The p53 Family Member Genes Are Involved in the Notch Signal Pathway*

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The p53 tumor suppressor is a transcription factor that regulates cell growth and death in response to environmental stimuli such as DNA damage. p63/p51 and p73 were recently identified as members of the p53 gene family. In contrast to p53 however, p63 and p73 are rarely mutated in human cancers. Mice that lack p53 are developmentally normal, while p63 and p73 appear to play critical roles in normal development. To determine how p63 and p73 are involved in normal development, we attempted to identify target genes that are specifically regulated by p63 and/or p73 but not by p53. We found that the Jagged1 (JAG1) and Jagged2 (JAG2) genes, encoding ligands for the Notch receptors, are up-regulated by p63 and p73. Furthermore, we identified a p63-binding site in the second intron of the JAG1 gene, which can directly interact with the p63 protein in vivo, as assessed by a chromatin immunoprecipitation assay. A heterologous reporter assay revealed that this p63-binding site is a functional response element and is specific for p63. We also found a target of Notch signaling, HES-1 was up-regulated in Jurkat cells, in which Notch1 is highly expressed, when co-cultured with p63-transfected cells, suggesting that p63 can trigger the Notch signal pathway in neighboring cells. Our findings show an association between the p53 family genes and Notch signaling and suggest a potential molecular mechanism for the involvement of the p53 family genes in normal development.

The involvement of the p53 tumor suppressor gene in cell growth and death is mediated by the transactivation of p53-target genes in response to environmental stimuli such as DNA damage (1–3). p63/p51 and p73 were recently identified as members of the p53 gene family and encode proteins that share considerable structural homology with p53 (4–6). p63 and p73 can bind to the p53-responsive elements and up-regulate some p53-target genes, which suggest that the p53 family members have a potential for functional overlap with p53 itself (7–12). However, in contrast to p53, p63 and p73 are rarely mutated in human cancers (13–15).

Different phenotypes between p63- or p73-deficient and p53-deficient mice were also reported (16–19). In contrast to p53-deficient mice, mice lacking the p73 genes show no increased susceptibility to spontaneous tumorigenesis. p73-deficient mice have neurological, pheromonal and inflammatory defects. p63-deficient mice have major defects in their limbs and craniofacial development, as well as a striking absence of stratified epithelia, suggesting that p63 is required for limb and epidermal morphogenesis. In humans, Li-Fraumeni syndrome patients have inherited mutations of the p53 gene and develop normally, but are predisposed to cancer (20), while heterozygous germline mutations in the p63 gene are the cause of ectrodactyly, ectodermal dysplasia, and facial clefts syndrome (21). These studies demonstrate a marked divergence in the developmental roles of p63 and p73 and further distinguished these p53 family genes from p53. Despite these revelations, little is known about target genes specifically regulated by p63 or p73. Identifying the gene targets of p63 and p73 that play a role in development is an important step to a better understanding of the roles of these proteins in normal development and developmental disorders.

To determine what mediates the function of the p53 family members on normal development, we attempted to identify genes that are specifically regulated by p63 and/or p73 but not by p53. As a result, we identified the JAG1 and JAG2 genes as targets for p63 and p73. Thus, there are likely to be differences among the p53 family members with regard to their optimal DNA-binding sequences. We also showed p63-mediated JAG1 induction can activate Notch signaling in neighboring cells. This study is the first to implic a link between the p53 family member genes and Notch signaling.

EXPERIMENTAL PROCEDURES

Cell Lines and Recombinant Adenoviruses—The human cancer cell lines used in this study were purchased from American Type Culture Collection or the Japanese Collection of Research Bioresources (Osaka, Japan). All cell lines were cultured under conditions recommended by their respective distributors. The endogenous p53 statuses in these lines are wild type for A172 and HCT116, mutant for DLD1, colo320, and PLC/PRF5, and p53 null for Saos2 and H1299. The generation and purification of replication-deficient recombinant adenoviruses containing p53, p73α, p73β, p63α, and p63γ genes or the bacterial lacZ gene were described previously (11).

Immunoblot and Northern Blot Analysis—The primary antibodies used in this study are as follows: mouse anti-human p53 monoclonal antibody (DO-7, Santa Cruz Biotechnology); mouse anti-human p73 monoclonal antibody (ER-15, Oncogene Research); mouse anti-human p63 monoclonal antibody (4A4, Oncogene Research); and rabbit anti-human JAG1 polyclonal antibody (H-114, Santa Cruz Biotechnology). For Northern blot analysis, total RNA (10 μg) was electrophoretically separated on a 1% agarose gel containing 2.2 M formaldehyde and blotted on a nitrocellulose membrane (Schleicher & Schuell). RNA was
visualized with ethidium bromide to ensure that it was intact and loaded in similar amounts and to confirm proper transfer. Hybridization was performed as described previously (11). cDNA probes for JAG1 (nucleotides (nt) 3531–4534), JAG2 (nt 419–1323), p21 (nt 11–429) and HES-1 (nt 286–642) were amplified by the reverse transcription-PCR from appropriate cDNA pools. PCR products were sequenced to verify their identity.

cDNA Microarray—For cDNA expression arrays, poly(A)+ RNA was isolated with the FastTrack 2.0 mRNA isolation system (Invitrogen) from adenovirus-infected A172 human glioma cells and used as a template for synthesis of Cy5- or Cy3-labeled cDNA probes. The probes were hybridized to cDNA microarrays containing 9216 genes. Microarray construction, hybridization procedures, and data analysis were described previously (22, 23).

Immunofluorescence Microscopy—Cells grown on glass coverslips were fixed in cold absolute acetone for 10 min. Following fixation, cells were washed in PBS then blocked in PBS plus 5% goat serum. Primary antibody (H-114) was diluted to 5 μg/ml in the presence of Triton X-100 and incubated for 2 h at room temperature. Cells were washed in PBS and then incubated with secondary antibody Alexa 488-conjugated goat anti-rabbit IgG (Molecular Probes) for 30 min at room temperature. The specimens were examined using a laser-scanning confocal microscope (Nikon Bio-Rad).

Chromatin Immunoprecipitation Assay (ChIP)—ChIP assay was performed using the Anti-Histone H3 Chromatin Immunoprecipitation Assay Kit (Upstate Biotechnology) as recommended by the manufacturer except that antibodies against p53 (DO-7), p63 (4A4), and FLAG tag peptide (M2, Sigma-Aldrich) were used in this study. 2 × 106 Saos2 cells were plated onto a 10-cm dish and infected with Ad-p53 or Ad-p63y. After 24 h, genomic DNA and protein were cross-linked by addition of formaldehyde (1% final concentration) directly to culture medium and incubated for 15 min at 37°C. Cells were lysed in 200 μl of SDS lysis buffer with a protease inhibitor mixture and sonicated to generate 300–800 bp DNA fragments. After centrifugation, the cleared supernatant was diluted 10-fold with the ChIP dilution buffer and incubated with the specific antibody against p53 at 4°C for 16 h. Immune complexes were precipitated, washed, and eluted as recommended. DNA-protein cross-links were reversed by heating at 65°C for 5 h, and DNA was phenol-extracted, ethanol-precipitated, and resuspended in 50 μl aTE. Five microliters of each sample were used as a template for PCR amplification. PCR amplifications of the second intron of the JAG1 gene containing the conserved p53-binding sequences (+5997, renamed the RE-JAG1 sequence and +5983) were performed on immunoprecipitated chromatin using the specific primers 5′-ACCTTTCACCATGCCCATCAC-3′ (forward) and 5′-GCCAAGGACAAATAGCGA-3′ (reverse), and 5′-CCATTGGTAACAGCTGTGCAAGCTG-3′ (forward) and 5′-CACTCTGGATTTGCGAT-3′ (reverse), respectively. PCR amplification of the p21 promoter was performed using oligonucleotides 5′-ACCTTTCCATCATCACGCTC-3′ (forward) and 5′-GCTAGATACTACATCTTTGGA-3′ (reverse), as described (24). To ensure that PCR was performed in linear range, template DNA was amplified for a maximum of 30 cycles. PCR products following ChIP assay were sequenced to verify the identity of the amplified DNA.

Luciferase Assay—A 51-bp fragment of the RE-JAG1 (5′-AGCCTCTTGTTGTCGGCTGTGTTGAAAGAGCGTGGTGGCTG-3′) and its mutant form (5′-AGGATTTCGTTGACCGAGTTGTTGTAAGCATACCAGGTACAGTG-3′) were synthesized and inserted upstream of a basal SV40 promoter in the pGL3-promoter vector (Promega), and the resulting constructs were designated pGL3-RE-JAG1 and pGL3-RE-JAG1-mut, respectively. 1 × 106 of H1299 cells in 6-cm dishes were co-transfected with 1.5 μg of either pGL3-RE-JAG1 or pGL3-RE-JAG1-mut, together with 1.5 μg of a pDNA3.1 control vector (Invitrogen) or a vector that expresses p53, p73β, or p63y using Lipofectin (Life Technologies). Cells were harvested 48 h after transfection followed by measurement of luciferase activity using the Luciferase Assay System (Promega). The ability to stimulate transcription was defined as the ratio of luciferase activity in the cells transfected with the pGL3-RE-JAG1 relative to the activity in the cells transfected with the non-responsive reporter plasmid, pGL3-RE-JAG1-mut. As a control experiment, pGL3-p53CBS containing three copies of the consensus p53-binding sequence or its mutant form (mut3) (11) were transfected into H1299 cells with a pcDNA3.1 control vector or a vector that expresses p53, p73β, or p63y. All experiments were performed in triplicate and repeated on at least three independent occasions.

RESULTS

p63 and p73 Induce Expression of the JAG1 and JAG2 Genes—To express the p53 family genes in human cancer cell lines, we used the replication-deficient adenoviral vector harboring human p53 (Ad-p53), p73β (Ad-p73β), and p63y/p51A (Ad-p63y/Ad-p51A) genes. To determine the relative efficiency of adenovirus-mediated gene transfer, cells were infected with adenovirus containing the bacterial lacZ gene (Ad-lacZ). We used seven human cancer cell lines that showed highly efficient gene transfer, with 90–100% of the cells staining for β-galactosidase activity at a m.o.i. of 50–100. A high-level p53 protein was observed in cells infected with Ad-p53 (examples in Fig. 1, upper panel). Infection with Ad-p73β and Ad-p63y resulted in expression of exogenous p73β and p63y proteins, respectively (examples in Fig. 1, middle and lower panels). We used p73β and p63y/p51A isoforms in this study because we and others have demonstrated that transcription of a p53-responsive reporter gene was activated more strongly in p73β and p63y/p51A than p73α and p63α/p51B (5, 11, 26).

In an effort to identify specific targets regulated by p73 and p63, we performed cDNA microarray analysis and compared expression patterns in a human glioma cell line A172 transfected separately with Ad-p53, Ad-p73β, and Ad-p63y. Using this approach, we detected several genes that were reproducibly activated in p73- and p63-transfected cells but not in p53-transfected cells. Two of the genes that were selectively induced by p63 and p73 were JAG1 and JAG2. Northern blot analysis using the JAG1 and JAG2 cDNAs as probes demonstrated that expression of the JAG1 gene was dramatically increased by infection with Ad-p63y and Ad-p73β in a time-dependent manner, but not by infection with Ad-p53 (Fig. 2). The JAG1 induction was seen as early as 9 h after Ad-p63y infection, similar to induction of p21, a well-defined target for both p53 and its family members (Fig. 2) (1, 4, 6). The early induction of JAG1 suggests that the JAG1 gene may be a direct target of transcriptional activation by p73 and p63.

To investigate whether JAG1 induction by p73 and p63 is specific to the cell-type used, we performed Northern blot analysis with seven human cancer cell lines, including DLD1, Colo320, and HCT116 (colorectal cancers), Saos2 (osteogenic sarcoma), H1299 (lung cancer), PLC/PRF5 (hepatocellular car-
Fig. 2. Time course of JAG1 and JAG2 induction following adenovirus-mediated transfer of p63 and p73 in A172 human glioma cells. A172 cells were infected with adenoviruses at a m.o.i. of 50, and the cells were harvested at the indicated times following infection. Total RNA were extracted and subjected to Northern blotting. Total RNA (10 μg) were loaded in each lane, and the same filter was re-hybridized with human JAG1, JAG2, and p21 cDNAs. Ethidium bromide staining of 28S ribosomal RNA (28S) in the lower panel shows that equal amounts of RNA were loaded in each lane.

cinoma), and A172 (glioma) (Fig. 3). JAG1 was highly induced by p63γ and by p73β in six of seven lines tested (no induction in PLC/PRF5 cells). In contrast, JAG1 induction by p53 was less dramatic and occurred in only three cell lines (DLD1, Saos2, and colo320 cells). JAG1 expression was reduced by p53 in H1299 and HCT116 cells. In addition, we examined JAG1 expression after infection with Ad-p73α and Ad-p63α/p51B in A172 and DLD1 cells (Fig. 3A). p73α was the strongest activator of JAG1 in A172 cells. JAG2 was highly induced by p73β in all seven cell lines tested (Fig. 3), though at a later time point relative to JAG1 (Fig. 2). JAG2 was also induced by p63γ in six of seven cell lines (Fig. 3, second panel). In the majority of cell lines tested, the strongest induction of JAG1 and JAG2 were observed following Ad-p63γ and Ad-p73β infection, respectively (Fig. 3). Ad-p53 induction of p21 was observed in all seven lines, but Ad-p63γ and Ad-p73β induced p21 only in a subset of these lines (Fig. 3, third panel).

We then examined the level of JAG1 protein by Immunoblot analysis using an antibody against the intracellular domain of human JAG1. Fig. 4A shows the high-level accumulation of endogenous JAG1 protein in Ad-p63γ-infected Saos2 cells and Ad-p73β- and Ad-p63γ-infected A172 cells, consistent with the Northern blot analysis (Fig. 3). Immunofluorescence staining using the same antibody also verified the expression of endogenous JAG1 in Ad-p63γ-infected Saos2 cells but not in control or Ad-lacZ-infected cells. The subcellular distribution of JAG1 coincided with that reported previously (27). In contrast, Ad-p53 infection resulted in only a slight increase in JAG1 expression (Fig. 4B). To determine whether expression of other Notch ligands and receptors are regulated by the p53 family genes, Northern blot analysis was performed. Neither Notch ligands (DLL1, DLL3, DLL4) nor Notch receptors (Notch1, Notch2, Notch3, Notch4) were significantly induced by p73, p63, or p53 in A172 and DLD1 cells (data not shown).

Identification of a Specific Target Sequence for p63 in the JAG1 Gene—The early and strong induction of JAG1 suggests that JAG1 may be a direct and specific target of transcriptional activation by p63. To address this hypothesis, we searched for a consensus p63-binding sequence in the JAG1 gene because the p63 and p73 proteins can also bind to the p53-binding sites (4–7, 28). We obtained the genomic sequence of the human JAG1 gene from the GenBank™ data base (accession number AL035456) and searched for a consensus p53-binding site(s) within 10 kb in and around exon 1 of the JAG1 gene. Ten candidate sequences were identified at the positions −5162, −3133, −2098, −1555, −1131, +2814, +5597, +5983, +7637, and +8102, where +1 represents the translation initiation site.

Fig. 3. Northern blot analysis shows JAG1 and JAG2 induction in human cancer cell lines. Seven human cancer cell lines were infected with adenoviruses at a m.o.i. of 50 or 100. Ten μg of total RNA isolated 24 h after infection was subjected to Northern blotting.

Fig. 4. The endogenous JAG1 protein is increased by transfection of Ad-p63 or Ad-p73. A, immunoblot analysis was performed on cell lysates from A172 and Saos2 cells 24 h following infection with Ad-lacZ (lanes 1 and 5), Ad-p53 (lanes 2 and 6), Ad-p73β (lanes 3 and 7), and Ad-p63γ (lanes 4 and 8). B, cell extracts (30 μg) were separated by electrophoresis on a 10% SDS/acylamide gel and analyzed by Western blotting using the JAG1 C-terminal antibody H-114. B, immunofluorescence analysis of JAG1 protein expression of control Saos2 cells (a) and Saos2 cells 24 h following infection with Ad-lacZ (b), Ad-p53 (c), or Ad-p63γ (d) is shown. Cells were stained with the JAG1 C-terminal antibody H-114. Omission of the primary antibody resulted in no staining (data not shown).

To determine whether the p63 protein can selectively bind to any of these candidate binding sites in vivo, we performed ChIP assays. The ChIP assay relies on the ability of specific antibodies to immunoprecipitate DNA-binding proteins along with the
associated genomic DNA. We used Saos2 cells infected with either Ad-p53 or Ad-p63y for ChIP assay, since the induction of JAG1 by p63y was strongest in this line (Figs. 3 and 4). Immunoprecipitation of DNA-protein complex using antibodies against p53 and p63 was performed on formaldehyde-cross-linked extract from Ad-p53- and Ad-p63y-infected Saos2 cells, respectively. We then measured the abundance of candidate sequences within the immunoprecipitate complexes by PCR. ChIP assay revealed that one DNA fragment containing a candidate sequence, +5987, was reproducibly present in a complex with p63y protein (Fig. 5B, middle panel, lane 3). We designated this p63-binding sequence RE-JAG1 (for responsive element in JAG1). RE-JAG1 consists of four copies of the consensus 10-bp motif separated by 3, 5, and 3 bp, respectively (Fig. 5A). In contrast, p53 protein binding to RE-JAG1 sequence was not detectable in p53-infected cells, as assessed by the ChIP assay with an anti-p53 antibody and subsequent PCR (Fig. 5B, middle panel, lane 6). The other nine candidates were amplified in the input-positive control for PCR, but not in the immunoprecipitated samples with an antibody against p53 or p63 (examples in Fig. 5B, lower panel). As a positive control for the ChIP assay, we analyzed the interaction of p53 and p63 with the p21 promoter. Both p53 and p63 proteins immunoprecipitated with the DNA fragment containing the p53-binding site in the p21 promoter (Fig. 5B, upper panel, lanes 3 and 6). Although we can not exclude a low level of p53 binding to the RE-JAG1 sequence, our ChIP data indicate that p63 is selectively associated with this site in vivo.

To determine whether RE-JAG1 possesses p63-dependent transcriptional activity, we performed a heterologous promoter-reporter assay using a luciferase vector prepared by cloning the oligonucleotide corresponding to RE-JAG1 sequence upstream of a basal SV40 promoter (see “Experimental Procedures”). A control reporter plasmid, pGL3-RE-JAG1-mut, was generated by altering potentially critical nucleotides of the RE-JAG1 sequence. H1299 cells were transiently co-transfected with pGL3-RE-JAG1 or pGL3-RE-JAG1-mut, together with the expression vector pcDNA3.1 containing the p53, p73β, or p63γ gene. The relative luciferase activity was defined as the ratio of luciferase activity in the cells transfected with the pGL3-RE-JAG1 relative to the activity in cells transfected with the non-responsive reporter plasmid, pGL3-RE-JAG1-mut. As a control experiment, pGL3-p63CBS containing three copies of the consensus p53-binding sequence (p53CBS × 3) or its mutant form was transfected into H1299 cells with the expression vector containing p53, p73β, or p63γ. All experiments were performed in triplicate and the means and standard deviation are indicated by the bars and brackets, respectively.

**Fig. 5.** Regulation of JAG1 expression by p63. A, schematic representation of part of the JAG1 gene. A potential responsive site, RE-JAG1, is located at the second intron of the JAG1 gene and consists of four copies of the consensus 10-bp motif of the p63-binding sequence. Lowercase letters identify disparities with the consensus. R represents purine; Y, pyrimidine; and W, adenine or thymidine. B, p63 interacts with the RE-JAG1 sequence in vivo. ChIP assay of a genomic fragment (nucleotide position +5499 to +5749, where +1 represents the translation initiation site) containing the RE-JAG1 sequence in Ad-p63y-infected (lanes 3–5) or Ad-p53-infected (lanes 6–8) Saos2 cells is shown (middle panel). Immunoprecipitation was performed using the gene-specific antibody against p63 (lane 3) or p53 (lane 6), followed by PCR amplification. Input chromatin represents a portion of the sonicated chromatin prior to immunoprecipitation (lanes 1 and 8). Immunoprecipitates with an anti-FLAG antibody (lanes 4 and 7) or in the absence of antibody (no antibody, lanes 5 and 8) were used for controls. DW indicates a no template control (lane 9). PCR amplification of the p21 promoter was performed using primers that flank the p53-binding site in the p21 promoter. PCR amplification revealed that a similar amount of p21 promoter sequence is present in p53- and p63-complexes extracted from each immunoprecipitate (upper panel). The RE-JAG1 was amplified in the immunoprecipitated samples with an antibody against p63 (middle panel, lane 3). One of the other candidate sequences, +5983, is amplified in the input control, but not in the immunoprecipitated samples with an antibody against p53 or p63 (lower panel). C, the RE-JAG1 in the JAG1 gene is responsive to p63y. H1299 cells were co-transfected with pGL3-RE-JAG1 or pGL3-RE-JAG1-mut, together with the expression vector pcDNA3.1 containing the p53, p73β, or p63γ gene. The relative luciferase activity was calculated as the luciferase activity in cells transfected with the pGL3-RE-JAG1 divided by the luciferase activity in cells transfected with the non-responsive reporter plasmid, pGL3-RE-JAG1-mut. As a positive control for transfection efficiency, H1299 cells were transiently co-transfected with pGL3-RE-JAG1 or pGL3-RE-JAG1-mut together with a pGL3-p53, p73β, or p63γ-expressing plasmid. The ability to stimulate transcription was calculated as the luciferase activity in cells transfected with the pGL3-RE-JAG1 divided by the activity in cells transfected with the non-responsive reporter plasmid, pGL3-RE-JAG1-mut. Fig. 5C shows that the increase in luciferase activity for pGL3-RE-JAG1 was higher for p63γ than for either p53 or p73β. These results are consistent with the strong induction of endogenous JAG1 by p63γ (Fig. 3). As a control, we demonstrate that the level of transcription from a
The Notch family of transmembrane receptors has been reported to play an important role in development by specifying cell fates (37, 38). To date, five Notch ligands have been identified, including JAG1, JAG2, Delta like-1 (DLL1), DLL3, and DLL4, all of which are transmembrane proteins having an extracellular domain important for receptor binding (39–43). Here we demonstrated physical but also functional involvement of p63 in the Notch/Jagged signal pathway. The induction of JAG1 correlated with the initiation of signaling downstream of the Notch receptor in co-cultivated cells. Endogenous HES-1 expression was up-regulated in Notch1-expressing Jurkat cells by co-culturing with Ad-p63γ-infected Saos2 cells. This agrees with a recent report showing that Rel/NFκB-mediated JAG1 expression can transactivate endogenous HES-1 gene expression in co-cultured Jurkat cells (25). Our findings indicate that p63 can trigger the Notch signal pathway in the neighboring cells and therefore raise the possibility that the members of the p53 family play a role in normal development through modulating Notch signal pathway.

Ligand-mediated activation of Notch induces the proteolytic release of the intracellular domain of Notch and transactivation of its target genes, which leads to modulation of cell proliferation and differentiation (44, 45). Mutations in human Notch ligands result in the disruption of the Notch signal pathway, leading to developmental abnormalities (46–48). Mutations in the JAG1 gene have been found in patients with Alagille syndrome, an autosomal dominant disorder characterized by abnormal development of heart, skeleton, liver, and eye, as well as a characteristic facial appearance. Our data are generally consistent with the similarity between the developmental defects associated with alteration in the p53 family members and those of Notch ligands (17–19, 21, 46–49). In particular, JAG2-deficient mice exhibit defects of limb and craniofacial development (49), closely resembling the phenotype of patients with ectrodactyly, ectodermal dysplasia, and facial clefts syndrome, in which p63 is mutated. Both p63 and p73 can transactivate the promoters of genes associated with neuronal or epidermal differentiation, and overexpression of these genes up-regulates neuronal or epidermal differentiation markers (50, 51). Together with our findings, these studies highlight the potential for an interplay between the p53 family genes and Notch signal pathway during ectodermal development.

In conclusion, we report that Notch ligands, JAG1 and JAG2, are selectively induced by p73 and p63, respectively, but not by p53. We also identified a specific binding site for the p63 protein in the second intron of the JAG1 gene and showed that p63 can activate Notch signaling to the neighboring cells. Our findings point to a potential role for p63 and p73 in normal development and cellular regulation mediated by Notch signaling.

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**REFERENCES**

1. el-Deiry, W. S. (1998) Semin. Cancer Biol. 8, 345–357
2. Tokino, T., and Nakamura, Y. (2000) Crit. Rev. Oncol. Hematol. 33, 1–6
3. Vogelstein, B., Lane, D., and Levine, A. J. (2000) Nature 408, 307–310
4. Kaghad, M., Bonnet, H., Yang, A., Creancier, L., Biscan, J. C., Valent, A., Minty, A., Chalon, P., Leilas, J. M., Dumont, X., Ferrara, P., McKeon, F., Minty, A., Chalon, P., Lelias, J. M., Dumont, X., Ferrara, P., McKeon, F.
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