An alternative model of H ferritin promoter transactivation by c-Jun

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c-Jun is a member of the activator protein 1 family, and its interaction with different nuclear factors generates a wide spectrum of complexes that regulate transcription of different promoters. H ferritin promoter transcription is tightly dependent on nuclear factor Y (NFY). Ferritin transcription is activated by c-Jun, although the promoter does not contain a canonical binding site. NFY, on the other hand, does not bind c-Jun *in vitro*, whereas *in vivo* c-Jun is found in the complex containing NFY. Moreover, a c-Jun–GCN4 chimaeric construct containing only the transactivation domain of Jun and the basic-region leucine-zipper domain of GCN4 stimulates the H ferritin promoter. A synthetic GAL4 promoter and the cognate activator,

the fusion protein NFY–GAL4, are potentially activated by c-Jun. Titration of p300 by co-expressing E1A abolishes the stimulatory effect. Moreover, another p300-dependent promoter, the cAMP-response element, can be superactivated by c-Jun using the same mechanism. These data indicate that c-Jun, when activated or overexpressed, is recruited to the H ferritin promoter by p300, which links NFY, bound to DNA, to the complex. These results add a new level of complexity to transcriptional regulation by c-Jun, which can activate p300-dependent promoters without binding directly to the target DNA.

Key words: CBP/p300, nuclear factor Y, transcription factors.

INTRODUCTION

Ferritin, the major iron storage protein, is composed of 24 subunits of two types, named H and L for heavy and light respectively [1]. The promoter of the gene coding for the H subunit consists of approx. 150 bp, upstream from the transcription start, in which are located two *cis*-elements [2]. The distal element (A box; at –132 bp from the transcription start) is a GC box and is recognized by Sp1. The proximal element (B box; at –62 bp from the transcription start) is an inverted CCAAT box and is recognized by a protein complex called the B-box binding factor (Bbf) [2]. Bbf is composed of the trimeric transcription factor nuclear factor Y (NFY), the co-activator molecule p300 and the histone acetylase p300/CBP-associated factor [pCAF; where CBP stands for cAMP-response element (CRE)-binding protein (CREB)-binding protein] [3–5].

We have recently reported that: (1) the NFY-B subunit binds, *in vivo*, the central domain of the co-activator CBP/p300; (2) cAMP signalling stimulates the formation of this complex, resulting in enhanced transcription [3,4]; and (3) the p300 N-terminus, in the Bbf complex, may interact with other transcription factors, recruiting them to the H ferritin promoter [3]. CBP/p300 is known to function as a general integrator of cellular metabolism that provides a link between transcription factors activated by exogenous stimuli and the basal transcriptional machinery. CBP/p300, in fact, interacts with several nuclear factors, such as c-Jun/v-Jun, c-Myc/v-Myc, CREB, nuclear hormone receptors, serum response factor

accessory protein 1A ('Sap-1A'), c-Fos and MyoD [6], inducing the transcription of the target genes.

In the present study we investigated the potential involvement of c-Jun in H ferritin gene transcription. c-Jun is a basic-region leucine-zipper (b-ZIP) DNA-binding protein and is a component of the activator protein 1 (AP-1) transcription factor complexes, together with other Jun proteins, or with members of the Fos and CREB families. We have found that c-Jun superactivates H ferritin transcription. c-Jun does not function as a typical AP-1 transactivator, since no canonical or spurious AP-1 sites are present in the H ferritin promoter. Moreover, a c-Jun–GCN4 chimaeric construct only bearing the c-Jun transactivation domain is still able to transactivate ferritin transcription.

In the present study we show that c-Jun is recruited to the H ferritin promoter by p300 without binding DNA. This novel mechanism appears to be general, since it also operates on a CRE-containing promoter.

EXPERIMENTAL

Cell culture

HeLa and 293A cells were cultured as monolayers in Dulbecco's modified Eagle's medium (DMEM; Life Technologies) supplemented with 10% (v/v) foetal calf serum (Sigma) and 100 units/l penicillin (HyClone, Logan, UT, U.S.A.). Cells were grown at 37 °C in a 5% CO₂ atmosphere. The A126 cell line, a derivative of PC12, defective in cAMP-induced transcription, was cultured

Abbreviations used: AP-1, activator protein 1; Bbf, B-box binding factor; b-ZIP, basic-region leucine-zipper; CAT, chloramphenicol acetyltransferase; CRE, cAMP-response element; CREB, CRE-binding protein; CBP, CREB-binding protein; DMEM, Dulbecco's modified Eagle's medium; JNK, c-Jun N-terminal kinase; NFY, nuclear factor Y; pCAF, p300/CBP-associated factor; RSV, rous sarcoma virus.

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in DMEM supplemented with 10 % (v/v) horse serum, 5 % (v/v) calf serum and 100 units/l penicillin.

DNA transfections and chloramphenicol acetyltransferase (CAT) assays

The H ferritin promoter/CAT constructs 5' HH/A, 5' HH/M, Bbf-TATAA and Bbf_{mut}-TATAA have been described previously [2,4]. The expression vectors for c-Jun, the c-Jun alanine mutant, GCN4, J1 and J4 were a gift from Dr S. Oliviero (Dipartimento di Biologia Molecolare, Università degli Studi di Siena via Fiorentina 1, Siena, Italy) [7]. The c-Jun N-terminal kinase (JNK) expression vector was kindly provided by Dr M. Santoro (Centro di Endocrinologia ed Oncologia Sperimentale del Consiglio Nazionale delle Ricerche, Naples, Italy) and the GAL4/NFY expression vectors were a gift from Professor R. Mantovani (Dipartimento di Biologia Animale, Università di Modena e Reggio, Modena, Italy). Transfections were carried out using the calcium-phosphate technique [8]. To complete the transfection mixture, the total amount of transfected DNA was taken up to 20 µg using pUC19 as carrier DNA. Variations in transfection efficiency were corrected by including the β-galactosidase gene under the control of the rous sarcoma virus (RSV) promoter in each experiment. Galactosidase activity was assayed as described previously [9].

Immunoprecipitations

HeLa-cell or 293A-cell total extract (1 mg) [3] was first incubated with 1 µg of anti-NFY antibody or with non-immune serum for 2 h at 4 °C, and then incubated for 1 h at 4 °C with Protein A–Sepharose. The beads were collected by centrifugation, washed four times with lysis buffer [3] and then loaded on to an SDS/12 % (w/v) polyacrylamide gel.

Western-blot analysis

Following electrophoresis, the proteins were transferred on to a nitrocellulose membrane. After addition of the blocking mixture, the membrane was incubated with a 1:5000 dilution of mouse anti-(c-Jun) antibody for 1 h at room temperature (18–20 °C). Bound antibody was detected using anti-mouse horseradish peroxidase-conjugated secondary antibody (1:5000 dilution) and ECL® (Amersham Biosciences).

Expression of recombinant proteins

NFY-A, -B and -C expression vectors were expressed and purified from the soluble fraction of *Escherichia coli* B121 (DE3), after induction with 0.4 mM isopropyl β-D-thiogalactoside for 2 h at 37 °C. Bacteria were lysed by sonication in lysis buffer [20 mM Hepes (pH 7.9), 30 mM KCl, 0.1 % Nonidet P40, 0.1 mM EDTA and 1 mM PMSF]. Following centrifugation, the supernatant was incubated with c-Jun recovered by affinity chromatography [10] and immunoprecipitated with anti-NFY antibody. The beads were collected by centrifugation, washed four times with the lysis buffer and then loaded on to an SDS/12 % (w/v) polyacrylamide gel.

RESULTS

The CCAAT element in the H ferritin promoter is the target of c-Jun

H ferritin promoter transcription is tightly dependent on the presence of the trimeric factor NFY. A synthetic promoter

Figure 1 c-Jun increases transcription of the H ferritin promoter

CAT activity driven by H ferritin promoter/CAT constructs [4], transiently transfected in HeLa cells with or without 4 µg of c-Jun expression vector (HH/M is the H ferritin promoter/CAT construct containing the TATAA box). The values are the means ± S.E.M. for three independent transfection experiments and are expressed as the percentage of the acetylated form of [¹⁴C]chloramphenicol.

bearing only the CCAAT sequence fused to a TATAA box is sufficient to drive the transcription of the downstream reporter [4]. Since *in vivo* ferritin transcription is activated by AP-1 stimulators [11], we wanted to know if c-Jun is able to trans-activate the promoter. Consequently, we transiently transfected HeLa cells with various H ferritin promoter/CAT constructs [4], either in the presence or absence of the c-Jun expression vector. Several constructs, from the 5' HH/A promoter (which contains the inverted CCAAT box at -62 and the TATAA box) to the minimal promoter element, carrying only a synthetic NFY site (CAAT) fused to the TATAA box (Bbf-TATAA), were used for these experiments. We also included in the transfections the mutated version of the NFY site (Bbf_{mut}-TATAA). The transfection efficiency was normalized by including the β-galactosidase gene under the control of the RSV promoter in each experiment. The results are shown in Figure 1. To rule out a potential competitive p300-binding effect of the E7 and E6 viral oncoproteins, expressed in HeLa cells, the same promoter constructs were also transfected in the human papillomavirus 18-negative A126 cell line, a derivative of PC12, which is defective in cAMP-induced transcription and does not differentiate in the presence of cAMP (results not shown).

The 5' HH/A plasmid was efficiently activated by c-Jun. This stimulatory effect was conserved in the minimal promoter carrying only the CAAT element fused to the TATAA box (Bbf-TATAA), whereas it was lost in the mutated version (Bbf_{mut}-TATAA). These data indicate that the target of c-Jun in the H ferritin promoter is the CAAT box.

The N-terminal region of c-Jun is essential for the induction of H ferritin transcription

To identify the protein domain of c-Jun responsible for the induction of ferritin transcription, we carried out domain-swapping experiments, by co-transfecting the 5' HH/A plasmid (as reporter plasmid) along with c-Jun–GCN4 chimaeric constructs. We used the J1 construct, which contains the GCN4 transactivation domain fused to the c-Jun b-ZIP region (corresponding to the C-terminus), and the J4 construct, in which the b-ZIP domain of GCN4 was fused to the N-terminus of c-Jun [7]. As controls, we used the full-length cDNAs for c-Jun and GCN4

Figure 2 The N-terminal region of c-Jun is essential to stimulate H ferritin transcription

(A) The top panel represents a schematic diagram of the structure of c-Jun-GCN4 fusion proteins. The bottom panel represents the CAT activity driven by the -100/+1 region of the H ferritin promoter (construct 5' HH/A) in HeLa cells co-transfected with 4 μ g of GCN4 expression vector and 4 μ g of c-Jun-GCN4 expression vector (J₁ or J₄). (B) CAT activity driven by the -100/+1 region of the H ferritin promoter (construct 5' HH/A) in HeLa cells co-transfected with 4 μ g of c-Jun, or with 4 μ g of c-Jun with the Ser⁶³ $\rightarrow$ Ala mutation (Jun/Ala) in the presence or absence of 4 μ g of JNK. The values are the means $\pm$ S.E.M. for three independent transfection experiments and are expressed as the percentage of the acetylated form of [¹⁴C]chloroamphenicol.

respectively. Figure 2(A) shows that only the J₄ construct was able to activate the transcription of the 5' HH/A reporter plasmid, thus indicating the functional role of the c-Jun N-terminus in enhancing ferritin transcription.

This region of c-Jun contains the transactivation domain, which contains the serine residues essential for the transcriptional activity of the oncoprotein [12]. To further validate the results obtained with the J₄ construct, we co-transfected the ferritin reporter plasmid along with a c-Jun construct in which Ser⁶³ was mutated to an alanine residue. Note that this residue is critical for the transcriptional activity of the protein [12]. Figure 2(B) shows that this mutant was ineffective in stimulating H ferritin transcription.

Ser⁶³, along with Ser⁷³, is phosphorylated by a specific kinase named JNK; following phosphorylation, the two residues are able to bind the co-activator p300, leading to enhancement of transcription of target genes. We wanted to determine whether the phosphorylation of Ser⁶³ might also play a role in the stimulation of transcription of the ferritin gene. To do this, we

Figure 3 NFY interacts with c-Jun *in vivo* but not *in vitro*

(A) His-tagged c-Jun was expressed in HeLa cells, recovered by affinity chromatography (lanes 1) and mixed with NFY expressed in *E. coli* (lanes 2 and 3). After incubation, the protein complexes were analysed by Western blotting with the anti-c-Jun antibody (lanes 1 and 2) or with the anti-NFY antibody (lane 3). (B) HeLa and 293A cells were transfected with the eukaryotic c-Jun expression plasmid (5 μ g), and with NFY-A, NFY-B and NFY-C expression vectors (1 μ g of each). Total HeLa and 293A cell extracts (CE; 1 mg) were immunoprecipitated with anti-NFY antibody (IP NFY; lanes 3 and 5 respectively) or with non-immune antiserum (IP SN; lane 2). Following SDS/PAGE and electroblotting, the membrane containing immobilized immunocomplex was analysed by Western blotting using anti-c-Jun antibody. Total HeLa and 293A extracts (50 μ g) were loaded as controls for the presence of c-Jun (lanes 1 and 4 respectively).

mutated Ser⁶³ to alanine and found that, under these conditions, the H ferritin promoter was not activated. On the other hand, the addition of an expression vector for JNK to the transfection mixture containing c-Jun and the ferritin construct resulted in a significant potentiation of CAT activity, which was abolished by the serine-to-alanine substitution at position 63 of c-Jun.

c-Jun is recruited by p300 to the transcriptional complex formed on the CCAAT box of the H ferritin promoter

The ferritin CCAAT element is recognized by a multiprotein complex, which we named Bbf. This multiprotein complex contains: (1) the histone acetylase pCAF [5]; (2) the co-activator molecule p300 [3,4]; and (3) the CAAT-binding protein NFY, which directly contacts the DNA [3]. We first asked

Figure 4 c-Jun requires p300 to transactivate the H ferritin promoter

CAT activity driven by the Bbf-TATAA construct in HeLa cells co-transfected with 4 μ g of c-Jun, 7 μ g of p300, 3 μ g of E1A and 7 μ g of p300del20 expression vectors. The values are the means $\pm$ S.E.M. for three independent transfection experiments and are expressed as the percentage of the acetylated form of [14 C]chloroamphenicol.

whether c-Jun interacts directly with the CAAT sequence. Recombinant c-Jun did not bind the synthetic oligonucleotide corresponding to the NFY recognition sequence on the H ferritin promoter. Under the same conditions, c-Jun was able to bind the thyroid hormone response element (results not shown).

We also determined whether c-Jun interacts with the NFY complex. To this end we expressed epitope-tagged c-Jun in HeLa cells, and purified the protein by affinity chromatography [10]. The NFY-A, -B and -C subunits were expressed in *E. coli*, recovered and then incubated with the affinity-purified c-Jun. The incubation mixture was immunoprecipitated for NFY-A, loaded on to an SDS/polyacrylamide gel and immunoblotted for c-Jun. As shown in Figure 3(A), c-Jun was not present in the NFY complex (lane 2). Lanes 1 and 3 were the controls showing the expression of c-Jun and NFY-A in HeLa cells and *E. coli* respectively. NFY-B and -C subunits were also expressed in *E. coli* (results not shown).

The NFY-c-Jun interaction was also probed by using c-Jun and NFY subunits expressed in HeLa cells. After transient transfection of c-Jun and NFY expression vectors, cells were recovered and total protein extract was prepared. The protein extract was immunoprecipitated with the NFY-A antibody, loaded on to an SDS/polyacrylamide gel and probed for c-Jun. The results, shown in Figure 3(B), indicated the presence of c-Jun in the complex immunoprecipitated with the NFY antibody (lane 3). c-Jun was present in the total extract from HeLa transfected cells (lane 1); lane 2 represents the control with non-immune serum. The expression of the NFY-B and -C subunits was also checked (results not shown). These findings were confirmed by repeating the co-precipitation assay in the 293A cell line, which constitutively express E1A. In the presence of this protein, which efficiently binds to p300, the anti-NFY antibody does not co-precipitate c-Jun (lane 5).

The discrepancy between *in vivo* and *in vitro* results may be explained by the absence of a critical molecule present *in vivo* but not *in vitro*. Since p300 binds NFY [3] and c-Jun [6], we tested whether p300 in the extracts of HeLa cells might function as a bridge between c-Jun [6] and NFY [3]. To determine if p300 recruits c-Jun on to the H ferritin promoter, we expressed c-Jun

Figure 5 c-Jun activates transcription of other p300-dependent promoters

(A) CAT activity driven by a GAL4 promoter (3 μ g) in HeLa cells (GAL4) co-transfected with 3 μ g of the NFY/GAL4 expression vector (NFY/GAL4), 4 μ g of the c-Jun expression vector (c-Jun), 7 μ g of the p300-expression vector (p300) and 3 μ g of the E1A expression vector (E1A). (B) CAT activity driven by a CRE construct in HeLa cells (CRE-CAT), co-transfected with 4 μ g of the c-Jun expression vector (c-Jun), 7 μ g of the p300-expression vector (p300) and 3 μ g of the E1A expression vector (E1A). The values are the means $\pm$ S.E.M. for three independent transfection experiments and are expressed as the percentage of the acetylated form of [14 C]chloroamphenicol.

in the presence of the oncoprotein E1A, and tested its ability to activate H ferritin transcription. Under these conditions, E1A titrates p300 from the NFY complex assembled at the -62 position on the H ferritin promoter and down-regulates the promoter efficiency [4]. HeLa cells were transiently transfected with the Bbf-TATAA plasmid as the reporter plasmid and with c-Jun, or with c-Jun and E1A expression plasmids. The results of the CAT assay are reported in Figure 4. In the presence of E1A, c-Jun was no longer able to activate transcription driven by the -62 element. c-Jun transactivation activity was restored only when the p300-expressing plasmid was added to the transfection mixture (Figure 4). As a control, the p300del20 expression vector, which encodes for a protein that is unable to bind E1A, was transfected in HeLa cells. Under these conditions, the H ferritin promoter was efficiently transactivated by c-Jun. Taken together, these results indicate that c-Jun requires the presence of the co-activator p300 to stimulate the NFY-dependent transcription driven by the H ferritin promoter. Analogous results were obtained by using another member of the Jun family,

namely Jun-D. A mutant isoform of this protein, lacking the p300-binding region, was unable to transactivate Bbf-TATAA (results not shown).

c-Jun induces the transcription of other p300-dependent promoters

p300 forms a multimeric complex with a variety of nuclear factors, inducing the transcription of target genes [6]. The data shown above suggest that c-Jun, when overexpressed, binds p300 and is recruited on to the H ferritin promoter, where it acts as a transcriptional activator. One implication of these findings is that p300 can recruit c-Jun to promoters that do not contain AP-1 binding sequences. We tested this prediction by transfecting the GAL4 promoter along with three fusion proteins carrying the GAL4 binding domain and the transactivation domain of NFY-A, -B or -C. HeLa cells were transfected with these constructs and a GAL4 promoter/CAT reporter. The activity of the GAL4 reporter plasmid was tested in the presence of c-Jun, E1A and p300 expression vectors in different combinations. Under these conditions, GAL4 transcription was dependent on the simultaneous expression of the three proteins. The CAT activity driven by the NFY-dependent GAL4 promoter was significantly stimulated by c-Jun. E1A expression reduced the activity of the reporter plasmid to the basal level (Figure 5). c-Jun transactivation of the NFY/GAL4 plasmid was fully restored in the presence of the p300-expressing vector (Figure 5A).

Next, we asked whether p300 is able to recruit c-Jun on to promoters regulated by transcription factors different from NFY. To do this we transiently transfected HeLa cells with the CRE/CAT construct, in which the transcription of the CAT gene is driven by the CRE derived from the somatostatin promoter [13]. The CRE/CAT construct was expressed in the presence of c-Jun and p300. The results, shown in Figure 5(B), demonstrate that c-Jun strongly activates the CRE/CAT construct. The addition of E1A to the transfection mixture inhibited the c-Jun stimulatory effect. This effect was reversed by the concomitant transfection of p300.

DISCUSSION

In the present study we have found that c-Jun acts as a positive regulator of the transcription driven by the minimal promoter element of the human H ferritin gene. Usually, c-Jun dimerizes with other Jun proteins or with members of the Fos and CREB families to create the AP-1 transcription factors [14,15]. AP-1 recognizes a DNA element, the AP-1 site, which only differs by one base from the CRE site. CRE and AP-1 sites are absent in the H ferritin promoter region stimulated by c-Jun.

An intriguing consequence of the above results is that c-Jun may be recruited to the target promoter by interactions with transcription factors already bound to the H ferritin promoter. The ferritin CCAAT element is recognized by the transcription factor NFY, composed of A, B and C subunits [3]. The NFY-B and -C subunits heterodimerize through their histone-fold motif and create the functional DNA binding complex, since subunit A only associates with the B/C heterodimer [16]. In the present study we provide evidence that c-Jun, when overexpressed, is able to potentiate the transcription of the H ferritin promoter without binding directly to the DNA, but through the NFY-p300 complex. Furthermore, we show that the protein p300 is the link between c-Jun and NFY, therefore acting as an 'anchor' molecule.

Our model does not appear to be in conflict with the general model of transcriptional regulation via direct binding of different factors to specific regions of the DNA.

In fact, we hypothesize that only under specific conditions, in which c-Jun expression is dramatically increased (e.g. physical, chemical and mechanical stress), this oncoprotein might exert an indirect transcriptional modulation without binding to AP-1 sites.

One possibility still open is that the interaction of c-Jun with p300 improves the binding of c-Jun to low affinity sites present in the minimal H ferritin promoter. However, this hypothesis is unlikely, since c-Jun induces the transcription of the two synthetic constructs GAL4/CAT and CRE/CAT. Thus p300 is able to recruit c-Jun on to different promoters, where it acts as a co-activator molecule in enhancing transcription.

Our results complement two earlier reports showing the role of c-Jun in inducing transcription of target genes via physical and functional interaction with transcription factors Sp1 and PU.1 respectively [17,18]. In both cases c-Jun does not bind to target sequences on the DNA.

Taken together, these results add a new level of complexity to the regulation of transcription by c-Jun and AP-1 in general. A large number of stimuli induce c-Jun transcription. UV irradiation, for example, is a very potent inducer of c-Jun transcription. Moreover, in response to UV irradiation, Ser⁶³ and Ser⁷³ are phosphorylated, resulting in enhanced c-Jun transcriptional activity. Tyrosine kinase receptors, stress and other conditions that activate the transcriptional ability of c-Jun can be targeted to several transcription units by the transcriptional activator p300, thereby dramatically increasing the number and type of expressed genes.

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REFERENCES

- 1 Arosio, P., Adelman, T. G. and Drysdale, J. W. (1978) On ferritin heterogeneity. *J. Biol. Chem.* **253**, 4451–4458
- 2 Bevilacqua, M. A., Giordano, M., D'Agostino, P., Santoro, C., Cimino, F. and Costanzo, F. (1992) Promoter for the human ferritin heavy chain-encoding gene (FERH): structural and functional characterization. *Gene* **111**, 255–260
- 3 Faniello, M. C., Bevilacqua, M. A., Condorelli, G., de Crombrughe, B., Maity, S. N., Avvedimento, V. E., Cimino, F. and Costanzo, F. (1999) The B subunit of the CAAT-binding factor NFY binds the central segment of the co-activator p300. *J. Biol. Chem.* **274**, 7623–7626
- 4 Bevilacqua, M. A., Faniello, M. C., Quaresima, B., Tiano, M. T., Giuliano, P., Feliciello, A., Avvedimento, V. E., Cimino, F. and Costanzo, F. (1997) A common mechanism underlying the E1A repression and the cAMP stimulation of the H ferritin transcription. *J. Biol. Chem.* **272**, 20736–20741
- 5 Bevilacqua, M. A., Faniello, M. C., Russo, T., Cimino, F. and Costanzo, F. (1998) pCAF/p300 complex binds the promoter for the heavy subunit of ferritin and contributes to its tissue-specific expression. *Biochem. J.* **335**, 521–525
- 6 Janknecht, R. and Hunter, T. (1996) A growing coactivator network. *Nature (London)* **383**, 22–23
- 7 Oliviero, S., Robinson, G. S., Struhl, K. and Spiegelman, B. M. (1992) Yeast GCN4 as a probe for oncogenesis by AP-1 transcription factors: transcriptional activation through AP-1 sites is not sufficient for cellular transformation. *Genes Dev.* **6**, 1799–1809
- 8 Graham, F. and van der Eb, A. J. (1973) A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* **52**, 456–467
- 9 Oliner, J. D., Pietsen, J. A., Thiagalingam, S., Gyuris, J., Kinzler, K. W. and Vogelstein, B. (1993) Oncoprotein MDM2 conceals the activation domain of tumour suppressor p53. *Nature (London)* **362**, 857–860
- 10 Papavassiliou, A. G., Treier, M. and Bohmann, D. (1995) Intramolecular signal transduction in c-Jun. *EMBO J.* **14**, 2014–2019
- 11 Wang, Y., Gong, B., Dai, W. and Lu, L. (1998) Identification of immediate early genes during TPA-induced human myeloblastic leukemia ML-1 cell differentiation. *Gene* **31**, 293–302

- 12 Bannister, A. J., Oehler, T., Wilhelm, D., Angel, P. and Kouzarides, T. (1995) Stimulation of c-Jun activity by CBP: c-Jun residues Ser63/73 are required for CBP induced stimulation *in vivo* and CBP binding *in vitro*. *Oncogene* **11**, 2509–2514
- 13 Sassone Corsi, P., Visvader, J., Ferland, L., Mellon, P. L. and Verma, I. M. (1988) Induction of proto-oncogene fos transcription through the adenylate cyclase pathway: characterization of a cAMP-responsive element. *Genes Dev.* **2**, 1529–1538
- 14 Rauscher, F. J., Voulalas, P. J., Franza, B. R. and Curran, T. (1988) Fos and Jun bind cooperatively to the AP-1 site: reconstitution *in vitro*. *Genes Dev.* **2**, 1687–1699
- 15 Benbrook, D. M. and Jones, N. C. (1990) Heterodimer formation between CREB and JUN proteins. *Oncogene* **5**, 295–302
- 16 Sinha, S., Maity, S. N., Lu, J. and de Crombrughe, B. (1995) Recombinant rat CBF-C, the third subunit of CBF/NFY, allows formation of a protein–DNA complex with CBF-A and CBF-B and with yeast HAP2 and HAP3. *Proc. Natl. Acad. Sci. U.S.A.* **92**, 1624–1628
- 17 Behre, G., Whitmarsh, A. J., Coghlan, M. P., Hoang, T., Carpenter, T., Zhang, D. E., Davis, R. J. and Tenen, D. G. (1999) c-Jun is a JNK-independent coactivator of the PU.1 transcription factor. *J. Biol. Chem.* **274**, 4939–4946
- 18 Kardassis, D., Papakosta, P., Pardali, K. and Moustakas, A. (1999) c-Jun transactivates the promoter of the human p21^{WAF1/Cip1} gene by acting as a superactivator of the ubiquitous transcription factor Sp1. *J. Biol. Chem.* **274**, 29572–29581

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