameshift mutation, greatly enhanced its stability, with loss of the effect of c-Jun on its stability. c-Fos synthesized in vitro was degraded by the 26S proteasome in a ubiquitin-dependent fashion. Simple association with c-Jun had no effect on the degradation of c-Fos, but the additions of three protein kinases, mitogen-activated protein kinase, casein kinase II, and CDC2 kinase, resulted in marked acceleration of its degradation by the proteasome-ubiquitin system, though only in the presence of c-Jun. In contrast, v-Fos and c-Fos with a truncated PEST motif were not degraded, suggesting that they escaped from down-regulation by breakdown. These findings indicate a new oncogenic pathway induced by acquisition of intracellular stability of a cell cycle modulatory factor.

The c-Fos proto-oncogene product is associated with its partner c-Jun to form the AP-1 transcriptional factor, which responds to a variety of extracellular stimuli by increasing the expression of a number of genes, in particular those related to cell cycle progression (1, 30, 32). AP-1 binds to the 12-*O*-tetradecanolphorbol-13-acetate-responsive element of target genes to increase their transcriptional activities (15). The cellular level of AP-1 proteins also changes dramatically during the mitotic cell cycle, indicating that AP-1 is an immediate-early gene elicited during the G_0-to-G_1 transition (18). Moreover, c-Fos and c-Jun are metabolically unstable, being degraded rapidly with half-lives of about 10 and 60 min, respectively. The instabilities of these proteins are presumably important for down-regulation of the transactivation of target genes by AP-1, which seems to be essential for regulating cellular functions or for normal cell cycle progression.

The nonlysosomal, ATP-dependent proteolytic pathway consists of two distinct, sequential steps, namely, ligation of ubiquitin (Ub) to target proteins and subsequent degradation of the ubiquitinated proteins, with metabolic energy being required for both steps (7, 12, 31). In the first step, Ub, a highly conserved 8,600-Da protein, is covalently attached to various target proteins to act as a signal for proteolytic attack through a reaction catalyzed by a multienzyme system, consisting of the Ub-activating enzyme E1, which is thought to require ATP, the Ub-conjugating enzyme E2, and the Ub-protein ligase (also called Ub recognition protein) E3 (12, 48). The subsequent degradation of the Ub-ligated proteins was found to be catalyzed by a novel, large, ATP-dependent intracellular protease with an apparent sedimentation coefficient of 26S, named the 26S protease complex (31) or the 26S proteasome (8, 42).

This Ub-proteasome pathway appears to be involved in selective removal of unnecessary proteins such as abnormal proteins generated in cells, of various transcriptional factors with a rapid turnover, and of key proteins that are closely related with cell cycle progression and metabolic regulation (for a review, see reference 3). Indeed, there is accumulating evidence that various nuclear oncoproteins such as c-Myc (4) and c-Jun (45), yeast MATα2 transcription factor (2), a key regulator of the meiotic cell cycle, c-Mos (24, 25), a regulator of M-phase-promoting factor, cyclin B (6), G_1 cyclins such as Cln2 (5) and Cln3 (49), and a tumor suppressor protein, p53 (37), are degraded by the Ub pathway. Although the enzyme(s) responsible for their breakdown was not identified until recently, the 26S proteasome was proved to be involved in ATP-dependent degradation of some proteins, such as c-Mos (16), yeast Clb2 (33), p53 (39), and IκB, which functions in the activation of NF-κB (28). Moreover, we reported that the 26S proteasome degrades ornithine decarboxylase, which has a very rapid turnover, by association with the specific inhibitory protein antizyme without ubiquitination (22). However, as individual proteins have different half-lives, there must be specific signals for the ubiquitinations of these proteins individually, as seen in the phosphorylation of c-Mos (24, 25), although the signals of most proteins are not yet known.

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c-Fos was first concluded to be degraded by the Ub pathway because immunodepletion of E1 from a reticulocyte proteolytic system suppressed its breakdown (4). However, although it is very unstable in living cells, its in vitro degradation was not rapid, suggesting the existence of some regulatory system responsible for its rapid degradation. In fact, the degradation of c-Fos was found to be promoted by c-Jun, suggesting the importance of formation of a dimeric complex with c-Jun for its targeted degradation (29). However, there have been no reports on the enzyme responsible for degradation of c-Fos or the structural basis for the instability of c-Fos. In this study, we show that the protease responsible for c-Fos degradation is the 26S proteasome and that the COOH-terminal PEST motif, a common sequence involved in the rapid destruction of short-lived proteins (34), is essential for the destabilization of c-Fos which is accelerated by c-Jun. We also demonstrate a coordinated role of multiple protein kinases in this proteolytic pathway, using an in vitro reconstituted proteolytic system.

MATERIALS AND METHODS

Construction of c-Fos mutants and transfection of cDNAs into HeLa cells. For generation of c-FosΔLZ cDNA, a wild-type c-Fos cDNA was digested with *Bfa*I. Truncated c-FosΔC21 and c-FosΔC47 cDNAs were synthesized by PCR with mutagenic primers to create stop codons. For construction of recombinant expression vectors encoding c-Fos, c-Fos mutants, v-Fos, and c-Jun, these cDNAs were ligated into the parent vector pactEF flanked by a β-actin promoter/EF-1 enhancer and the polyadenylation site of simian virus 40. Samples (10 μg) of the cDNAs ligated into the expression vector were transfected into approximately 10^6 HeLa cells by the calcium phosphate precipitation method (9), and the transfected cells were cultured for 3 days. The transfection efficiency was tested by measuring the activity of β-galactosidase by using 3 μg of an internal standard, pCH110 (Pharmacia LKB Biotechnology), bearing the β-galactosidase gene under the control of the simian virus 40 early promoter.

Immunoblot analysis. Immunoelectrophoretic blot analysis was carried out by the method of Towbin et al. (44). Samples of the cell extracts (100 μg of protein) prepared by lysis with 0.5% Nonidet P-40 were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE), transferred electrophoretically to Immobilon membranes (Millipore), and treated with an anti-c-Fos polyclonal antibody (Medac). Immunostaining was carried out with an enhanced chemiluminescence immunoblotting system (Amersham).

Preparation of labeled proteins in an in vitro transcription-translation system. Human c-Fos and murine c-Jun mRNAs were transcribed, using an SP6/T7 transcription kit (Boehringer Mannheim), from the corresponding full-length clones in pGEM4 vector with SP6 RNA polymerase (38). The mRNAs were translated in the presence of [^{35}S]methionine in a rabbit reticulocyte lysate (Amersham).

In vitro proteolysis assay in a reconstituted system. For assay of c-Fos degradation, 1 μl (about 5,000 cpm) of ^{35}S-labeled c-Fos was incubated at 37°C for 1 to 2 h in a total volume of 100 μl of reaction mixture containing 100 mM Tris-HCl (pH 9.0), 5 mM $MgCl_2$, 2 mM ATP, 1 mM dithiothreitol, 2 U of PP_i per ml, 2.5 μg of Ub, and 1 μg of purified 26S proteasomes in the presence or absence of the Ub protein ligation enzymes E1-E2 and E3. Reaction products precipitated with cold acetone were subjected to SDS-PAGE, and the disappearance of ^{35}S-labeled c-Fos was monitored fluorographically. Highly purified E1-E2 and partially purified E3, which were added to the assay mixture in amounts of 12 and 30 μg, respectively, were obtained from fraction II of a rabbit reticulocyte extract by Ub-Sepharose affinity chromatography as described previously (41). The 26S proteasome was purified from rat liver as reported previously (47). The casein kinase II (CKII), mitogen-activated protein kinase (MAPK), and CDC2 kinase (cyclin B-associated form; CDC2K) used were highly purified by reported methods from bovine liver (14), *Xenopus* oocytes (10), and starfish oocytes (27), respectively.

RESULTS

Destabilization of c-Fos by cotransfection with c-Jun. First, we compared the relative stabilities of c-Fos and v-Fos in living cells. For this, expression vectors encoding these cDNAs were transfected into HeLa cells, and protein levels were measured by immunoblot analysis. v-Fos was found to be highly stable, unlike c-Fos. Interestingly, cotransfection of c-Jun cDNA resulted in almost complete disappearance of c-Fos but not v-Fos (Fig. 1a). This effect of c-Jun was not observed with c-Fos lacking the leucine zipper, an essential domain for binding with

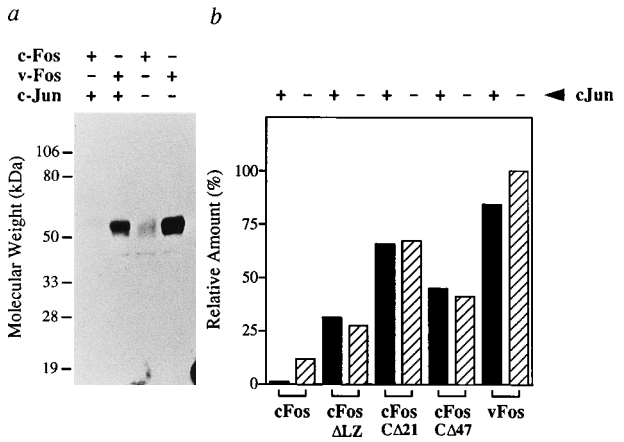

FIG. 1. Stabilities of c-Fos, c-Fos mutants, and v-Fos in HeLa cells with or without coexpression of c-Jun. (a) Effects of cotransfection of c-Jun cDNA on the stabilities of c-Fos and v-Fos. (b) Effects of cotransfection of c-Jun cDNA on the stabilities of c-FosΔLZ, c-FosΔ21, and c-FosΔ47. c-FosΔLZ has a deletion of the leucine zipper domain corresponding to amino acid residues 179 to 193. c-FosΔC21 and c-FosΔ47 are truncated forms with deletions of the last 21 and 47 amino acid residues, respectively: the C-terminal PEST sequence is deleted in the former, and the region differing in c-Fos and v-Fos by frameshift mutation is deleted in the latter. For preparation of these c-Fos mutants, see Materials and Methods. Values obtained by densitometric scanning are expressed as percentages of the maximal intensity and are averages of values in three independent experiments. Note that the transfection efficiency did not alter greatly, being below 10% in each experiment, and thus the data are presented without normalization.

c-Jun (35), and the level of the mutant c-Fos was higher than that of wild-type c-Fos (Fig. 1b), indicating the necessity of association with c-Jun for c-Fos degradation.

Why is v-Fos more stable than c-Fos despite their close structural similarities? The C-terminal region of c-Fos contains the PEST sequence, which may be a degradation signal in most rapidly degraded proteins (34), whereas v-Fos lacks this motif as a result of frameshift mutation together with deletions of some other nucelotides (1). We examined whether the C-terminal PEST region is responsible for the instability of c-Fos. For this, we constructed two c-Fos mutants with deletions of the last 21 and 47 amino acid residues, corresponding to the PEST motif (c-FosΔC21) and the region of c-Fos altered in v-Fos (c-FosΔC47), respectively. As shown in Fig. 1b, the two c-Fos mutants in which the C-terminal PEST region was deleted showed stability as high as that of v-Fos. Moreover, coexpression of c-Jun did not appreciably accelerate the degradation of these truncated c-Fos mutants, unlike wild-type c-Fos.

In this study, we measured the stability of c-Fos by immunoblot analysis without measuring its degradation rate directly. However, as we used the same expression vector with a β-actin promoter/EF-1 enhancer and carried out all experiments at the same time, the rates of synthesis of various transfected genes could be assumed to be similar. Even though the 5′-end structure of mRNA affects its translational activity, the rates of synthesis of wild-type c-Fos and the mutant c-Fos with a truncated COOH-terminal side are presumably the same, irrespective of cotransfection of c-Jun. Therefore, it is clear that c-Jun promotes degradation of c-Fos, but not v-Fos, and that the COOH-terminal PEST region of c-Fos is important for destabilization of c-Fos in living cells.

Acceleration by c-Jun of degradation of c-Fos by the 26S proteasome in a cell-free system. Next we examined whether c-Fos is degraded by the Ub-proteasome pathway in vitro. No

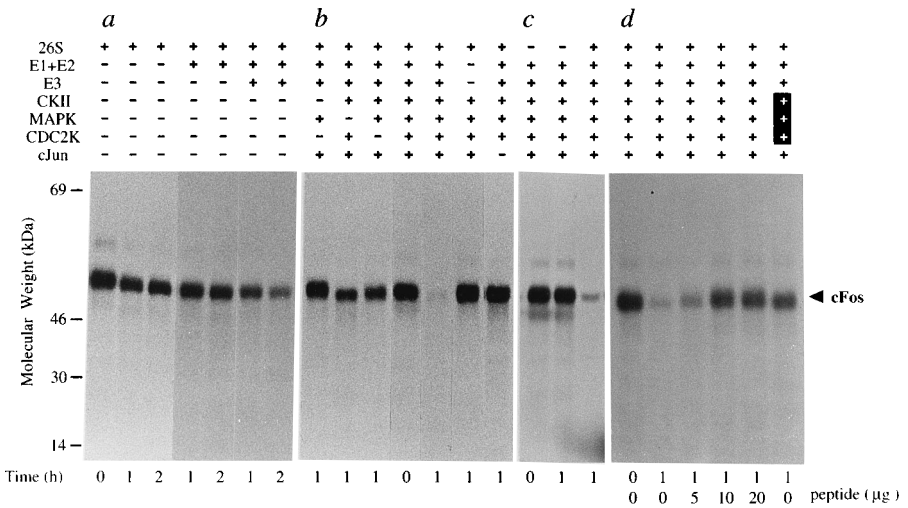

FIG. 2. Accelerated degradation of c-Fos by the Ub-proteasome pathway in the presence of c-Jun and multiple protein kinases. (a) Ub-dependent degradation of c-Fos by the 26S proteasome. ^{35}S-labeled c-Fos synthesized in vitro was incubated with the 26S proteasome, Ub, and the Ub ligation enzymes E1-E2 and/or E3. (b) Degradation of c-Fos in the presence of c-Jun, CKII, MAPK and CDC2K (cyclin B-associated form). (c) Requirement of the 26S proteasome for degradation of c-Fos. (d) Effects of the peptide substrate, RRREEETEEE, for CKII (19) and heat denaturation (boiling for 5 min) of the protein kinases (boxed in black) on c-Fos degradation. Reactions were carried out at 37°C for 1 or 2 h. For panels b to d, the reaction mixtures contained c-Fos with or without an equal amount of unlabeled c-Jun, which had been translated in a reticulocyte lysate, in the presence or absence of CKII, MAPK, and CDC2K. These protein kinases were added in amounts with activities to incorporate 100 pmol of P_i into casein per min, 150 pmol of P_i into myelin basic protein per min, and 50 pmol of P_i into histone H1 per min, respectively. The approximate molar ratios of synthesized proteins were determined with a BAS2000 imaging analyzer (Fuji film) from fluorograms of SDS-polyacrylamide gels.

significant degradation of ^{35}S-labeled c-Fos was observed with the 26S proteasome alone, but the addition of a Ub system (12) consisting of Ub and the Ub ligation enzymes (E1, E2, and E3) resulted in appreciable breakdown of c-Fos in 2 h (Fig. 2a). The degradation of c-Fos was largely E3 dependent. No band of degraded intermediates of c-Fos was detected, suggesting exhaustive degradation of c-Fos by the 26S proteasome.

An interesting question is whether c-Jun affects the in vitro stability of c-Fos. No appreciable difference was found in the degradations of c-Fos by the 26S proteasome-Ub system in the presence and absence of unlabeled c-Jun (data not shown). Recently, the levels of various nuclear oncoproteins, including c-Fos and c-Jun, have been shown to be strictly regulated by the phosphorylation cascade system (15, 17), and CKII, MAPK, and cyclin B-associated CDC2K (M-phase-promoting factor) have been suggested to be the most likely enzymes involved in the cell cycle-dependent phosphorylation of these cell proteins (20, 23, 26). We therefore examined the effects of these protein kinases on the in vitro stability of c-Fos. Addition of these three protein kinases together markedly accelerated the degradation of c-Fos, resulting in its almost complete degradation within 1 h (Fig. 2b). The additions of all three protein kinases were required for marked degradation of c-Fos, their additions individually or in pairs having no significant effect. Moreover, no stimulation of c-Fos degradation by multiple protein kinases was observed in the absence of a Ub system or c-Jun (Fig. 2b). In addition, deletion of the leucine zipper resulted in no appreciable degradation of c-Fos (see Fig. 4b). These findings suggest that the accelerated degradation of c-Fos by c-Jun together with multiple protein kinases occurs via a Ub-dependent process. Moreover, no significant degradation of c-Fos was observed upon omission of the 26S proteasome from the complete in vitro reconstituted proteolytic system, indicating that the 26S proteasome is responsible for the degradation of c-Fos (Fig. 2c). It is noteworthy that the three protein kinases had no effect on the activities of ^{125}I-lysozyme-Ub conjugation catalyzed by E1, E2, and E3 used in this study, suggesting that the Ub pathway is not a target for these protein kinases (data not shown). Similarly, the ATP-dependent breakdowns of the ^{125}I-lysozyme-Ub conjugates were 8.73 and 8.65%/h, respectively, in the presence and absence of the three protein kinases used in this work. Thus, these protein kinases did not affect the overall ATP-dependent proteolytic activity of the 26S proteasome but possibly affected the degradations of specific proteins.

Protein kinase CKII is known to be present at high concentration in the reticulocyte lysate (11) used for in vitro syntheses of c-Fos and c-Jun. To clarify the involvement of CKII in the degradation pathway of c-Fos, we carried out a competition experiment with RRREEETEEE as a peptide substrate for CKII (19). As shown in Fig. 2d, this peptide almost completely suppressed the degradation of c-Fos in a dose-dependent manner. Moreover, heat inactivation of the protein kinases used also resulted in almost complete inhibition of degradation of c-Fos (Fig. 2d). These results suggest that the phosphorylations catalyzed by these three protein kinases are required for c-Fos degradation promoted by c-Jun and that the action of CKII is probably coupled with those of MAPK and/or CDC2K. Furthermore, these protein kinases accelerated the degradation of c-Fos in a time-dependent manner, causing extremely rapid destruction of c-Fos with a half-life of 5 to 10 min (Fig. 3), which is similar that in vivo (1, 18).

The RRREEETEEE peptide and the three protein kinases had no effect on the activities of in vitro ubiquitination measured by ^{125}I-Ub or ^{125}I-lysozyme conjugation (data not shown), suggesting that they do not significantly affect the activity of the Ub-protein ligation system used in this study. We examined the effect of the peptide on the activity of the 26S proteasome. The ATP-dependent hydrolyses of ^{125}I-lysozyme-Ub conjugates by the purified 26S proteasome were 9.08 and 10.53%/h, respectively, in the presence and absence of the RRREEETEEE peptide. Thus, the RRREEETEEE peptide did not affect the overall ATP-dependent proteolytic activity of the 26S proteasome.

Stabilities of v-Fos and mutant c-Fos lacking PEST. We examined whether v-Fos is also degraded in vitro by the Ub-

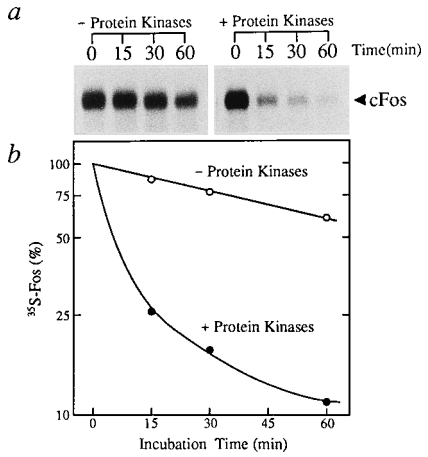

FIG. 3. Time courses of c-Fos degradation catalyzed by the 26S proteasome in the presence of the c-Jun and Ub system with or without the three protein kinases. The assay system used for panel a was as that for Fig. 2 except that reactions were carried out for the indicated times. The arrowhead shows the position of c-Fos. Decay of ^{35}S-labeled c-Fos observed in fluorograms was monitored quantitatively with a BAS2000 imaging analyzer (b).

proteasome system. As shown in Fig. 4a, v-Fos was not degraded appreciably by the 26S proteasome during incubation for 1 or 2 h in the presence or absence of a Ub system and/or c-Jun and the three protein kinases. Moreover, no significant degradations of the two c-Fos mutants with a truncated PEST motif (c-FosΔC21 and c-FosΔC47) were observed in the presence of c-Jun and the three protein kinases (Fig. 4b), indicating that the C-terminal PEST sequence of c-Fos is essential for its breakdown by the Ub-proteasome pathway. These findings strongly suggest that the oncoprotein v-Fos is resistant to breakdown by the proteolysis system as a result of the absence of a C-terminal PEST sequence. The high stability of v-Fos would result in continuous signaling for transactivation of a variety of genes and so may be responsible for the tumorigenic action of this oncogene.

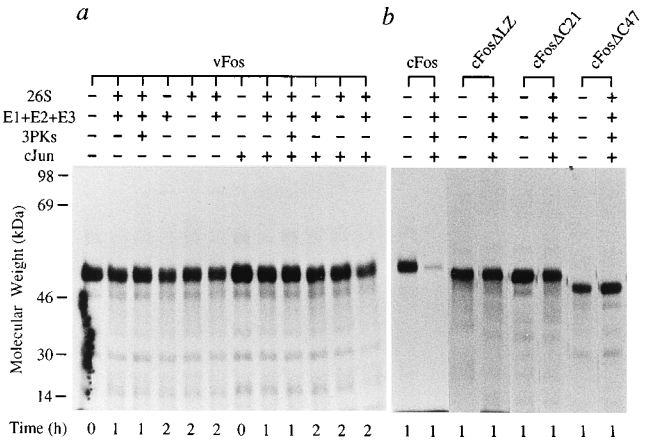

FIG. 4. Insensitivities of v-Fos and the c-Fos mutants c-FosΔLZ, c-FosΔC21, and c-FosΔ47 to degradation by the 26S proteasome-Ub system. ^{35}S-labeled v-Fos (a) and c-Fos mutants (b; see Fig. 1b) synthesized in vitro were incubated at 37°C for 1 or 2 h with 26S proteasomes in the presence or absence of Ub ligation enzymes, unlabeled c-Jun, and/or the three protein kinases (3 PKs). The assay system and analysis of the reaction products were as for Fig. 2 except that v-Fos and c-Fos mutants synthesized in an in vitro transcription-translation system were used. v-Fos cDNA was obtained from GenBank, and the 5′ region encoding the Gag protein was deleted. The plasmid was named pSGv-Fos. The mutant c-Fos cDNAs c-FosΔLZ, c-FosΔC21, and c-FosΔ47 (see the legend to Fig. 1b) were ligated to pBluescript II.

DISCUSSION

Roles of transrecognition signal and phosphorylation in selective proteolysis. In this study, we showed that the degradation of c-Fos was accelerated by its association with c-Jun in vivo (Fig. 1) and in vitro (Fig. 4), because c-Jun had no effect on the stability of c-Fos with deletion of its leucine zipper domain, which binds to c-Jun. This finding was not surprising, because the degradation of the tumor suppressor protein p53 is well known to be dramatically accelerated by association with an oncoprotein, E6, encoded by papillomaviruses (36, 37) and because the degradations of *Xenopus* cyclins A and B2 (40) and yeast Cln2 (5) and Cln3 (49) have been shown to require their binding to p34^{cdc2} and p34^{cdc28}, respectively. Moreover, we have reported that the 26S proteasome degraded ornithine decarboxylase upon its association with the specific inhibitory protein antizyme without ubiquitination (22). Thus, a transrecognition signal by formation of a heterodimeric protein complex seems to be a general system for determining the metabolic stabilities of various short-lived proteins irrespective of whether they are degraded by a Ub-dependent or a non-Ub pathway.

However, simple association of c-Jun with c-Fos is not sufficient for presentation of the signal for degradation of c-Fos, and covalent modification of c-Jun by multiple phosphorylations could also be required (Fig. 2 and 3), suggesting that AP-1 is modulated in a complex way in vivo. There is accumulating evidence that phosphorylation or dephosphorylation is a key signal for the metabolic stabilities of various short-lived nuclear proteins such as c-Mos (25), cyclin B (13), Cln2 (5), Cln3 (49), and a V(D)J recombination activator protein, RAG-2 (21). Although c-Mos and cyclin B are known to be phosphorylated at multiple sites, the modification of a single Ser or Thr residue of c-Mos or RAG-2 is sufficient as a degradation signal. Papavassiliou et al. (29) reported that phosphorylation of c-Jun is required for promotion of degradation of c-Fos, although they did not identify the site(s) of phosphorylation in c-Jun for this effect. Here we found that the degradation of c-Fos catalyzed by the 26S proteasome in a Ub-dependent pathway was accelerated by its association with c-Jun and the coordinated actions of the protein kinases CKII, MAPK, and CDC2K, suggesting the importance of coordinated phosphorylations. It is not yet known which serine or threonine residues are phosphorylated in c-Fos and/or c-Jun. In addition, the possibility that other components, including the 26S proteasome and/or a Ub system, are phosphorylated by the added protein kinases cannot be ruled out completely, although we show here that these protein kinases did not affect the ubiquitination and ATP-dependent degradation by the 26S proteasome of a model substrate, ^{125}I-lysozyme.

Escape of oncoproteins from degradation by the ubiquitin-proteasome pathway. It is interesting that v-Fos was not affected by this desensitization pathway mediated by the 26S proteasome. This finding may explain tumorigenic resistance to down-regulation of AP-1 activity in the pathway of phosphorylation-dependent signal transduction. The resistance of v-Fos to proteolysis may be because, by a frameshift mutation, it has lost the COOH-terminal PEST motif present in c-Fos, a region that is essential for rapid destruction of c-Fos by the Ub-proteasome pathway as demonstrated in this work. This finding is in accord with a report that deletion of the COOH-terminal PEST region of yeast Cln3 induced stabilization of the Cln3 protein, causing cell cycle arrest in the M phase (46,

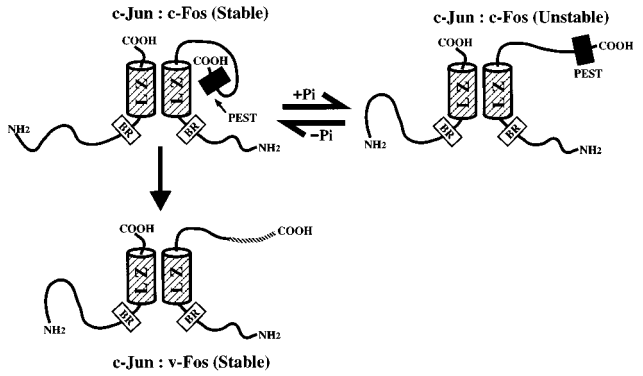

FIG. 5. Schematic representation of a possible mechanism for c-Jun-accelerated destabilization of c-Fos. LZ, leucine zipper; BR, basic region; +Pi, phosphorylation; −Pi, dephosphorylation. The stabilities of Fos proteins associated with c-Jun are shown in parentheses. The COOH-terminal PEST region of c-Fos without phosphorylation may be hidden within the molecule, whereas posttranslational modifications of c-Fos and/or c-Jun by multiple phosphorylations with various protein kinases may expose the COOH-terminal PEST domain as a degradation signal. Note that v-Fos lacks the COOH-terminal PEST motif by frameshift mutation. See text for details.

49). Ub-dependent c-Jun degradation was recently found to be mediated by a 27-amino-acid stretch, termed the δ domain, located in the NH_2-terminal part of the molecule, and v-Jun was shown to lack the δ domain as a result of an in-frame deletion (45). In the latter report, a novel route of oncogenesis was proposed, on the basis of the correlation between transforming activity and escape from Ub-dependent degradation of c-Jun. This mechanism closely resembles the structure-destabilization relationship between c-Fos and v-Fos described here. It is noteworthy, however, that the elements and locations responsible for destabilization, namely, the COOH-terminal PEST motif of c-Fos and the NH_2-terminal δ domain of c-Jun, differ. This may be related to the differences in stabilities of these two proteins in vivo, c-Fos showing much more rapid turnover (18). In addition, the E2 and E3 used here did not contain the novel species found recently in fraction I to be responsible for ubiquitination of p53 (36, 39) and cyclin B (13), which catalyze the non-N-end rule pathway, suggesting the existence of multiple pathways for ubiquitination, which may be important for determinations of the different half-lives of individual proteins.

Even after long exposure, the fluorogram of the reaction products without the 26S proteasome showed no detectable bands of ^{35}S-labeled c-Fos of high molecular weights, corresponding to ubiquitinated forms of c-Fos. Similarly, no high-molecular-weight form was detected in the Ub-dependent degradation of c-Myc (4). This is in contrast to the findings of multiple ubiquitinated forms of c-Mos (16, 24, 25) and p53 (37, 39) before their breakdowns. Thus, in the case of c-Fos, the ubiquitination process may be linked to subsequent rapid proteolytic attack by the 26S proteasome. Alternatively, high-resolution techniques might be required for detection of ubiquitinated c-Fos, because an increase in the sensitivity of detection of ubiquitination by using Ub fused with a hemagglutinin tag resulted in clear detection of multiple ubiquitinated forms of c-Jun before its degradation (45).

A possible mechanism for destabilization of c-Fos. On the basis of the observations presented above, we propose the model for acquisition of instability of c-Fos, but not v-Fos, shown schematically in Fig. 5. In the stable form of c-Fos, the COOH-terminal PEST region may be hidden within the molecule and so escape degradation mediated by the Ub-proteasome system. But posttranslational modifications of c-Fos and/or c-Jun by multiple phosphorylations through various protein kinases may expose the COOH-terminal PEST domain, presenting it as a degradation signal recognized by the Ub-proteasome system. On the other hand, as v-Fos has no COOH-terminal PEST motif as a result of a frameshift mutation, it may be stable because it is resistant to the Ub-proteasome degradation pathway. However, it is still not known how the COOH-terminal PEST region is recognized in the Ub-proteasome pathway.

The 26S proteasome probably catalyzes the degradations of various oncoproteins localized in the nucleus as well as proteins, such as ornithine decarboxylase, present in the cytoplasm. This is consistent with the presence of the proteasome in both compartments (43). Thus, we conclude that the 26S proteasome catalyzes the ATP-dependent, selective degradations of naturally occurring short-lived proteins related to the cell cycle, irrespective of differences in their degradation signals and irrespective of whether these proteins are located in the nucleus or the cytoplasm.

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