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To cite this version:
Anne Chauchereau, Maria Georgiakaki, Mallory Perrin-Wolff, Edwin Milgrom, Hugues Loosfelt. JAB1 Interacts with Both the Progesterone Receptor and SRC-1. Journal of Biological Chemistry, American Society for Biochemistry and Molecular Biology, 2000, 275 (12), pp.8540-8548. 10.1074/jbc.275.12.8540. hal-03211045

HAL Id: hal-03211045
https://hal.archives-ouvertes.fr/hal-03211045
Submitted on 28 Apr 2021

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JAB1 Interacts with Both the Progesterone Receptor and SRC-1

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JAB1 (Jun activation domain-binding protein-1) has previously been described as a coactivator of AP1 transcription factor. We show here, by yeast and mammalian two-hybrid analyses and by pull-down experiments, that JAB1 also interacts with both the progesterone receptor (PR) and the steroid receptor coactivator 1 (SRC-1) and that it stabilizes PR-SRC-1 complexes. We also show that JAB1 potentiates the activity of a variety of transcription factors known to associate with SRC-1 (nuclear receptors, activator protein-1, and nuclear factor κB). This occurs without any modification of PR or SRC-1 concentration. JAB1 is a subunit of a large multiprotein complex that has been called the COP9 signalosome. The latter is present in plant and animal cells and has been shown to be involved in a variety of cellular mechanisms including transcription regulation, cell cycle control, and phosphorylation cascades. We now show that it is also involved in the mechanisms of action of nuclear receptors and of their coactivators.

Recent studies have shown that a JAB1–PR–SRC1 complex is present in large multiprotein complexes in several cancer cell lines. The data suggest a link between the JAB1 complex and the downstream transcriptional regulation of PR by the JAB1 complex.

EXPERIMENTAL PROCEDURES

Construction of Vectors—The yeast (pAS2, pAS2–1, and pACT2) and mammalian (pM, pVP16, and pG5CAT) two-hybrid system vectors were obtained from CLONTECH. The DBD-D12-PR-150 vector was obtained initially by ligating the rabbit PR cDNA (29) (nucleotides 3 to 2889) into the pAS2 vector containing the yeast trp1 gene marker. This vector encodes a DBD-D12-PR-150 fusion protein. The DBD-D12-PR-150 and DBD-D12-PR-300 vectors were constructed from DBD-D12-PR-150 vector by enzymatic deletion of DNA fragments encoding amino acids 45–896 or 1–516 of PR, respectively. A human placenta cDNA library, fused to the G418 selection vector (AD4N1), and present in the yeast pACT2 vector encoding the Leu2 gene marker was used for the two-hybrid screening. DBD-D12-PR-150, DBD-D12-PR-150, and DBD-D12-PR-300 vectors used for two-hybrid experiments in mammalian cells were constructed by subcloning the inserts of pAS2–PR corresponding mutants into the pG5CAT vector.

Isolation of a truncated JAB1 cDNA by the two-hybrid screen (see below) led us to clone the full-length cDNA of JAB1. This was performed by three independent rounds of polymerase chain reaction from the human placenta cDNA library (CLONTECH) using F6/ S DNA polymerase (Stratagene) with the sense primer 5′-ATTAAGAATTCCTTCTCGGGGATTGCC and the 3′ reverse primer 5′-ATAAAGAATCTGCTTCTGGAGAGTTT. The resulting EcoRI/BamHI fragment encoding the full-length sequence of JAB1 (nucleotides 11 to 1602) was

* This work was supported by INSERM, the Association pour la Recherche sur le Cancer, the Ligue contre le Cancer, the Faculté de Médecine Paris-Sud, and the Fondation pour la Recherche Médicale. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† Recipient of a fellowship from the A. Onasis Foundation of Greece.
‡ Recipient of a grant from the Association pour la Recherche sur le Cancer.

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1 The abbreviations used are: AP1, activator protein-1; SRC, steroid receptor coactivator; CBP, CREB response element-binding protein; PR, progesterone receptor; HA, hemagglutinin epitope; DBD, DNA-binding domain; LBD, ligand-binding domain; AD, activation domain; GST, glutathione S-transferase; ERE, estrogen-responsive element; PREG, progesterone-responsive element; SC medium, synthetic complete medium; 3T, 3-aminotriazol; CAT, chloramphenicol acetyltransferase; TPA, 12-O-tetradecanoylphorbol 13-acetate; GR, glucocorticoid receptor; NFκB, nuclear factor-κB; R50520, 17,21-di-methyl-17α-norpregn-4,9-dien-3,20-dione; RUGS6, 17α-hydroxy-11β-(4 dimethylaminophenyl)-17α-(1-propynyl)octa-4,9-dien-3-one; ZRK829, 11β-(4-dimethylaminophenyl)-17α-hydroxy-17p4-flat; RX5637, 11β-(4-(1-methyl-4-9 gemadene-5-one; PAOE, polyarylamine gel electrophoresis.
cloned into the mammalian expression vector pSGS (Stratagene) to create pSGS-JAB1. The same fragment was also subcloned into the same sites of a modified pSGS vector (pSGS-HA) resulting in the N-terminal in-frame fusion of JAB1 with the HA epitope (pSGS-HA-JAB1). Two differences were observed with the JAB1 sequence reported by Karin and co-workers (11). There was a change in the Val181 codon which was silent. The second one was a GAV change that transformed the aspartic acid H128 into an arginine. It should be noted that Arg128 is conserved in the sequence of JAB1 homologs pad1 and POF1 (2, 3). The full-length JAB1 cDNA was also subcloned from the pSGS-JAB1 into the yeast pYES2–1 and pACT2 vectors using the yeast 2-hybrid system (41) and the full-length JAB1, respectively. The same DNA fragments were introduced into pM and pVP16 vectors resulting in the expression of DBD—pGAL1 and AD—pGAL1 fusion protein in mammalian cells, respectively. Finally, the full-length JAB1 was expressed in E. coli with an N-terminal GST fusion protein by inserting the same DNA fragment into the pGEX-KX vector (Amersham Pharmacia Biotech) pGEX-JAB1 vector.

The pSGS-HA vector was obtained from polymerase chain reaction from the human placenta cDNA library as three overlapping fragments of similar size. The upper primer and lower primer were CCG GAACGGTACCATTAATGACCGTACATTAGAGCGGAGA and AAGGATCTGCTACGATTTCATTTATTTTATTATT, respectively. The entire open reading-frame of SRC-1 (nucleotides 1−118 to 118−359) was finally reconstituted in the yeast 2-hybrid vector of the yeast 2-hybrid system (20) and the full-length SRC-1 cDNA sequence was confirmed by sequencing. The full-length SRC-1 cDNA was entirely sequenced on both strands and corresponds to the sequence of the SRC-1 published by Takehita et al. (15) as a 3′-untranslated region (3′-UTR) of the rat SRC-1 gene. The full-length SRC-1 cDNA was further subcloned from pSGS-HA-SRC-1 into the pSGS-HA vector. This construction was then transformed into yeast and the yeast 2-hybrid system was used. The yeast 2-hybrid system was used to detect interaction with the G-actin-binding domain of SRC-1 (nucleotides 1−118 to 118−434) with the HA epitope. The yeast AD-DBD vector was obtained by inserting the pSGS-HA vector into the pACT2 vector. The construct AD-DBD SRC-1 for the mammalian two-hybrid system was obtained by cloning the same DNA fragment into the pVP16 vector.

The expression vectors for SRC-1 were generated from the pSGS-HA-SRC-1 vector. The SRC-1 mutants A (pSGS-HA-SRC-182−1−118−118−434), E (pSGS-HA-SRC-182−1−118−118−434), and C (pSGS-HA-SRC-1−118−118−434) were obtained by replacing the BamHI/HindIII fragment of the blunted pSGS-HA-SRC-1 vector with the BamHI digest of the respective mutants. The SRC-1 mutants D (pSGS-HA-SRC-1−118−118−434), E (pSGS-HA-SRC-1−118−118−434), and F (pSGS-HA-SRC-1−118−118−434) were obtained by replacing the 3′ MscI/BglII fragment by a double-stranded oligonucleotide containing a stop codon in SRC-1 mutants B and C and in wild-type SRC-1, respectively.

The expression vector encoding the full-length rat progesterone receptor (pSGS-PR) and the reporter plasmid PRK-TATA-CAT have previously been described (31). The reporter plasmid ERE-TATA-CAT was a gift from Dr. J. F. Tazi and contains the mouse MMTV-LTR promoter driving the expression of a CAT reporter gene. The expression vector for the TATA-CAT vector with the synthetic oligonucleotide containing two EREs and a TATA box GATCCGCTTCAGCTTGTCTCAGTCGTTACGTCAGTTAATATTATTATGCTTATAAGG. The plasmids encoding the other nuclear receptors and their respective response element have been previously described (31). The 11,4-fold enriched exons of the 5′ region of the human c-myc gene were amplified by PCR with primers 5′-CCCGCMGGTCTAGCAAGGCTGAGAAGAGACCTATTCCTTCCAGACTC-3′ and 5′-CCCGACGCTCTTCCAGACTCAGGCTGAGAAGAGACCTATTCCTTCCAGACTC-3′ and subcloned into the pBluescript SK+ vector. The construct pBluescript SK+ was linearized at the unique XhoI site and used to transform competent cells. The plasmid was isolated and sequenced for orientation and anti-sense primer orientation for the presence of either 1 µM R5020 or 10 µM RU468. Plasmids were isolated from the corresponding clones initially selected on 50 µM 3AT medium. The placenta library plasmids were segregated by transformation of the E. coli strain M1H206 strain (Leu+, Trp+, Ura+) (37) by plating on M9−Leu minimal medium. The plasmids were then purified (Qiagen resin) and sequenced (ABI-Perkin 373A automated sequencer) using primers complementary to pCT23-Galactosidase Assays in Yeast Cultures—The Y326 yeast strain (Mata, trp1-901, leu2-3, 112, ura3-52, his3-200, ade2-101, lys2-801, gal4-542, gal80-538, URA3::GAL1p-Lac2) was transformed with various plasmids (see figure legends) and plated on SC-Trp−Leu−medium. Cultures derived from 3−6 batches of three isolated colonies were grown in SC-Leu−medium for 48 h, and then diluted into 24-well plates at 1.5 ml of the same medium containing 1 µM R5020, 10 µM RU468, or no steroid. After growing to the end of the log phase, the β-galactosidase activity of the cells was measured as described by Transy and Legrain (34).

Transient Transfections of Mammalian Cells—CV-1 cells or HeLa cells were maintained in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) supplemented with 10% fetal calf serum (Seromed). 3 × 105 cells were seeded per well of a six-well-multiwell dish in Dulbecco’s modified Eagle’s medium containing 10% charcoal-stripped serum, and cells were transiently transfected using the calcium phosphate coprecipitation procedure as previously described (38). All transfections were performed with 330 µl of calcium-phosphate precipitate/well containing 1 mg of plasmid DNA and 300 µl of calcium phosphate precipitate. The mixture was incubated for 2 h at room temperature, and then diluted into 24-well plates with 1.5 ml of the same medium containing 1 µM R5020, 10 µM RU468, or no steroid. In Vitro Protein Binding Assays—The pGEX-JAB1 vector was used to express the BL21 strain of E. coli to synthesize the GST-JAB1 fusion protein. The manufacturer’s instructions (Amersham Pharmacia Biotech) were followed except that ethylene glycol (10%) (39) and sarcosyl (1%) (40) were added to the extract buffer to increase the solubilization of the aggregated protein. After centrifugation, Triton X-100 (2%) was added (buffer A), and the cleared lysate was incubated with GST Sepharose-4B for 1 h at 20°C. The gel beads were then poured into a column, washed with 20 ml of buffer A, and finally with 20 ml of buffer B (500 mM KCl, 50 mM Tris-HCl, pH 8). Binding of the TNT was determined using the TNT each experiment, and total DNA was normalized to 20 µg of precipitate using salmon sperm DNA. The CAT activity was measured with the CAT enzyme-linked immunosorbent assay kit (Boehringer Mannheim). Protein concentrations were determined by using the BCA protein assay kit (Pierce). The CAT activity of the protein fraction was determined using the TNT binding assay. The amount of GST-JAB1 present in the supernatant was quantified by Laemmli-PAGE followed by Coomassie Blue staining. More than 50% of the solubilized fusion protein contained in the crude extract was finally bound to the affinity gel. The gel was stored in aliquots at −20°C.

1H- and 2H-radioabeled proteins were synthesized by transcription of pBluescript SK+ expressing of 2H-labeled proteins and subsequent translation using the TNT T7 coupled reticulocyte lysate system (Promega) as described by the manufacturer. Protein-protein interactions were studied as described (31) using binding buffer (20 mM Tris, 100 mM NaCl, 1 mM EDTA, 0.1% Nonidet P-40, pH 8.0). 5 µl of the in vitro-translated lysate were incubated with 1 µg of purified protein of interest. The mixture was centrifuged in a Spin-X ultracentrifuge at 100,000 × g for 2 h, and supernatants were collected. The supernatant was incubated in binding buffer or presence or absence of 1 µM R5020. Representative gels were stained with Coomassie Blue before being subjected to autoradiography to ensure that equal amounts of GST fusion proteins were included in each reaction. 35S radioactivity was quantified using the Instant Imager (Packard).

To study the formation of the ternary complex PR/SRC-1/JAB1, PR and SRC-1 were synthesized respectively as 1Hlucenine- and 3Hmethionine-labeled proteins, whereas JAB1 was unlabeled. Translation was stopped by adding buffer C (buffer B containing 10 mM EDTA, 0.1 mg/ml bovine serum albumin) and 50 µg/ml RNase A. After ultracentrifugation for 1 h at 100,000 × g (4°C), [3H]P or [35S]SRC-1 (10 µl of lysate), and various concentrations of JAB1 or control (lysate) (transcription-translation in presence of empty pP65 vector) were mixed together to a 50-µl final volume in buffer C and incubated overnight at 4°C. The complexes were then incubated with 1 µg of protein A-Sepharose antibodies and, after incubation for 2 h at 4°C, precipitated by rabbit anti-mouse IgG immunoglobulins (Sigma). Resins were washed in the absence of PR. The proteins of the resin were then performed. Non-specific absorption was then measured by immunoprecipitation with anti-PR antibody. These values were subtracted from the results obtained in presence of [3H]P.
RESULTS

Interaction between JAB1 and the Progesterone Receptor—

The amino acid sequence 897–930 of the PR contains helix 12 of its ligand-binding domain (LBD). This region has been shown to be of great importance in the transcription activation properties of nuclear receptors (43–47). We thus searched for proteins interacting with this region using it as a bait in a yeast two-hybrid screen. PR amino acids 897–930 were linked to Gal4 DNA-binding domain. Yeast cells were transformed with this plasmid and with a library of placental cDNAs linked to Gal4 activation domain (Gal4AD). Reporter genes responsive for Gal4 were present encoding a histidine synthesis enzyme (His3) and an adenine synthesis enzyme (Ade2).

Several cDNA sequences were isolated by their property to allow yeast cells growth in the presence of PR<sub>897–930</sub> and in medium lacking histidine and adenine. They were further studied with the help of a reporter gene encoding ß-galactosidase. Among these clones two corresponded to a fragment of JAB1 as shown by DNA sequencing. JAB1 was cloned in totality, and it was verified that it interacted with PR amino acids 897–930 in yeast cells (Fig. 1A).

It could be argued that the use of a short amino acid segment of a protein does not relate to the physiological situation because this segment might not be accessible in the full-length protein. We thus analyzed the interaction of JAB1 with the totality of PR cDNA-LBD (amino acids 847–930) using the two-hybrid methodology. PR cDNA-LBD by itself exerted a hormone-dependent transactivation; however, the latter was markedly enhanced by the addition of AD<sub>Gal4</sub>-JAB1 (Fig. 1B). This interaction between PR and JAB1 was only observed in the presence of hormone.

We then used the <i>in vitro</i> pull-down methodology and the mammalian two-hybrid system to establish that the JAB1<sub>PR</sub> interaction was not restricted to yeast. [<sup>35</sup>S]PR was synthesized <i>in vitro</i> and incubated with either the fusion protein GST-JAB1 or GST alone. Only the former retained a major fraction of the [<sup>35</sup>S]PR (Fig. 2). There was no clear effect of the hormone on this interaction. A limited (25%) effect of hormone was observed when using high ratios of [<sup>35</sup>S]PR versus GST-JAB1. Similar discrepancies in hormone dependence between <i>in vivo</i> experiments and <i>in vitro</i> pull-down experiments have been described for several coactivators and corepressors (13, 41, 48, 49). It is possible that PR synthesized <i>in vitro</i> is in the active conformation even in the absence of the hormone (13).
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**A**

![Graph showing relative CAT activity](image1)

**B**

![Graph showing relative CAT activity](image2)

**Fig. 3.** JAB1 interacts with PR in mammalian cells. The mammalian two-hybrid system was used to analyze the interaction between JAB1 and PR (A) or PR (B). A, CV-1 cells were transfected with vectors encoding the DNA-binding domain of Gal4 fused to JAB1 (ADgal4-JAB1) and the reporter plasmid pG5CAT (5 μg/ml) containing Gal4 upstream activating sequences. Nonfused ADgal4 or ADgal4 were used in control experiments. B, PR (PRwt) was replaced by PR (PRmut). The cells were treated with 10 nM R5020 (black bars), 10 nM RU486 (gray bars), or buffer only (empty bars). The reporter plasmid was pG5CAT. The results are shown as the means ± S.E. of three separate experiments.

The DBDGal4-PR927-990 construct was cotransfected along with the AdPr10 construct into CV-1 cells with the corresponding reporter gene. A low level of transcription was observed. When the ADPr10-JAB1 (activation domain) construct was cotransfected with the reporter gene, there was a slight increase of the basal level of transcription. However, when DBDGal4-PR927-990 and ADPr10-JAB1 were cotransfected with the reporter gene, a marked increase in CAT activity was observed, indicating an interaction between PR helix 12 and JAB1.

The experiment was repeated with DBDGal4-PR897-990 corresponding to the DBD-LBD domains of the receptor. A hormone-dependent transactivation of the reporter gene was observed with this construct (Fig. 3B). It was enhanced by cotransfection with ADPr10-JAB1. The latter interaction was hormone-dependent and was not observed in the absence of the agonist RU486.

**JAB1 Potentiates Nuclear Receptor-mediated Gene Transactivation**—Because JAB1 was shown to interact with PR, it was important to determine whether it modulated the biological activity of PR. We thus cotransfected cells with PR, a PR-driven reporter gene, and increasing amounts of JAB1. As shown in Fig. 4A, JAB1 markedly increased hormone-induced reporter gene transcription. The effect of JAB1 on PR-mediated transcription was strictly hormone-dependent because there was no effect of JAB1 in the absence of hormone nor in the presence of the antagonists RU486 or ZK82999.

JAB1 binds to PR LBD. A PR molecule devoid of its LBD is known to exert a constitutive activity (38). As expected, when such a modified PR molecule was cotransfected with JAB1, the latter did not potentiate its activity (Fig. 4B).

The effect of JAB1 on other nuclear receptors was then examined. As shown in Fig. 5, JAB1 potentiated the transactivation properties of most receptors. The strongest activity was exerted on steroid hormone receptors (glucocorticoid, mineralocorticoid, androgenic, and estrogenic receptors). Only limited activity was observed on thyroid hormone, vitamin D, and retinoic acid receptors.

**Effect of JAB1 on PR- and GR-mediated Inhibition of AP1 Activity**—Glucocorticoid and progesterone receptors are known to inhibit the activity of the AP1 complex (50–54). Because JAB1 binds to both of these transcription factors, its effect on this repressive activity was examined. As shown in Fig. 6, JAB1 enhanced phorbol ester (TPA) activity on the collagenase promoter. PR and GR incubated with the corresponding hormone inhibited TPA-induced transcription. Cotransfection with JAB1 only partially relieved this inhibition. The experiment was repeated with various concentrations of JAB1 (3–10 μg/ml) yielding similar results. JAB1 thus does not seem to play a major role in the PR or GR inhibitory effect on AP1 transactivation.

**JAB1 Interacts with SRC-1**—SRC-1 has been shown to be a common coactivator for nuclear receptors and AP1 (8). We thus considered the possibility that the effects of JAB1 on transactivation by both nuclear receptors and AP1 were mediated by SCR-1.
Fig. 5 JAB1 enhances the hormone-dependent transactivation by nuclear receptors. CV-1 cells were cotransfected with expression vectors encoding the different receptors (0.5 μg/ml), the corresponding reporter genes (5 μg/ml), and increasing amounts of pSG5-JAB1. The cells were treated with the corresponding hormone (●) or untreated (○). Plasmids encoding the human glucocorticoid receptor (pSG-hGR), the human androgen receptor (pSG-hAR), and the human mineralocorticoid receptor (pSG-MR) were cotransfected with the PRE2-TATA-CAT reporter gene in the presence or absence of dexamethasone (10 nM), dihydrotestosterone (50 nM), and aldosterone (50 nM), respectively. The plasmid encoding the human estrogen receptor (pKSV-mER) was cotransfected with the ERE-TATA-CAT reporter gene in the presence or absence of estradiol (10 nM). The plasmid encoding the mouse thyroid hormone receptor α (pKSV-mTRα1) was cotransfected with the triiodothyronine-RE-TK-CAT reporter gene in the presence or absence of triiodothyronine (10 nM). The plasmid encoding the human vitamin D receptor (pAM-VDR) was cotransfected with the VDRE-TK-CAT reporter gene (PUT-KAT3) in the presence or absence of 1,25-dihydroxycholecalciferol (100 nM). The plasmids encoding the retinoic acid receptors RARβ (pSG-RAR) (0.5 μg/ml) and hRXRα (0.5 μg/ml) were cotransfected with the RARE-TK-CAT reporter gene (AMTV-TRp-CAT) in the presence or absence of all-trans-retinoic acid (1 μM). The vectors encoding the receptors and the corresponding reporter genes are described either under "Experimental Procedures" or by Guiochon-Mantel et al. (31). CAT activities were normalized (1 = CAT activity in the presence of hormone and in the absence of JAB1). Similar results were obtained in three independent experiments.

Before testing this hypothesis directly, we examined the effect of JAB1 on another transcription factor also known to interact with SRC-1: NFXB (55, 56). As shown in Fig. 7, co-transfection with JAB1 strongly enhanced NFXB activity on a target gene.

The question was thus raised as to whether JAB1 directly contacted SRC-1 or rather that its activity was due to some indirect mechanism. To answer this question, initial experiments were performed with the two-hybrid system in yeast (Fig. 8A). The DBD domain of JAB1 construct alone enhanced reporter gene transcription to some extent. However, cotransfection of yeast cells with AD domain of SRC-1 provoked a markedly stronger transcription activation, demonstrating that an interaction can occur between JAB1 and SRC-1. Similar results were obtained using a mammalian two-hybrid system in CV-1 cells (Fig. 8B).

A pull-down experiment with the fusion protein GST-JAB1 and in vitro translated 35S-labeled SRC-1 confirmed this interaction (Fig. 9). This method was used to map the site of interaction on SRC-1 using a variety of deletion mutants (Fig. 9B). As shown in Fig. 9 (C and D), a major binding region for JAB1 was localized in the C-terminal part of SRC-1 (amino acids 1139–1440).

JAB1 Stabilizes the Interaction between PR and SRC-1—Because JAB1 interacts with both PR and SRC-1, it appeared possible that its mechanism of action on PR-mediated transactivation might result from stabilization of PR-SRC-1 complexes. To examine this possibility, we used a mammalian three-hybrid system. Cells were cotransfected with a reporter
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Fig. 6. Effect of JAB1 on PR and GR-mediated inhibition of AP1 activity. A, HeLa cells were cotransfected with the reporter plasmid p6Col-TK-CAT (2 μg/ml), the vector pSOS-PR (0.5 μg/ml) and pSOS-JAB1 (5 μg/ml). The cells were treated (+) or not (−) with R5020 (10 nM) and TPA (80 ng/ml). B, identical to A except that no receptor was transfected (GR is present in HeLa cells) and dexamethasone (10 nM) was used instead of R5020. CAT values were normalized (100% = CAT activity in the presence of TPA and in the absence of hormone and JAB1). Similar results were obtained in three separate experiments.

Fig. 7. JAB1 enhances NFκB-mediated transcription. CV-1 cells were cotransfected with the plasmid encoding the p65 Rel-A NFκB transcription factor (0.5 μg/ml), with the corresponding NFκB-CAT reporter plasmid (5 μg/ml), and with increasing concentrations of pSOS-JAB1. Similar results were obtained in three separate experiments.

gene driven by Gal4 upstream activation sequence and a DBDGal4-PR/PR expression vector (Fig. 10). Under the effect of the hormone, there was enhanced transcription from the reporter gene. If a vector encoding SRC-1-VP16 activation domain was also cotransfected, the interaction between PR and SRC-1 enhanced reporter gene transcription. Finally, addition of JAB1 further increased this transcription, showing a stabilization of PR-SRC-1 complexes.

To confirm this result we studied in vitro the stabilization of PR-SRC-1 complexes by JAB1 (Fig. 11). [3H]PR, [35S]SRC-1, and unlabelled JAB1 were translated separately in rabbit reticulocyte lysate. Complexes were preformed with fixed quantities of [3H]PR and [35S]SRC-1 and increasing concentrations of unlabelled JAB1. After immunoprecipitation with an anti-PR monoclonal antibody, the complexes were washed and counted for [3H] and [35S] radioactivity. There was a clear cut increase in SRC-1 coimmunoprecipitation with PR when increasing concentrations of JAB1 were added.

JAB1 Does Not Regulate PR or SRC-1 Concentration—Many members of the JAB1 family of proteins are present in proteasomes. It has been proposed that the activity of JAB1 is related to a disruption of proteasome activity and to the ensuing increased concentration of transcription regulatory factors (4). We thus examined the possibility that in the above experiment JAB1 was increasing target gene transcription by increasing either the concentration of the corresponding nuclear receptor or of SRC-1. Cells were cotransfected with JAB1 and PR or SRC-1. The cells were either treated by the agonist R5020 or the antagonist RU486 or left untreated. Western blots were performed using either anti-PR or anti-SRC-1 antibodies (Fig. 12).

As described previously (57), treatment by R5020 provoked a decrease of receptor migration (called upshift) that is related to receptor phosphorylation. A similar but less marked effect was observed after RU486 treatment. In all cases, cotransfection with JAB1 did not change receptor concentration (Fig. 12A). SRC-1 concentration was also independent of JAB1 overexpression (Fig. 12B).
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**DISCUSSION**

JAB1 has been shown to potentiate AP1-mediated transcription activation of its target genes (1). The mechanism of this effect remained unclear. It was proposed that JAB1 stabilized AP1-TRE (TPA-response element) complexes. However gel shift experiments failed to demonstrate the presence of DNA complexes containing both AP1 and JAB1. We show here that JAB1 contacts not only AP1 but also SRC-1 and may thus act by stabilizing their interaction. The activity of JAB1 is not restricted to AP1 but also applies to other transcription activation factors acting, at least in part, through SRC-1 and including nuclear receptors and NFkB.

JAB1 (molecular mass, 37.5 kDa) has very recently been shown to be a subunit of a large (~450 kDa) multiprotein complex called JAB1-containing signalsome (5) or COP9 signalsome (6). The signalsome has been characterized in plants (58) where it was initially called COP9 complex and in animals (5). It is apparently absent from the unicellular eukaryote *Saccharomyces cerevisiae*. The eight subunits of the signalsome have been cloned. They exhibit homology to components of the regulatory 19 S particle of the proteasome. The latter is composed of a base containing all the ATPases and of a lid (7, 59). All the proteins homologous to subunits of the signalsome are present in the lid. Moreover there is a one-to-one homology.
Fig. 10. JAB1 stabilizes PR-SRC-1 complexes in vitro. The effect of JAB1 on PR-SRC-1 complexes was analyzed by a three-hybrid system in CV-1 cells. The cells were cotransfected with the pG5CAT reporter plasmid (5 μg/ml), the vector encoding AD_{JAB1} SRC-1 (1 μg/ml), the vector encoding DBD-Gal4 fused to the PR (DBD-Gal4-PR (0.2 μg/ml), along with increasing concentrations of pSG5-JAB1 vector. The cells were incubated with R5020 (10 nM). In control experiments AD_{JAB1} SRC-1 was replaced by AD_{null} (○). The results are the means ± S.E. of three separate experiments.

Fig. 11. JAB1 stabilizes PR-SRC-1 complexes in vitro. [3H]PR, [35S]SRC-1, and unlabeled JAB1 were prepared by transcription-translation as described in the Methods chapter. Fixed concentrations of [3H]PR and [35S]SRC-1 were incubated with increasing concentrations of reticulocyte lysate containing unlabeled JAB1 (■). The complexes were immunoprecipitated with the anti-PR monoclonal antibody (L26) and anti-mouse IgG immunoglobulins. Washed pellets were counted for radioactivity. A control lysate without JAB1 (prepared by transcription translation of empty pSG5 vector) was also used (○). The results are expressed as cpm of immunoprecipitated [35S]SRC-1.

between subunits of the COP9 signalosome and components of the lid. This observation suggests that both structures have a common evolutionary origin and have diverged to assume different functions. The signalosome has been found to contain a kinase activity that phosphorylates c-Jun, inhibitor αB, and p105, the precursor of NFκB. Various functions have been described for the different subunits of the signalosome. Subunit 1, also called GPS1, inhibits JNK1 (Jun N-terminal kinase) and represses Jun-dependent promoter activity (60). Subunit 2 has independently been described as TRIP15 (thyroid hormone interacting protein 15) (61). It interacts with the ligand-binding domain of the thyroid hormone receptor and of the retinoic acid receptor RXR. This interaction is inhibited by the addition of ligand. The TRIP15 gene is highly homologous to the Drosophila alien gene, which is expressed in the muscle attachment sites during embryogenesis (62). It has recently been shown that alien is a corepressor of members of the nuclear receptor family (63). Subunit 5 of the signalosome is JAB1, whereas subunit 6 is VIP (human HIV-1 Vpr protein interacting protein), which is involved in the viral protein Vpr-induced cellular differentiation and growth arrest (64). Cells expressing antisense VIP are blocked in the G2-M phase of the cell cycle. Because the description of the signalosome is very recent and minor constituents may also associate with the particle, further description of other functions is highly probable. In the plant Arabidopsis thaliana the COP9 signalosome complex is involved in light-dependent morphogenesis (7, 58, 65).

Interestingly, whereas the lid of the 19 S proteasome particle and the signalosome have diverged structurally and functionally, other components of the proteasome system have evolved to assume functions often related to transcription regulation in addition to their function in proteolysis. Indeed TRIP1/SUG1, which is a component of the proteasome, interacts with the ligand-binding domain of nuclear receptors and potentiates their transcriptional activity (66, 67). MSS1 (mammalian suppressor of sv40) plays a role in HIV-1 Tat-mediated transcription (68, 69). E6-AP (E6-associated protein) is an ubiquitin-protein ligase involved in the Angelman syndrome. It is also a coactivator of the progesterone receptor (70). Ubiquitinylation may be involved in gene transcription regulation because histone-ubiquitin conjugates are concentrated in nucleosomes of transcribed genes (71, 72). JAB1 has recently been shown to interact with cyclin-dependent kinase inhibitory protein p27kip1, to provoke its translocation from the nucleus to the cytoplasm and to enhance its degradation (73).

JAB1 belongs to a new category of transcription regulators that act by bridging coactivators to receptors. This is also the case for cyclin D1, which binds both estrogen receptor and SRC-1 (74). However, cyclin D1 exerts this effect on both hormone-bound and unliganded estrogen receptor, whereas JAB1 interacts only with hormone-PR complexes.

Nuclear receptors, their coactivators and coregulators are present in large multimeric complexes (75). JAB1 is localized in signalosomes. It is thus possible that both types of multiprotein complexes cross-talk in the cells. Indeed larger structures, associating weakly or transiently with the COP9 signalosome, have been observed (7). It has been shown that the COP9 signalosome contains kinase activities that phosphorylate API and NFκB on sites overlapping with those implicated in transcriptional activation. Further work will be necessary to establish whether the signalosome plays a role in the phosphorylation of nuclear receptors and of their coactivators.

Acknowledgments—We are grateful to Dr. P. Legrain (Institut Pasteur Paris, France) for advice and technical help with the yeast two-hybrid system. We thank Dr. P. James (University of Wisconsin, Madison, USA) for the kind gift of the yeast strain P/69-4A. We also thank Dr. J. Berteloot (INSERM, Chatenay-Malabry, France) for the reporter

Fig. 12. Overexpression of JAB1 does not change the concentration of PR and SRC-1. A, CV-1 cells were transfected with pSG5-RPR (10 μg/ml) in the presence or absence of pSG5-HA-JAB1 (10 μg/ml). The cells were treated with either R5020 (10 nM) or R2486 (10 nM). The cells were harvested in Laemmli buffer, and aliquots were electrophoresed as described under “Experimental Procedures.” PR was revealed with the monoclonal 125I-labeled antibody. B, CV-1 cells were transfected with pSG5-RPR (5 μg/ml) in the presence or absence of pSG5-HA-JAB1 (10 μg/ml) and of pSG5-HA-SRC-1 (10 μg/ml). The cells were treated or not with R5020 (10 nM). The cells were harvested in RIPA buffer, and aliquots were electrophoresed as described under “Experimental Procedures.” SRC-1 was revealed with the monoclonal anti-HA antibody.
GENES: STRUCTURE AND REGULATION:
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J. Biol. Chem. 2000, 275:8540-8548.
doi: 10.1074/jbc.275.12.8540

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