AP-1: A DOUBLE-EDGED SWORD IN TUMORIGENESIS

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The AP-1 transcription factor is a dimeric complex that contains members of the JUN, FOS, ATF and MAF protein families. AP-1 proteins are primarily considered to be oncogenic, but recent studies have challenged this view — some AP-1 proteins, such as JUNB and c-FOS, have been shown to have tumour-suppressor activity. Here, we focus on the JUN and FOS proteins and aim to offer a new perspective on the molecular mechanisms that regulate the oncogenic and anti-oncogenic effects of AP-1 in tumour development.

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The AP-1 (activator protein 1) transcription factor is a dimeric complex that comprises members of the JUN, FOS, ATF (activating transcription factor) and MAF (musculoaponeurotic fibrosarcoma) protein families. The AP-1 complex can therefore form many different combinations of heterodimers and homodimers, and this combination determines the genes that are regulated by AP-1 (REFS 1–3; FIG. 1).

AP-1 proteins are known as basic leucine-zipper (bZIP) proteins because they dimerize through a leucine-zipper motif and contain a basic domain for interaction with the DNA backbone[1] (FIG. 1). The main AP-1 proteins in mammalian cells are FOS and JUN, and JUN has a yeast homologue, the Gcn4 protein, that can also form dimers to regulate gene expression — in this case, that of enzymes involved in amino-acid synthesis. Gcn4 and AP-1 recognize the same DNA response element, the TPA-responsive element (TRE) — so called because it is strongly induced by the tumour promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA). This element was first identified in the promoter and enhancer elements of the metallothionein I gene and simian virus 40 (SV40)[4]. In addition to tumour promoters, the DNA binding of the AP-1 complex to the TRE sequence is rapidly induced by growth factors, cytokines and oncoproteins, which are implicated in the proliferation, survival, differentiation and transformation of cells[4].

AP-1 function in tumorigenesis

FOS and JUN proteins were first identified as the viral oncoproteins v-Fos and v-Jun in the Finkel–Biskis–Jinkins osteosarcoma virus and avian sarcoma virus 17, respectively[3] (BOX 1). When the cellular counterparts of the viral oncoproteins were discovered, the upregulation of AP-1 proteins by overexpression or by oncogenic RAS was found to correlate with a positive effect on cell transformation. Other JUN (JUNB and JUND) and FOS (FOSB, FRA1 and FRA2) proteins have since been identified, all of which are components of AP-1. The functions of the individual AP-1 proteins in normal development have been elucidated using genetically modified mice[4] (TABLE 1).

Several of the AP-1 proteins — such as c-FOS, FOSB and c-JUN — can transform cells efficiently in culture, and these all have potent transactivation domains[5], which were identified by their ability to induce target-gene transcription. When widely overexpressed in mice, c-Fos causes osteosarcoma formation by the transformation of chondroblasts and osteoblasts, which identifies these two cell types as cellular targets of c-Fos-induced tumorigenesis[6,7] (FIG. 2a). c-Jun is more important in the development of skin and liver tumours, as reducing c-Jun/AP-1 activity using a dominant-negative c-Jun (TAM67) in basal keratinocytes or conditional inactivation of c-Jun in the liver interferes with the development of chemically induced papillomas and liver tumours, respectively[8,9] (FIG. 2b, c). By contrast, AP-1 proteins that lack potent transactivation domains have either a weak transforming activity (for example, Fra1 and Fra2 (REFS 10,11)) or no transforming activity (for example, JunB and JunD[12]). Overexpression of Fra1 and Fra2 in transgenic

Summary

- AP-1 is a dimeric transcription factor that contains members from the JUN, FOS, ATF and MAF protein families.
- AP-1 activity can be regulated by dimer composition, transcription, post-translational modification and interactions with other proteins.
- Two of the components of AP-1 — c-JUN and c-FOS — were first identified as viral oncoproteins, so their role in tumorigenesis is well established.
- However, some JUN and FOS family proteins can suppress tumour formation. The decision as to whether AP-1 is oncogenic or anti-oncogenic depends on the cell type and its differentiation state, tumour stage and the genetic background of the tumour.
- AP-1 can exert its oncogenic or anti-oncogenic effects by regulating genes involved in cell proliferation, differentiation, apoptosis, angiogenesis and tumour invasion.
- AP-1 might be a good target for anticancer therapy.

mice leads to the development of lung tumours and epithelial tumours, respectively[13] (TABLE 1). Although the mechanism of Fra-induced tumorigenesis is unclear, it is possible that dimerization of Fra1 and Fra2 with AP-1 proteins that have an intact transactivation domain is required.

AP-1 in tumour suppression

Not only do some JUN and FOS proteins lack transforming activity, but some can also suppress tumorigenesis[14]. Whereas c-JUN is oncogenic, JUNB and JUND can have anti-oncogenic effects. The c-JUN/JUNB antagonism in oncogenic transformation was first described in rodent fibroblasts *in vitro*[15]. The anti-oncogenic activity of JunB was confirmed recently *in vivo* using JunB-deficient mice that carry a *JunB* transgene. The transgene rescued the embryonic lethality of JunB-deficient fetuses, but its expression was epigenetically silenced in cells of the myeloid lineage[16]. This resulted in progressive myeloid leukaemia, with increased proliferation of granulocytic progenitor cells[16] (FIG. 2d). Conditional inactivation of JunB in the epidermis of c-*Jun*$^{+/-}$ mice also promotes spontaneous papilloma formation, which indicates that JunB has tumour-suppressor activity in several tissues[17] (R. Zenz and E. F. W., unpublished observations).

In contrast to JUNB, the anti-oncogenic activity of JUND is less well defined. Some studies have shown that JUND is a negative regulator of cell proliferation[18] (A. Meixner and E. F. W., unpublished observations). Interestingly, mice that lack JunD are viable and do not form tumours spontaneously[19]. Inhibition of JunD by direct interaction with the tumour suppressor menin might be involved in the suppression of neoplastic growth of some tumours, which indicates that JunD can also function as an oncoprotein[20].

The decision as to whether AP-1 is oncogenic or anti-oncogenic might depend on the antagonistic activity of different JUN proteins, but it is probably also influenced by tumour type, tumour stage and the genetic background of tumours. For example, deletion of c-*Fos* in *Trp53*-null mice results in the formation of RHABDOMYOSARCOMAS with high penetrance. This tumour type is rarely seen in *Trp53*-null mice, which indicates that even '*bona fide*' oncogene products such as c-Fos can function anti-oncogenically. Preliminary data suggest that c-Fos can function anti-oncogenically in combination with p53 by regulating apoptosis-inducing genes or by suppressing survival genes (A. Fleischmann and E. F. W., unpublished observations).

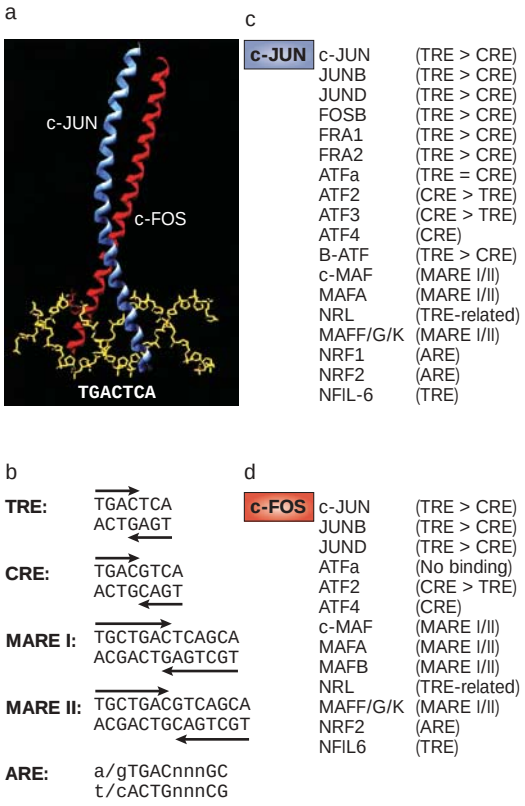

Figure 1 | **The AP-1 transcription factor.** AP-1 (activator protein 1) proteins include the JUN, FOS, ATF (activating transcription factor) and MAF (musculoaponeurotic fibrosarcoma) protein families, which can form homodimers and heterodimers through their leucine-zipper domains. The different dimer combinations recognize different sequence elements in the promoters and enhancers of target genes. **a** | The leucine-zipper domain and the adjacent basic domain form an X-shaped α-helical structure in the AP-1 complex, which binds to the DNA backbone[75]. **b** | The main DNA response-element is the TPA-responsive element (TRE), but different dimers preferentially bind to elements such as the cAMP-RESPONSE ELEMENT (CRE), the MAF-recognition elements (MAREs) and the antioxidant-response elements (AREs). **c** | c-JUN binding partners and the response elements that the dimers bind. **d** | c-FOS binding partners and the response elements that the dimers bind. NFIL-6, nuclear factor interleukin-6; NRF, nuclear respiratory factor; NRL, neural retina leucine zipper. **a** is modified with permission from REF. 2 (2001) © Nature Publishing Group.

Specific functions of AP-1

The pro-oncogenic and anti-oncogenic activities of AP-1 are manifested through their effects on several 'hallmarks' of cancer[21].

RHABDOMYOSARCOMA
A common soft-tissue sarcoma of childhood, which arises from rhabdomyoblasts (primitive muscle cells). As rhabdomyoblasts are located throughout the body, the tumours can arise at numerous locations, including around the eyes, in the genitourinary tract, at the extremities and in the chest and lungs.

cAMP-RESPONSE ELEMENT
This sequence element is present in the promoters and enhancers of genes that are inducible by cyclic AMP. It is bound by two classes of transcription factors — CREB (CRE-binding) proteins and AP-1 proteins.

Box 1 | Comparison between mouse c-Jun and c-Fos and their viral counterparts v-Jun and v-Fos

Jun. The protein encoded by the avian sarcoma virus 17 oncogene v-*Jun* shows increased transforming activity compared with c-Jun, its normal cellular counterpart. v-Jun differs from c-Jun in three important ways that might explain its transforming potential. One determinant is an in-frame deletion of the delta domain, which is near the amino terminus of the protein. As a consequence of this deletion, v-Jun does not require Jnk (Jun amino-terminal kinase) signalling for its activation, which is believed to underlie the oncogenic effects of the delta-domain deletion. However, a recent study has questioned this hypothesis and indicates that disruption of the Jun–Jnk interaction is not the mechanism by which the delta-domain deletion enhances transforming activity[74]. The two other differences are single amino-acid substitutions that change a phosphorylation site that is recognized by the kinases glycogen-synthase-kinase-3β (Gsk-3β), casein kinase II (CkII) and Erk (extracellular-signal-regulated kinase) (Ser 247 in c-Jun is equivalent to Phe 199 in v-Jun) and a site that is recognized by the redox factor Ref1 (Cys 273 in c-Jun is equivalent to Ser 225 in v-Jun).

Fos. The main difference between the protein encoded by the Finkel–Biskis–Jinkins osteosarcoma virus oncogene v-*Fos* and that encoded by its cellular counterpart c-*Fos* is a 104-base-pair deletion in the carboxyl terminus of the v-Fos protein. As a consequence, a different codon usage leads to completely unrelated sequences in the carboxyl termini of the two proteins and ablates two phosphorylation sites for Erk and Rsk2 (ribosomal S6 kinase 2) as well as an Erk-docking site in v-Fos (FTYP; the DEF domain).

The mechanisms that have increased the oncogenic potential of v-Jun and v-Fos during evolution are mainly unknown. It seems that a common principle that underlies oncogenic mutations is to escape regulation by kinases or other modifying enzymes, thereby leading to constitutive activity.

DNA-PK, DNA-dependent protein kinase; Frk, Fos-related kinase; Pka, protein kinase A; Pkc, protein kinase C.

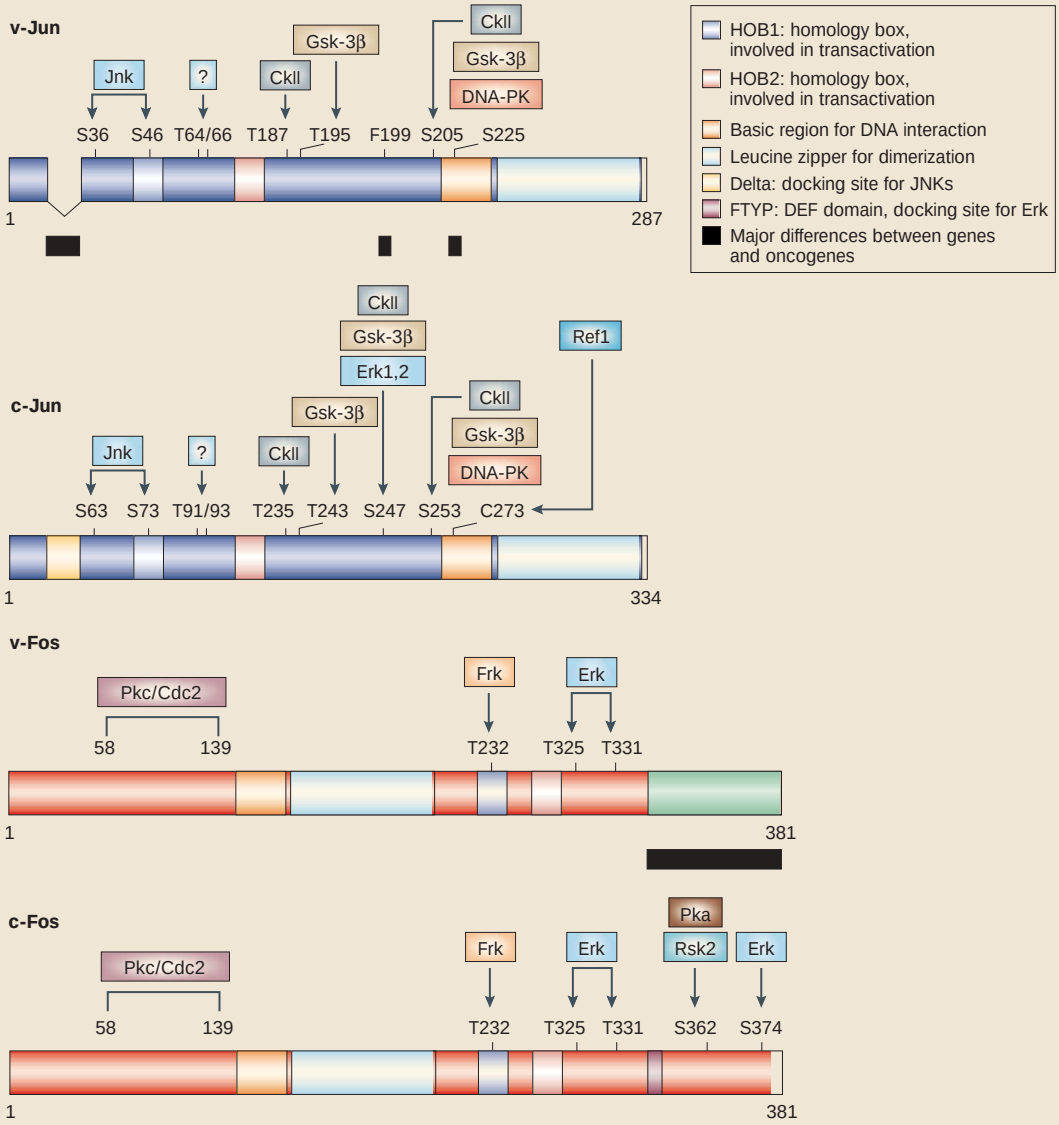

Table 1 | Functions of Jun and Fos proteins in mice

Genetic manipulation	Phenotype	Organs and cell types affected
Knockout		
c-Jun	Embryonic lethality at E12.5	Liver, heart, hepatoblasts, neural crest
JunB	Embryonic lethality at E10	Extraembryonic tissue, placenta
JunD	Male sterility	Testis, spermatids
c-Fos	Osteopetrosis	Bone, osteoclasts
FosB	Nurturing defect	Brain, hypothalamus
Fra1	Embryonic lethality at E9.5	Extraembryonic tissue, placenta
*Fra2**	Lethal at birth	Bone, heart, gut
Knock-in		
JunB for *c-Jun*	Rescues embryonic lethality of *c-Jun* knockout until birth	Anterior eye structure, heart
JunD for *c-Jun**	Rescues embryonic lethality of *c-Jun* knockout until birth	Anterior eye structure, heart
Fra1 for *c-Fos*	Rescues osteopetrosis of c-*Fos* knockout	None
Promoter–transgene		
H2Kb–c-Jun	None	None
UbC–JunB	None	None
CD4–JunB	Enhanced T-helper 2 cell maturation	Thymus, CD4 thymocytes
*UbC–JunD**	Peripheral T and B cells reduced	Lymphocytes, colon
H2Kb–c-Fos	Osteosarcoma	Bone, osteoblasts
H2Kb–FosB	None	None
Tcrb–ΔFosB	Impaired T-cell differentiaton	Thymus, immature thymocytes
NSE–ΔFosB	Osteosclerosis	Bone, osteoblasts
H2Kb–Fra1	Osteosclerosis	Bone, osteoblasts
CMV–Fra2	Ocular malformations	Anterior eye structure
*H2Kb–Fra2**	Tumour formation	Pancreas, thymus, lung

Surprisingly, the substitution of c-Jun with JunB rescued the embryonic lethality of the *c-Jun* knockout mice[73], revealing overlapping functions between c-Jun and JunB during development despite the c-Jun/JunB antagonism described for cell proliferation and cell transformation. Gain-of-function experiments have been carried out with different promoters, which leads either to ubiquitous expression (for example, with the *H2Kb*, ubiquitin C (*UbC*) and cytomegalovirus (CMV) promoters) or to tissue-specific expression (for example, with the *CD4*, *Tcrb* (T-cell-receptor-β) and *NSE* (neurone-specific enolase) promoters) of the transgenes. See REF. 5 for original data. *Unpublished observations.

KAINATE
This neuroexcitotoxin has been used to model the aetiology of several neurodegenerative disorders. Kainate binds to kainate receptors — a class of ionotropic glutamate receptors — which mediate excitatory synaptic transmission through ligand-induced opening of transmembrane ion channels. Activation of these receptors by kainate can induce neuronal apoptosis, which occurs preferentially in the hippocampal region.

Tumour-cell proliferation. The regulation of cell proliferation by AP-1 might be of crucial importance for the multi-stage development of tumours[22,23]. However, AP-1 does not always promote cell proliferation — it also has anti-proliferative activities. The limiting components that guide the decision seem to be the JUN proteins, with the FOS proteins having little effect[24].

c-JUN is primarily a positive regulator of cell proliferation, as c-JUN-deficient fibroblasts have a marked proliferation defect *in vitro*[25,26] and the proliferation of c-Jun-deficient hepatocytes is severely impaired during liver regeneration *in vivo*[27]. To fully promote cell-cycle progression, the c-JUN protein has to be activated by JUN amino-terminal kinases (JNKs)[28]. Subsequently, the activated c-JUN-containing AP-1 complex induces the transcription of positive regulators of cell-cycle progression — such as cyclin D1 — or represses negative regulators — such as the tumour suppressor p53 and the cyclin-dependent-kinase inhibitor INK4A (also known as p16; REFS 25,299; TABLE 2).

JUNB and JUND are often considered to be negative regulators of cell proliferation. Fibroblasts derived from mice overexpressing JunB show reduced proliferation, whereas JunD-deficient immortalized fibroblasts show increased proliferation[30,31]. However, primary JunD-deficient fibroblasts also show reduced proliferation[31], which indicates that JunD can both positively and negatively regulate cell-cycle progression, depending on the cellular context. JUNB and JUND can change the c-JUN-mediated activation or repression of crucial regulators of cell-cycle progression[18] (TABLE 2).

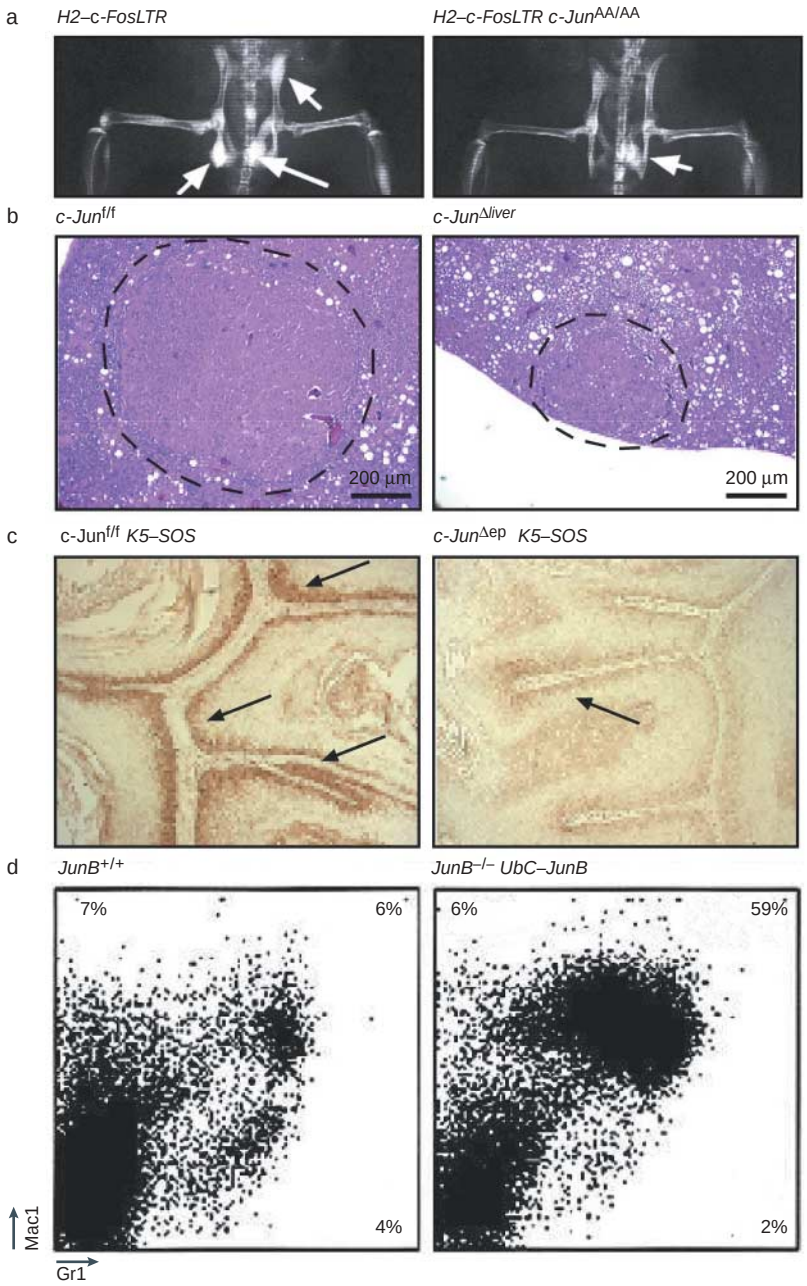

Figure 2 | **Opposing functions of AP-1 in tumorigenesis. a** | Ectopic expression of c-*Fos* from the ubiquitously active *H2* promoter induces bone tumours[7] (arrows in left panel). c-*FosLTR* cooperates with activated c-Jun in bone tumorigenesis, as bone-tumour formation induced by c-Fos is impaired in c-*Jun*$^{AA/AA}$ mice, which express a c-Jun mutant that is not activated by JNKs[58] (right panel). **b** | Conditional deletion of *c-Jun* in the liver using the flox (f) system impairs chemically induced liver-tumour formation. Tumours that develop in c-Jun-deficient livers (right panel) are smaller than control tumours that contain flox sites but from which c-*Jun* has not been deleted (left panel). The oncogenic activity of c-Jun in liver tumours is mediated by antagonizing the pro-apoptotic activity of p53 (REF. 9). **c** | Similarly, formation of skin tumours induced by the expression of the *K5–SOS* transgene is reduced in mice with a skin-specific deletion of c-*Jun* (c-*Jun*$^{\Delta ep}$; right panel), which is due to the downregulation of epidermal growth factor receptor (EGFR)[17] (arrows indicate EGFR expression). **d** | By contrast, JunB functions as a tumour suppressor. Epigenetic silencing of the *UbC* (ubiquitin C)–*JunB* transgene (right panel) — used to rescue the embryonic lethality of *JunB*-null fetuses — leads to loss of JunB expression in myeloid cells. This results in myeloid leukaemia and higher numbers of granulocyte (Gr1)/macrophage (Mac1) double-positive splenocytes than *JunB*$^{+/+}$ fetuses (left panel), as shown by fluorescence-activated cell-sorting analysis[16]. LTR, long terminal repeat. **a** Reproduced with permission from REF. 58 © (2000) Nature Publishing Group. **b** Reproduced with permission from REF. 9 © (2003) Elsevier. **c** Reproduced with permission from REF. 17 © (2003) Elsevier. **d** Reproduced with permission from REF.16 © (2001) Elsevier.

Overexpression of JunB seems to antagonize the c-Jun-mediated induction of cyclin D1 in fibroblasts[30], and JunD-deficient primary fibroblasts undergo premature senescence, which requires p53 and increased Arf expression[31]. As well as this competitive inhibition of c-JUN-mediated gene activation, JUNB-containing AP-1 complexes directly regulate the expression of cell-cycle modulators that are independent of c-JUN, such as INK4A (REF. 30; TABLE 2). Therefore, it is likely that c-JUN-containing complexes are the main contributors to AP-1 DNA-binding activity in highly proliferative tumours, whereas JUNB and JUND might be selectively downregulated due to their anti-proliferative ability.

Apoptosis and survival of tumour cells. It is generally accepted that programmed cell death (apoptosis) suppresses oncogenic transformation — a principle that drug developers are trying to exploit in anti-cancer therapies. Early *in vitro* studies indicated that increased AP-1 activity can lead to apoptosis in specific cell types, including human tumour cells[32]. However, oncogenic AP-1 can antagonize apoptosis in liver tumours[9]. The differential activity of AP-1 in apoptosis is best exemplified by the dual role of c-Jun in neuronal cells and hepatocytes. Increased c-Jun activity promotes apoptosis in neuronal cells *in vitro*. When the activation of c-Jun is impaired, either in *Jnk3*-null or *c-Jun*$^{AA/AA}$ mice, which express a c-Jun mutant that cannot be activated by JNKs, hippocampal neurons are protected from KAINATE-induced cytotoxicity[28,33,34]. By contrast, c-Jun is required for the survival of fetal hepatocytes, which undergo apoptosis in c-Jun-deficient mouse embryos[35] (TABLE 1).

The cell-specific consequence of AP-1 activity for apoptosis is probably due to differential regulation of pro-apoptotic and anti-apoptotic target genes (TABLE 2). In neuronal cells, c-Jun regulates the expression of Bim, a pro-apoptotic BCL2 FAMILY member that is crucial for neuronal apoptosis[36]. In T cells, c-Jun and c-Fos regulate the gene that encodes Fas ligand (FasL), which can trigger apoptosis through the Fas receptor[37]. The crucial pro-apoptotic target gene that is repressed by c-Jun in tumours might be *Trp53*. This has been shown for liver tumours that lack c-Jun, in which p53 is upregulated and consequently induces apoptosis[9]. Genes that encode Bcl2 family members also feature among the anti-apoptotic targets that are regulated by AP-1. In T cells, Jun proteins exert a protective signal through the induction of Bcl3 (REF. 38), and inactivation of JunB in myeloid cells leads to reduced apoptosis with increased expression of the anti-apoptotic *Bcl2* and *Bcl-xl* genes[16]. In addition, c-Jun and JunD might regulate genes that protect cells from cell death induced by tumour necrosis factor-α (Tnf-α)[39], as c-Jun-deficient and JunD-deficient hepatocytes show increased sensitivity to Tnf-α[9,31].

The differential regulation of pro-apoptotic and anti-apoptotic genes indicates that AP-1 can promote apoptosis in some tumour types, whereas it induces survival in others[9].

Table 2 | AP-1 target genes in tumour development and suppression

Gene product	Activity	Main regulator	References
DNMT1	DNA methylation	c-FOS (upregulates)	76
EGFR	Stimulates proliferation	c-JUN (upregulates) JUNB (upregulates)	17
HB-EGF	Stimulates proliferation	c-JUN (upregulates)	77
GM-CSF	Stimulates proliferation	c-JUN (upregulates) JUNB (downregulates)	78
KGF	Stimulates proliferation	c-JUN (upregulates) JUNB (downregulates)	79
Cyclin D1	Stimulates proliferation	c-JUN (upregulates) JUNB (downregulates)	29
WAF1	Inhibits proliferation	c-JUN (downregulates)	79
p53	Inhibits proliferation Stimulates apoptosis	c-JUN (downregulates)	25
ARF	Inhibits proliferation Stimulates apoptosis	JUND (downregulates)	31
INK4A	Inhibits proliferation Stimulates apoptosis	c-JUN (downregulates) JUNB (upregulates)	30
FASL	Stimulates apoptosis	c-JUN (upregulates) c-FOS (upregulates)	37
FAS	Stimulates apoptosis	c-JUN (downregulates)	80
BIM	Stimulates apoptosis	c-JUN (upregulates)	36
BCL2	Inhibits apoptosis	JUNB (downregulates)	16
BCL-XL	Inhibits apoptosis	JUNB (downregulates)	16
BCL3	Inhibits apoptosis	c-JUN (upregulates)	38
VEGFD	Angiogenesis	c-FOS (upregulates)	42
uPA	Angiogenesis	FRA1 (upregulates)	41
uPAR	Angiogenesis	FRA1 (upregulates)	41
Proliferin	Angiogenesis	c-JUN (upregulates) JUNB (upregulates)	44
MMP1	Invasiveness	c-FOS (upregulates) FRA1 (upregulates)	40
MMP3	Invasiveness	c-FOS (upregulates) FRA1 (upregulates)	40
CD44	Invasiveness	c-FOS (upregulates) c-JUN (upregulates)	49
Cathepsin L	Invasiveness	c-FOS (upregulates)	81
MTS1	Invasiveness	c-FOS (upregulates)	81
KRP1	Invasiveness	c-FOS (upregulates)	82
TSC36/FRP	Invasiveness	c-FOS (upregulates)	81
Ezrin	Invasiveness	c-FOS (upregulates)	83
Tropomyosin 3	Invasiveness	c-FOS (upregulates)	83
Tropomyosin 5b	Invasiveness	c-FOS (upregulates)	83

JUN proteins preferentially regulate genes that are implicated in proliferation and apoptosis, whereas FOS proteins are often required for angiogenesis and tumour invasion by malignant tumours. DNMT, DNA methyltransferase; EGFR, epidermal growth factor receptor; FASL, FAS ligand; GM-CSF, granulocyte–macrophage colony stimulating factor; HB-EGF, heparin-binding EGF; MMP, matrix metalloproteinase; uPA, urokinase-type plasminogen activator; uPAR, uPA receptor; VEGFD, vascular endothelial growth factor D.

Invasive growth and angiogenesis. Some target genes that are regulated by AP-1 have putative roles in angiogenesis and tumour metastasis (TABLE 2). Both processes require the degradation of extracellular-matrix components to allow blood-vessel formation and cell migration, respectively. c-Fos and Fra1 regulate the expression of matrix metalloproteinases (MMPs) — such as Mmp3 and Mmp1 (REF. 40) — and proteases of the urokinase plasminogen-activator system[41], which promote angiogenesis and the invasive growth of cancer cells. In addition to these, the gene that encodes the angiogenic factor vascular endothelial growth factor D (VegfD) has been identified as a c-Fos target gene[42]. The expression of some angiogenic factors might be regulated by c-Jun and JunB. Both are upregulated during fibrosarcoma tumour progression and increase angiogenesis in a cell-culture model of fibrosarcoma by activating the angiogenic factor proliferin[43,44]. Moreover, the expression of proliferin is downregulated in the placenta of JunB-deficient fetuses, which impairs proper vascularization and causes embryonic lethality[45].

AP-1 also regulates genes that are required for tumour metastasis (TABLE 2). One hallmark of metastasis and invasive growth is the transition of tumour cells from an epithelial to a mesenchymal morphology, known as the epithelial–mesenchymal transition (EMT). Both c-Fos and c-Jun can induce EMT, which is associated with loss of cell polarity, in mammary epithelial cells[46,47]. However, only the overexpression of c-Fos is able to promote invasive growth in collagen gels, which indicates that c-Fos might have a more important role than c-Jun during late-stage tumorigenesis[46]. The importance of c-Fos in tumour invasion has been supported *in vivo*, as the progression of chemically induced papillomas to invasive squamous-cell carcinomas is impaired in c-Fos-deficient mice[48]. Ectopic expression of the dominant-negative c-Jun mutant TAM67 inhibits the invasiveness of several cell types, which indicates that c-Jun–c-Fos complexes might regulate genes that are important for metastasis[49].

Regulation of AP-1 activity

The regulation of AP-1 activity is complex and occurs at different levels, including dimer composition, transcriptional and post-translational events, and interaction with ancillary proteins.

Dimer composition. The activities of AP-1 are thought to be modulated through the differential expression of its individual components, which determines the dimer composition. The significance of AP-1 composition in tumorigenesis was tested using AP-1 monomers that were joined by a flexible polypeptide tether to force specific pairing. This 'single-chain approach' showed that c-Jun–Fra2, but not c-Jun–Fra1 or c-Jun–c-Fos, inhibits the growth arrest of immortalized fibroblasts at confluence and under low-serum conditions[50]. The ectopic expression of single-chain molecules in mice will determine the relevance of specific AP-1 dimers to tumour formation.

The oncogenic potential of defined AP-1 dimers has also been studied using dimer-specific mutants of AP-1 proteins, in which manipulation of the leucine-zipper domain allows only specific dimers to form[51]. These experiments indicate that the c-Jun-induced transformation programme can be separated into two distinct pathways: c-Jun–Atf2 activity triggers growth-factor independence and c-Jun–c-Fos activity causes anchorage-independent growth[51]. Therefore,

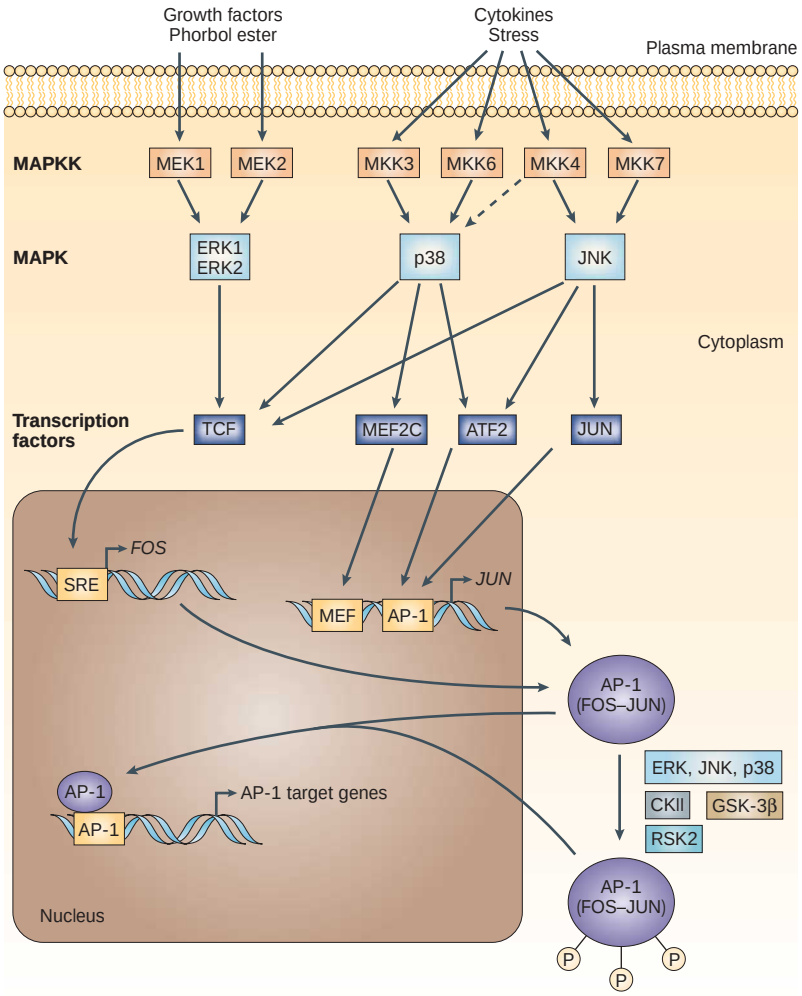

Figure 3 | **Transcriptional and post-translational activation of AP-1.** AP-1 (activator protein 1) activity is stimulated by a complex network of signalling pathways that involves external signals (for example, growth factors) and mitogen-activated protein kinases (MAPKs) of the extracellular-signal-regulated kinase (ERK), p38 and JUN amino-terminal kinase (JNK) families. The dashed arrow indicates that phosphorylation of p38 by MKK4 is controversial. MAPKs activate various transcription factors (ternary-complex factors (TCFs), myocyte-enhancer factor 2C (MEF2C), activating transcription factor 2 (ATF2) and JUN) that induce the transcription of *FOS* and *JUN* genes, thereby increasing the number of AP-1 complexes and activating AP-1 target genes. Post-translational phosphorylation by various kinases regulates AP-1 activity, which includes its transactivating potential, DNA-binding capacity and the stability of AP-1 components. CKII, casein kinase II; GSK-3β, glycogen synthase kinase-3β; MAPKK, MAPK kinase; RSK2, ribosomal S6 kinase 2; SRE, serum-response element.

various AP-1 dimer combinations are required at different stages of tumorigenesis and some dimers can suppress tumorigenesis.

Transcriptional and post-translational regulation. AP-1 is induced by several external stimuli that increase mitogen-activated protein kinase (MAPK) activity. First and foremost, the expression of c-FOS is induced by ternary complex factors (TCFs), which are activated through phosphorylation by the ERK (extracellular-signal-regulated kinase) MAPKs (FIG. 3). Subsequently, c-FOS and MYOCYTE-ENHANCER FACTOR 2 (MEF2) transcription factors induce c-JUN expression. When the AP-1 complexes are present in larger glycogen-synthase-kinase-3β and RSK2 (ribosomal S6 kinase 2) — phosphorylate FOS and JUN proteins, thereby regulating their transactivation potential and DNA-binding activity (FIG. 3). The effect of these kinases on AP-1 activity in tumorigenesis is not well-defined. ERKs are persistently activated by growth factors and oncogenic RAS in tumours and are positive regulators of tumorigenesis[52]. They contribute substantially to the increased expression and activation of AP-1 members in many tumour types. The roles of JNK and p38 MAPKs in tumorigenesis are double-edged. p38 is activated by many conditions that are known to suppress tumour growth[53], whereas JNK activity — although increased in bone[54] and brain tumours[55] — can antagonize RAS-mediated transformation[56] and induce cell death[57]. However, N-terminal phosphorylation of c-JUN by JNKs strongly augments c-JUN activity, which is required for son of sevenless (SOS)-induced skin tumorigenesis and c-FOS-induced osteosarcoma formation[58], but not for the chemically induced formation of liver tumours[9]. This indicates a differential requirement for N-terminal c-JUN phosphorylation in tumorigenesis.

Genetic interaction with other oncoproteins. To fully elicit their oncogenic potential, most AP-1 components need the activity of 'cooperating' oncoproteins, which often induce the expression of JUN and FOS proteins but also support AP-1-mediated cell transformation by post-transcriptional mechanisms. The main 'cooperating' partner of AP-1 is the RAS pathway[59,60], as cell transformation by activated RAS or MEK1 (MAPK kinase) induces AP-1 protein expression[61,62]. It seems that FRA1 and c-JUN are the main AP-1 components that are induced by activated RAS[61]. Ras-mediated cell transformation is largely suppressed in fibroblasts that lack c-Jun[63]. The oncogenic cooperation of c-JUN with RAS and other oncoproteins that function upstream of RAS might require N-terminal phosphorylation of c-JUN by JNKs[64,65]. This hypothesis was tested *in vivo* using mice that carry the *K5–SOS* TRANSGENE, in which the activated human *SOS* gene is expressed from a keratin-specific promoter. In these mice, skin tumours develop after constitutive activation of the Ras pathway[66]. *K5–SOS*-induced skin tumorigenesis was impaired in *c-JunAA/c-JunAA* mice, which carry c-JunAA proteins that cannot be activated by JNKs, supporting the significance of c-Jun N-terminal phosphorylation in Ras-induced tumorigenesis[58]. In contrast to c-Jun, JunB and JunD are not efficient substrates for JNKs[67]. They are less potent collaborators of Ras in cell transformation than c-Jun, which correlates with their lower transcriptional activity[68]. In fact, overexpression of JunB in mouse fibroblasts reduces transformation by Ras or Src, which indicates that JunB antagonizes the activity of c-Jun in oncogene-induced cell transformation[30].

Interaction with ancillary proteins. The number of ancillary proteins that have been found to interact with AP-1 components and regulate their transcriptional ability is both large and constantly increasing. Among the ancillary proteins that interact with AP-1 and have clinical importance are nuclear receptors

BCL2 FAMILY
BCL2 family members are implicated in programmed cell death, but can be either pro-apoptotic or anti-apoptotic. The anti-apoptotic members prevent the release of apoptogenic molecules from mitochondria, whereas pro-apoptotic family members function as sentinels for cellular damage — cytotoxic signals induce their translocation to organelles where they bind to their pro-survival relatives, promote organelle damage and trigger apoptosis.

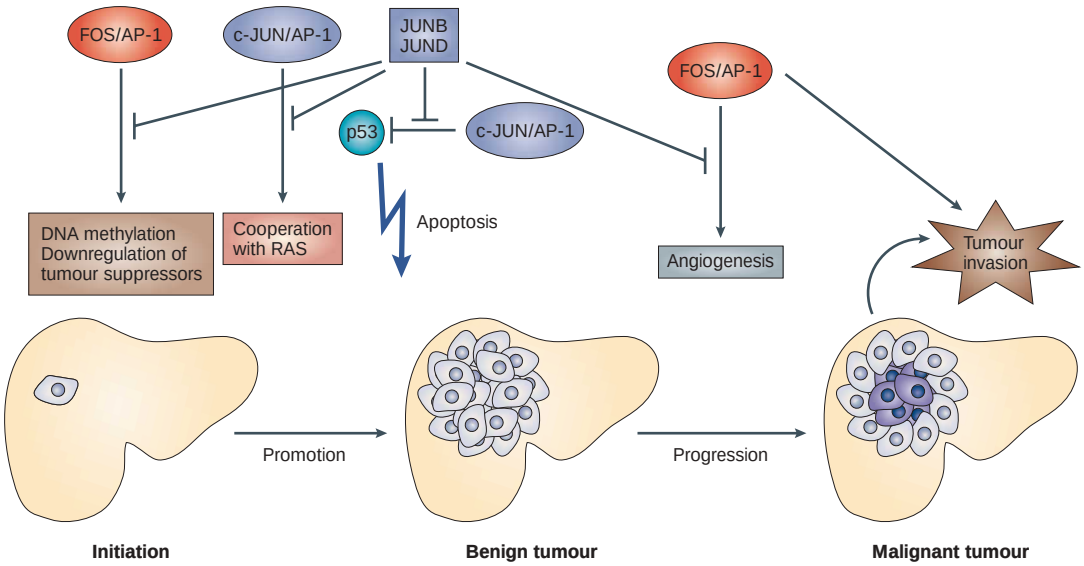

Figure 4 | **Pro-oncogenic and anti-oncogenic activities of AP-1 in multi-stage tumorigenesis during liver-tumour formation.** At the initiation stage of liver carcinogenesis, c-FOS-containing AP-1 (activator protein 1) complexes might change the DNA methylation pattern by regulating the DNA 5-methylcytosine transferase gene. This can lead to the downregulation of tumour-suppressor genes, which favours tumour development. In addition, c-JUN–c-FOS and c-JUN–activating-transcription-factor-2 (ATF2)-containing complexes contribute to primary-tumour formation by cooperating with the products of other oncogenes, such as RAS. JUNB and JUND have to be downregulated to allow the formation of these oncogenic dimers. At later stages c-JUN/AP-1 complexes are essential to prevent p53-mediated apoptosis, although these become dispensable for tumour progression, presumably because p53 is inactivated by somatic mutations. In advanced tumours, c-FOS/AP-1 complexes induce the expression of genes that are implicated in angiogenesis and tumour invasiveness. In addition to these cell-autonomous effects, AP-1 activity in tumour-surrounding tissues is able to stimulate the secretion of paracrine factors, thereby influencing tumour growth in a non-cell-autonomous manner (not shown).

such as the glucocorticoid receptor (GR) and the retinoic-acid receptor (RAR)[1,2,69]. Both can inhibit AP-1 target-gene transcription by TRANSREPRESSION. However, in certain tissues or cell types, AP-1 and GR can synergize and activate common target genes through composite DNA response elements. The composition of AP-1 determines whether the crosstalk with GR is positive or negative. Glucocorticoids, the activating ligands of GR, are important drugs that are used for the treatment of acute lymphoid leukaemia. Glucocorticoids induce apoptosis in leukaemic cells and the transrepression of genes through AP-1 and NF-κB interactions is probably essential for their apoptotic potential[69].

In addition to glucocorticoids, retinoic acids — the ligands of retinoic-acid receptors (RARs and RXRs) — have tumour-suppressive abilities. The ability to induce tumour-cell differentiation and apoptosis offers great promise for cancer therapy, which has been shown impressively in the case of acute promyelocytic leukaemia. Moreover, retinoic acid also blocks tumorigenesis of other cancer types, such as chemically induced skin cancer. In at least some of these cases, the tumour-suppressive activity of retinoic acids is mediated by transrepression of AP-1 (REF. 70). Although the mechanistic basis of the anti-AP-1 activity of retinoids remains elusive, the importance of this crosstalk for cell proliferation is increasingly being recognized.

Conclusion

The textbook description that AP-1 functions primarily as an oncogenic complex in tumorigenesis has to be revised on the basis of recent studies in mice and humans. AP-1 clearly has a double-edged activity — it can be anti-oncogenic by inducing apoptosis and it can be oncogenic by signalling cell survival. The final outcome of AP-1 activity in tumours seems to depend on AP-1 dimer composition as well as the cellular and genetic context. The genetic link between AP-1 and p53 seems to be of particular importance in liver tumorigenesis, but might well determine in general whether tumour formation is induced or suppressed (FIG. 4). It is worth mentioning that, until now, no activating mutations, deletions or amplifications of any *JUN* or *FOS* gene have been identified in human tumours. However, many AP-1 components — for example, JUN proteins — are expressed at high levels, and the pathways that lead to increased DNA binding by AP-1 are often constitutively activated in human tumour cells[71].

As well as these rather general assumptions, how do we envisage the generation of specific AP-1 dimers during development and oncogenesis? We favour a dynamic model, with continuous remodelling of AP-1 complexes, over a static model of stable, predetermined dimers. This model might have little impact on developmental processes, in which gene substitutions in mice — for example, *Fra1* for c-*Fo*s or *JunB* for c-*Jun* — have shown that the overall regulation of AP-1

MYOCYTE-ENHANCER FACTOR 2
The four MEF2 transcription factors (MEF2A to MEF2D) regulate calcium-dependent gene expression in muscle cells and have a pivotal role in the differentiation of cardiac and skeletal muscle. The expression of muscle-specific genes is often accomplished through MEF2-responsive elements (MEF2 sites) and recognition elements for other muscle-specific transcription factors, such as MyoD and myogenin.

K5–SOS TRANSGENE
This transgene directs the ectopic expression of the constitutively active human SOS protein in keratinocytes as it is under the control of the keratin-5 promoter. SOS functions upstream of RAS in the receptor tyrosine kinase MAPK pathway and induces skin papillomas in *K5–SOS* transgenic mice.

TRANSREPRESSION
Mutual negative interference between transcription factors. Examples are the interactions between the glucocorticoid receptor (GR) and other transcription factors, such as CREB, AP-1, NF-κB and OCT1. The GR attenuates the activity of these transcription factors by direct protein–protein interaction, without the need for DNA-binding. AP-1 is also transrepressed by retinoic-acid receptors, the oestrogen receptor, the thyroid receptor and the fusion receptor PML–RAR.

seems to be more important than its specific composition[72,73]. However, AP-1 composition undoubtedly matters for the initiation, maintenance and progression, or suppression, of tumorigenesis. The recently discovered anti-oncogenic functions of some AP-1 proteins might provide a new opportunity for anticancer treatments that are based on modulating AP-1 complexes in specific tumour types. This approach would not only inhibit oncogenic AP-1 activity, but might also actively interfere with different stages of tumour development. It is certainly a goal for the future to induce the formation of specific dimers for the selective and efficient killing of cancer cells and thereby make use of the deadly sword of AP-1 in antagonizing tumour development.

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