Multiple Functions of Jab1 Are Required for Early Embryonic Development and Growth Potential in Mice*

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Jab1 interacts with a variety of signaling molecules and regulates their stability in mammalian cells. As the fifth component of the COP9 signalosome (CSN) complex, Jab1 (CSN5) plays a central role in the deneddylation of the cullin subunit of the Skp1-Cullin-F box protein ubiquitin ligase complex. In addition, an AIP-dependent function of Jab1 is suggested but is less well characterized. To elucidate the function of Jab1, we targeted the Jab1 locus by homologous recombination in mouse embryonic stem cells. Jab1-null embryos died soon after implantation. Jab1+/− embryonic cells, which lacked other CSN components, expressed higher levels of p27, p53, and cyclin E, resulting in impaired proliferation and accelerated apoptosis. Jab1 heterozygous mice were healthy and fertile but smaller than their wild-type littermates. Jab1+/− mouse embryonic fibroblast cells, in which the amount of Jab1-containing small subcomplex, but not that of CSN, was selectively reduced, proliferated poorly, showed an inefficient down-regulation of p27 during G1, and was delayed in the progression from G0 to S phase by 3 h compared with the wild-type cells. Most interestingly, in Jab1+/− mouse embryonic fibroblasts, the levels of cyclin E and deneddylated Cul1 were unchanged, and p53 was not induced. Thus, Jab1 controls cell cycle progression and cell survival by regulating multiple cell cycle signaling pathways.

The successful identification and characterization of the COP9 signalosome (CSN) complex, Jab1 complex from yeast (1–3) to mammals (4) and higher plants (5, 6) revealed that the function of CSN is not restricted to light/dark-mediated signal transduction in plants but is connected to divergent biological responses (7–11) such as development (12, 15), oogenesis (14, 16, 17), immune response (18, 19), DNA metabolism (20), apoptosis (21), checkpoint control (22), DNA repair (23), and cell cycle (1, 24, 25). The core CSN complex is composed of eight subunits (4), and disruption of one component results in the loss of the whole complex (13, 26–28). Protein kinases capable of phosphorylating c-Jun, NF-κB, and p53 are associated with CSN (4, 29, 30). p53 is destabilized by CSN-mediated phosphorylation at Thr-155 in proliferating cells, and disruption of CSN leads to accumulation of p53 and eventual cell cycle arrest/apoptosis (31). CSN interacts with the Skp1-Cullin-F box protein (SFB) ubiquitin ligase and removes a ubiquitin-like polypeptide, Nedd8, from the Cul subunit (deneddylation) (32), thereby regulating the ligase activity (34, 35). The JAMM domain within the Jab1/CSN5 subunit plays an essential role in this reaction (32), but the monomeric form of the Jab1/CSN5 polypeptide alone failed to manifest the activity. The disruption of the CSN complex in Drosophila results in accumulation of the hyper-deneddylated Cul subunit and cyclin E polypeptide (one of the substrates of SFB) and failure of oogenesis (17). It is not clear whether all biological responses correlated with CSN are mediated either by phosphorylation or deneddylation (27). Other biochemical functions associated with CSN include regulation of the subcellular localization of the target protein (24, 36, 37) and recruitment of the deubiquitination enzyme (38), but the mechanisms involved remain to be investigated. In addition to the component of the intact 450-kDa CSN complex, CSN subunits are found as a small complex or a monomeric form (12, 13, 26, 28, 39). Because disruption of one CSN subunit does not necessarily result in the same phenotype as nullification of the others (28), it seems likely that each CSN subunit has its own unique function (40) in addition to being a component of the CSN complex.

Jab1 (also known as the fifth component of the CSN complex, CSN5) has been shown to interact with and control multiple intracellular signaling molecules (41), including c-Jun (42), p27 (24), LFA-1 (integrin) (43), MIF (18), HIF1α (44), Smad4 (45), Bcl3, IκB α, p53 (31), and Cul1 (SFB) (33, 46). Although Jab1 was shown to play a critical role in other organisms such as Caenorhabditis elegans (14) and Drosophila (13, 16), some Jab1 targets are unique to mammalian cells, and it is important to know how these targets are regulated in living organs. Jab1 was also found to be a smaller form not part of the CSN complex in various species (12, 13, 26, 39). It was originally found as a monomeric form in Arabidopsis (26) and later as a smaller cytoplasmic complex in mammalian cells (39). Although Jab1 plays an essential role in phosphorylation, deneddylation, and translocation, it remains to be determined how these activities are regulated by the large CSN complex and possibly by the small complex. Furthermore, Jab1 was found to be highly expressed in human cancers (47–56), which, in some cases, correlates with a poor prognosis and low level expression of the
CDK inhibitor p27. To understand better the function of Jab1 in development, cell proliferation, and oncogenesis, we targeted the Jab1 locus by homologous recombination in ES cells. Jab1-null embryos did not survive, whereas Jab1 heterozygous mice were viable and fertile but smaller than the wild-type littermates. Jab1+/− cells lacked other CSN components and expressed higher levels of p27, p53, and cyclin E, resulting in inefficient down-regulation of p27 during G1. Most interestingly, in Jab1+/− MEFs, the levels of cyclin E and neddylationed Cul1 were unchanged, and p53 was not induced. Thus, Jab1 controls cell proliferation and survival in mice through multiple cell cycle regulatory pathways in both CSN-dependent and -independent ways.

EXPERIMENTAL PROCEDURES

Targeted Disruption of the Mouse Jab1 Gene—The gene structure of mouse Jab1 was determined by PCR and DNA sequencing2 and subsequently confirmed by Blast (NCBI) analysis of the complete cDNA sequence (AP066829) and the genomic sequence (NT039169). The Jab1 targeting vector was constructed by subcloning a 1-kb genomic DNA fragment containing the sequence upstream from the initial methionine and a 5-kb genomic DNA fragment downstream of exon 6, both of which had been amplified by genomic PCR and confirmed by sequencing, into the ploxPNT vector at the EcoRI and XhoI sites, respectively. The targeting vector was linearized with XhoI and was electroporated into mouse RF8 ES cells (57). ES clones selected in 200 µg/ml G418 and 0.2 µm FIAU were subjected to Southern blot analysis by using probes external to both the 5′ and 3′ end of the targeting construct (Fig. 1, a and b). We did not detect truncated polypeptides from the putative open reading frame (corresponding to amino acids 266–334) in exons 7 and 8 by Western blotting by using antibody recognizing the C-terminus of the Jab1 protein, indicating that the mutant allele is truly a null locus. Jab1+/− ES cells were microinjected into blastocyst stage C57BL/6 mouse embryos. Chimeric males were crossed to C57BL/6 females, and offspring were genotyped by genomic PCR using Jab1-specific primers as follows: a (5′-CTC TCT GTC CTG GGC TTT CAT TAC CAT TTC-3′), b (5′-GCT CTC CAC ACC CTT CAT CTC CCA CCC CTC-3′), and a neo gene-specific primer c (5′-CTG GGC TGA TCT GCT GTA TCA CA-3′) (Fig. 1, a and c). p53+/− mice were purchased from Taconic Farms. p27+/− and p27−/− mice were generated basically according to the method described previously (58).

Histology and Immunohistochemistry—Uteri from pregnant females were dissected, fixed overnight in 4% paraformaldehyde, embedded in paraffin, cut into 4-µm sections, and stained with hematoxylin and eosin. For antibody staining, sections were deparaffinized, rehydrated, and placed in a 3% solution of hydrogen peroxide for 20 min. This was followed by blocking in 5% bovine serum albumin for 30 min. After incubation with primary antibodies overnight at 4 °C, peroxidase-conjugated secondary antibody was applied (Histofine Simple Stain MAX PO, Nichirei Co.). The staining was visualized with diaminobenzidine, and the sections were counterstained with hematoxylin. The antibodies used included rabbit polyclonal antibodies to Jab1/CSN5 (1:1000) (24), CSN1 (1:50) (39), cyclin E (M-20 Santa Cruz Biotechnology, 1:50), and Cul1 (Zymed Laboratories Inc., 1:50), and mouse monoclonal antibodies to p27 (Transduction Laboratories, 1:50) and p53 (Oncogene Science, 1:50 and Calbiochem, 1:250). We incubated sections with either no primary or no secondary antibodies to control for nonspecific staining (data not shown). For detection of apoptosis, TUNEL staining of the sections was carried out according to the manufacturer’s instructions (ApopTag Red, Integenx).

Blastocyst Outgrowth and Immunofluorescence Analysis—Blastocysts were isolated from the uterus at embryonic day 3.5 (E3.5), cultured in ES medium in 5% CO2 at 37 °C, and photographed. Cells cultured on a Lab-TekII Chamber (Nalge Nunc) were fixed in 3% paraformaldehyde, permeabilized in 0.5% Triton X-100, stained with primary antibodies, and incubated with fluorescein isothiocyanate-linked anti-mouse and Texas Red-linked anti-rabbit IgG (Amersham Biosciences). For the determination of BrdUrd incorporation, cells were incubated in 10 µM bromodeoxyuridine for 24 h, stained with anti-Jab1 rabbit polyclonal antibody followed by Texas Red-linked anti-rabbit IgG, treated with 1.5 M HCl, and stained with anti-BrdUrd mouse monoclonal antibody (Amersham Biosciences) and fluorescein isothiocyanate-linked anti-mouse IgG. The TUNEL assay was performed with Jab1-stained cells according to the manufacturer’s instructions (see above). The cell samples were viewed by phase-contrast or fluorescence microscopy. The genotype of the cultured embryos was determined by anti-Jab1 immunofluorescence staining and by genomic PCR using primers a, b, and c (Fig. 1, a and c).

MEF Assays—Primary MEFs were isolated from E13.5 embryos and cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum (FBS). For growth curve assays, only early passage MEFs (passage 2–4) were seeded at 105 cells on 6-cm plates and quantified at given time points. The 3T6 protocol was employed by plating 2 × 106 cells on 10-cm plates and replating at the same cell density every 3 days. To analyze S phase entry, 2 × 106 cells per 6-cm dish were starved in Dulbecco’s modified Eagle’s medium supplemented with 0.1% FBS for 48 h before being stimulated with 10% FBS for given periods. Collected cells were suspended in a 1-ml solution of 0.1% sodium citrate and 0.1% Triton X-100 containing 50 µg/ml of propidium iodide and treated with 1 µg/ml of RNase for 30 min at room temperature. Fluorescence from the propidium iodide-DNA complex was measured with a FACScan flow cytometer (BD Biosciences), and the percentages of cells in phases G1, S, and G2/M of the cell cycle were determined with CellFit cell cycle software.

Protein Analyses—Cell lysis, gel electrophoresis, and immunoblotting were performed by using standard procedures (24, 39, 59). Developed films were quantitatively analyzed with a densitograph (ATTO, Japan). Rabbit polyclonal antibodies against γ-tubulin, p27, Cul1, cyclin E, Cdk4, Skp2, p21, p53, MDM2, and p16 and mouse monoclonal antibodies to cyclin D1 were purchased from Santa Cruz Biotechnology. Mouse monoclonal antibody to mouse p27 was obtained from Transduction Laboratories. For nonadenating gel electrophoresis, cells were lysed in modified EBC buffer containing 0.1% digitonin as a detergent. Lysates were separated in a pre-made nonadenating gel (Biocraft) without SDS and analyzed by immunoblotting. The fractions from the glycerol gradient centrifugation (39) containing only the large CSN complex or the small Jab1 complex were separated by nonadenating gel electrophoresis, and the positions of each complex were determined. In this assay, the amount of the small Jab1 subcomplex was 10–20% of total Jab1 protein (equivalent to the results of the glycerol gradient centrifugation analysis), and the CSN complex migrated slower and appeared as a broad band, which contained all eight CSN subunits (CSN1–8), suggesting multiplicity of the modified CSN complex.2 The in vitro kinase assay for Cdk2 and Cdk4 was performed as described (59) using a recombinant retinoblastoma protein as a substrate. The phosphorylated retinoblastoma protein was separated by SDS-PAGE and quantified for 32P incorporation by a Fuji BAS-2500 analyzed instrument. To obtain the quantitative results, we routinely used several different MEF lines (usually five lines or more) prepared from different mice for each experiment. The averages and the standard deviations were calculated and are shown in the text. We show only the representative data in the figures.

RESULTS

Targeted Disruption of the Jab1 Gene and Requirement of Jab1 in Early Embryogenesis—To examine the physiological requirement of Jab1 in development, cell proliferation, and cell survival, we attempted to disrupt the Jab1 gene in mice. The murine Jab1 gene spans about 13 kb, containing eight coding exons (Fig. 1a). Our strategy was to delete coding exons 1–6, including the start codon, and replace them with a neo marker gene through homologous recombination in ES cells. Correct targeting was confirmed in two independent ES clones by Southern blot hybridization analysis with 5′ and 3′ probes, respectively (Fig. 1b). One ES clone was injected into blastocysts from C57BL/6 mice to generate chimeras, and germ line transmission was established. Jab1+/− heterozygous mice were fertile and were intercrossed to produce Jab1+/− mice. No nullizygous mice were born live among

2 K. Tomoda, A. Fukumoto, N. Yoneda-Kato, and J.-y. Kato, unpublished observations.
Elevated Levels of Cyclin E, p27, and p53 soon after implantation without undergoing gastrulation.

In vitro analysis of genomic DNA isolated from blastocysts cultured for 4 days B), and hybridized with probes A and B. The sizes of wild-type (7.5 and 8.0 kb) and disrupted (2.0 and 6.0 kb) alleles are indicated. c, PCR analysis of genomic DNA isolated from blastocysts cultured for 4 days in vitro. The positions of wild-type (WT, 2047 bp) and mutant (KO, 2464 bp) amplification products are indicated.

186 offspring of different heterozygous intercrosses, and the ratio of heterozygous mice to wild-type mice was 2.0 to 1 (Table I), indicating that loss of Jab1 was embryonic lethal. Embryos were isolated from timed heterozygous intercrosses from embryonic day 8.5 to as early as E3.5 (Table I) and were genotyped by genomic PCR (Fig. 1c) or histochemical staining with antibody to Jab1 (Fig. 2). No viable Jab1+/− embryos were found at E8.5, and Jab1−/− embryos at E7.5 and E6.0 exhibited a disrupted development compared with wild-type and heterozygous littermates (Fig. 2). Normal E7.5 embryos underwent gastrulation and were transformed into a multi-layered, three-chambered conceptus containing mesoderm, whereas nullizygous embryos were severely growth-retarded, smaller, disorganized, and started to be resorbed, although extra-embryonic cells were retained. E6.5 Jab1−/− embryos were slightly smaller in size and abnormal in shape (Fig. 2), although trophoblast giant cells were seen. At E3.5, however, nullizygous embryos of normal appearance were evident (Table I and Fig. 3, see below). These results indicate that Jab1−/− embryos survived to the blastocyst stage and died soon after implantation without undergoing gastrulation.

Loss of Jab1 Results in Accelerated Apoptotic Cell Death and Elevated Levels of Cyclin E, p27, and p53—To assess the effect of homozygous disruption of Jab1 on early embryos, an immunohistological analysis using specific antibodies was carried out on E6.5 embryos (Fig. 2). CSN components (CSN1 and CSN5/Jab1) were ubiquitously expressed in the early stage embryos, and levels were markedly reduced in Jab1−/− embryos. Among putative targets of the Jab1/CSN pathway, the tumor suppressor p53 (31) and Cdk inhibitor p27 (24) were completely absent in wild-type embryos and markedly induced in Jab1−/− embryonic cells. Cyclin E was expressed in wild-type cells, and its level was higher in mutant embryos, and cyclin E up-regulation was more prominent in extra-embryonic cells. The total level of Cul1 expression was the same regardless of the Jab1 genotype. In addition, TUNEL staining of the section showed that the apoptotic process was accelerated in nullizygous embryos.

To investigate further the function of Jab1 by an alternative approach, we cultured blastocysts in vitro and examined their outgrowth (Fig. 3). Newly isolated Jab1−/− blastocysts were viable and morphologically indistinguishable from blastocysts of the wild-type and the heterozygous mice (Table I). Both wild-type and Jab1−/− blastocysts, hatched from the zona pellucida, attached onto the culture dish and produced apparently normal trophoblast giant cells. The inner cell mass (ICM) cells in Jab1−/− embryos grew similarly to those of the normal littermates for 3 days, but the number of mutant ICM cells was greatly reduced after 5 days of culture. The BrdUrd incorporation assay shows that vigorous DNA synthesis occurred in normal ICM and trophoblast cells throughout the entire outgrowth, whereas in Jab1−/− blastocysts, ICM and trophoblast giant cells ceased to proliferate by the 4th day. In addition, the TUNEL assay revealed that nuclear fragmentation was enhanced in Jab1−/− ICM cells.

![Figure 1](image1.png)

**Fig. 1.** Targeted disruption of the Jab1 gene. a, Jab1 gene targeting strategy. The coding exons are shown as filled boxes; open boxes denote the noncoding portions. Probes A and B used for Southern blot analysis and PCR primers a–c are indicated. NEO, the neomycin phosphotransferase gene; TK, thymidine kinase gene. Restriction sites are shown as single letters as follows: H, HindIII; N, NheI; V, EcoRV. b, Southern blot analysis of genomic DNA was extracted from ES cell clones, digested with HindIII (for probe A) and NheI + EcoRV (for probe B), and hybridized with probes A and B. The sizes of wild-type (7.5 and 8.0 kb) and disrupted (2.0 and 6.0 kb) alleles are indicated. c, PCR analysis of genomic DNA isolated from blastocysts cultured for 4 days in vitro. The positions of wild-type (WT, 2047 bp) and mutant (KO, 2464 bp) amplification products are indicated.

![Figure 2](image2.png)

**Fig. 2.** Histological analysis of normal and mutant embryos. Sections of uteri from pregnant females at E7.5 (a and b) and E6.5 (c–r) were stained with hematoxylin and eosin (a–d) and immunostained with antibodies to Jab1 (e and f), CSN1 (g and h), cyclin E (i and j), p27 (k and l), p53 (m and n), and Cul1 (o and p). A TUNEL assay was performed on sections of E6.5 embryos (q and r). Only the magnified embryonic portions are shown (c–r). The signal around the rim of the embryo stained with antibodies to p27 (k and l) and p53 (m and n) is the background staining due to the nonspecific binding of the mouse monoclonal antibody. Up-regulation of cyclin E was more prominent in extra-embryonic cells (j). Marked down-regulation of CSN3 and CSN8 was also seen in E6.5 Jab1−/− embryos (data not shown).
Fig. 3. Blastocyst analysis in vitro. Wild-type (α–e and k–q) and Jab1−/− (f–j and r–x) E3.5 blastocysts were cultured in vitro for 24 (a and f), 72 (b and g), and 120 h (c and h). Blastocysts were incubated in the presence of BrdUrd for 24, 96, and 120 h, fixed, and immunostained with anti-BrdUrd antibody (d and i). Seventy-two-hour cultures were fixed (k and r) and assayed for apoptosis (e and j) or immunostained with antibodies to Jab1 (l and s), CSN1 (m and t), cyclin E (n and u), p27 (o and v), p53 (p and w), and Cul1 (q and x). Genotypes were determined by PCR after 3–5 days of culture (Fig. 1c) and by immunostaining with antibody to Jab1 (l and e).

after day 3 of culture. Immunofluorescent staining of the cultured blastocysts indicated basically the same results as obtained in the immunohistochemical analysis of the embryonic sections in Fig. 2, a reduction in CSN1 and an increase in cyclin E, p27, and p53. Thus, loss of Jab1 resulted in a disruption of the CSN complex, an increase in the level of cyclin E, p27, and p53, cell cycle arrest, and enhanced cell death. So far, a nullizygous genetic background at the p27 loci has failed to rescue the embryonic lethality markedly reduced (by 59.4%) whereas the amount of the Jab1-containing small complex was 5.9% in Jab1−/− as compared with wild-type cells (Fig. 4a).

Fig. 4. Impaired growth of Jab1−/− cells. a, photograph of a Jab1+/− mouse and a control littermate at 15 weeks of age. b, body weights of representative Jab1+/− mice and control littersmates. Mouse genotypes were determined by genomic PCR as described in Fig. 1c. c, growth curves of primary wild-type (black circles) and Jab1+/− (red circles) MEFs. Cells (1 × 10^5) were plated onto a 6-cm dish and enumerated at the indicated time points. Data shown are means ± S.D. derived from four independent clones. d, immunoblot analysis of wild-type and Jab1+/− MEFs with antibodies directed against Jab1 (total Jab1, CSN, and small complex), γ-tubulin (γ-Tub), p27, Cul1, cyclin E, p53, p21, MDM2, cyclin D1, Cdk4, Skp2, and p16. Cell lysates were separated by standard SDS-PAGE (for total Jab1, γ-tubulin, p27, Cul1, cyclin E, p53, p21, MDM2, cyclin D1, Cdk4, Skp2, and p16) and by nondenaturing PAGE (for CSN and small complex). The representative results of the in vitro kinase assay for Cdk2 and Cdk4 are also shown (for Cdk2-kinase and Cdk4-kinase, respectively). e, kinetics of S phase entry after restimulation of serum-starved, wild-type (black circles) and Jab1+/− (red circles) MEFs. Data are means derived from three independent clones. f, immunoblot analysis of p27 in wild-type and Jab1+/− MEFs after restimulation of serum-starved cells. An antibody against γ-tubulin was used as a loading control.

22.7 ± 5.0% in Jab1+/− cells (Fig. 4d). Because Jab1 is present both in the CSN complex and in a smaller form (12, 13, 26, 39), we separated these two forms by a native-PAGE method (see “Experimental Procedures” for details), and we found that the level of the CSN complex was reduced only by 19.1 ± 5.9%, whereas the amount of the Jab1-containing small complex was markedly reduced (by 59.4 ± 1.6%) (for representative data, see Fig. 4d, and for quantitative measurement, see “Experimental Procedures” for the details). Among the cell cycle regulators, we observed no major detectable differences in expression levels of Cul1, cyclin E, cyclin D1, Cdk4, Skp2, p21, MDM2, p16, and p53. Furthermore, the amount of neddylated Cul1 subunit (the
slower migrating form) was equivalent between wild-type and Jab1 heterozygous cells (Fig. 4d). One exception was that the Cdk inhibitor p27 was significantly up-regulated in Jab1+/− cells (2.66 ± 0.89-fold) (Fig. 4d). Consistent with this observation, the quantitative in vitro kinase assay revealed that Cdk2 and Cdk4-associated kinase activities in Jab1+/− MEF cells were reduced by 31.1 ± 3.1 and 29.7 ± 3.9%, respectively (for representative data, see Fig. 4d). Serum-starved Jab1+/− MEFs entered S phase with delayed kinetics (~3 h) compared with their wild-type counterparts after serum stimulation (Fig. 4e). In these cells, the down-regulation of p27 during G1 was markedly impaired (Fig. 4f). These results suggest that Jab1 specifically participates in the regulation of p27 in vivo.

**DISCUSSION**

In this study, we showed that Jab1 plays an important role in early embryonic development and cell proliferation in mice. Jab1/CSN5 is essential in other multicellular organisms such as Drosophila (12) and C. elegans (14), and because other CSN subunits are also essential in these organisms (Drosophila (13), Arabidopsis (15), and mice (21, 60)), one may presume that the whole CSN complex is required for the development and maintenance of multicellular organisms. In the case of Arabidopsis, the situation is more complicated because of the duplication of the CSN5/Jab1 gene (AhJH1 and -2), and double mutation is required to reveal the phenotype. Embryonic lethality at a similar developmental stage with activation of p53 and/or dysregulation of cyclin E is commonly seen in mice deficient in Uba3 (61) and Cull1 (62, 63), in addition to CSN2 (60), CSN3 (21), and CSN5/Jab1, indicating that the integrity of the NEDD8-SCF-CSN pathway is critical during early embryogenesis. The cause of embryonic death is not fully uncovered. Up-regulation of cyclin E seems to accelerate cell proliferation rather than provoking cell death, and induction of p53 may only partly be involved in embryonic lethality, because the p53−/−/CSN5/Jab1−/− genetic background did not rescue lethality in Jab1−/− embryos, and knockdown of Jab1 in human cancer-derived cell lines resulted in cell death regardless of the p53 genotypes.4 Furthermore, the cause of embryonic death could be different between mice lacking different components; no substantial increase in the TUNEL signal was observed in CSN2−−/− mice, whereas a marked enhancement of apoptosis was seen in CSN3−−−/− and Jab1−−−/− mice. The loss of subunit-specific function may contribute to the difference.

Expression of p53 was commonly seen in mice with a defective NEDD8-SCF-CSN pathway, but the inductive mechanism is not clear because the SCF complex is unlikely to be the ubiquitin ligase for p53. It is possible that embryos that failed to develop adequately may be discriminated by the induction of p53 expression. Alternatively, a recently discovered p53-ubiquitin ligase, COP1 (64), may participate in this process, and loss of the CSN subunit possibly inactivates COP1 resulting in activation of p53, analogous to the case in Arabidopsis in which loss of CSN precludes COP1 from entering the nucleus and thereby activating the transcription factor Hys in the nucleus (37).

Embryonic lethality is common in nullizygous mice missing different CSN subunits (21, 60), whereas the up-regulation of p27 in both Jab1−−/− and Jab1+/− cells and the impaired cell proliferation in heterozygous mice are features unique to Jab1/CSN5. Among the CSN components, it is often observed that disruption of one subunit does not necessarily result in the same phenotype as the loss of other subunits (28). This could be because each subunit plays a slightly different role in CSN-mediated deneddylation and phosphorylation. Alternatively, each subunit may form unique complexes other than the CSN complex to exert their specific functions. Several researchers including ourselves have found that CSN subunits exist as a smaller form (a smaller complex or a monomeric form) outside the CSN complex in a variety of organisms (12, 13, 26, 28, 39).

In the case of Jab1, a monomeric form was originally found in Arabidopsis, and most interestingly, its appearance was regulated by other gene products, COP1 and DET1 (26). We reported previously that Jab1 forms a smaller complex in mouse fibroblasts beside CSN, which contains only one subset of CSN components (39). We recently found that Jab1 forms multiple different subcomplexes, which do not necessarily contain other CSN subunits, in mammalian cells.4 The precise function of Jab1-containing subcomplexes, their relationship to the large CSN complex, and their regulations should be investigated in detail in the near future.

The intracellular abundance of the Cdk inhibitor p27 is known to be regulated by cyclin E-Cdk2-mediated phosphorylation, Skp2-SCF-mediated ubiquitination, and proteasome-mediated degradation in the late G1-S phase in mammalian cells (65, 66). It is also suggested that there are additional mechanisms that regulate the intracellular abundance of p27, especially in early to mid-G1 (67). The participation of Jab1 (24) and CSN (25) in p27 regulation was shown previously, and both the small Jab1-containing subcomplex (39) and the large CSN complex (25) are suggested to be involved, but the precise mechanism has yet to be fully uncovered. In a mouse reverse genetic approach, Skp2−/− mice exhibit up-regulation of p27 (68), whereas Uba3−/− (61) and Cull1−−/− (62, 63) embryos do not seem to contain higher levels of p27 (Uba3−/− embryos were shown to express higher levels of p57, a family member of p27-related Cdk inhibitors). This could be because these embryos died long before the effect on p27 became manifest. The results of this study showed that the Jab1 gene-targeted embryos and cells contained up-regulated p27, and this will help us to understand the overall mechanism of p27 regulation in the G1 phase. It is a tempting hypothesis that the Jab1-containing subcomplex is a part of the p27 regulatory mechanism in early to mid-G1, and CSN controls p27 through Skp2-SCF in late G1. Furthermore, high expression levels of Jab1 are observed in human cancer cells, with a poor prognosis and a low level of p27 (47–56). Jab1−−/− cells and animals may help determine the role of Jab1 in the regulation of potential target molecules in cancer such as p27 and the pathologies that accompany their dysregulation.

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**REFERENCES**

1. M. K. F. Porte, J. Murray, J. M., Brikos, C. Christensen, P. U., Caspary, T., Hagan, I. M., M. Miller, J., Simanik, V., Hofmann, K., and Arr, A. M. (1999) *Curr. Biol.* 9, 1427–1430.

2. Maytal-Kiviti, V., Piran, P., Pick, E., Hofmann, K., and Glickman, M. H. (2002) *EMBO J.* 3, 1215–1221.

3. Wee, S., Hefed, R., Dubiel, W., and Wolf, D. A. (2002) *BMC Genet.* 3, 15–22.

4. Seeger, M., Krapf, R., Ferrell, K., Bech-Otschir, D., Demuley, R., Schade, R., Gordon, C., Neumann, M., and Dubiel, W. (1998) *FASEB J.* 12, 469–478.

5. Wei, N., Chamovitz, D. A., and Deng, X. W. (1998) *Curr. Biol.* 7, 1187–1194.

6. Wei, N., Tsuge, T., Serino, G., Doohane, N., Takio, K., Matsu, M., and Deng, X. W. (1996) *Curr. Biol.* 7, 177–182.

7. Bech-Otschir, D., Seeger, M., and Dubiel, W. (2002) *J. Cell Sci.* 115, 467–473.

8. Wei, N., and Deng, X. W. (2003) *Annu. Rev. Cell Dev. Biol.* 19, 261–286.

9. Wei, N., and Deng, X. W. (1999) *Trends Genet.* 3, 129–133.

10. Chamovitz, D. A., and Glickman, M. N. (2002) *Curr. Biol.* 12, 232.

11. Seeger, M., Gordon, C., and Dubiel, W. (2001) *Curr. Biol.* 11, 843–846.

12. Freilich, S., Oron, E., Kapp, Y., Nevo-Gaspi, Y., Orgad, S., Segal, D., and Chamovitz, D. A. (1995) *Curr. Biol.* 9, 1187–1190.

13. Oron, E., Mannervik, M., Rencus, S., Haran-Stemberg, O., Neuman-Silberberg, S., Segal, D., and Chamovitz, D. A. (2002) *Dev. Biol.* 249, 4399–4409.
