AP-1 subunits: quarrel and harmony among siblings

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Summary

The AP-1 transcription factor is mainly composed of Jun, Fos and ATF protein dimers. It mediates gene regulation in response to a plethora of physiological and pathological stimuli, including cytokines, growth factors, stress signals, bacterial and viral infections, as well as oncogenic stimuli. Studies in genetically modified mice and cells have highlighted a crucial role for AP-1 in a variety of cellular events involved in normal development or neoplastic transformation causing cancer. However, emerging evidence indicates that the contribution of AP-1 to determination of cell fates critically depends on the relative abundance of AP-1 subunits, the composition of AP-1 dimers, the quality of stimulus, the cell type and the cellular environment. Therefore, AP-1-mediated regulation of processes such as proliferation, differentiation, apoptosis and transformation should be considered within the context of a complex dynamic network of signalling pathways and other nuclear factors that respond simultaneously.

Key words: AP-1, Transcription, Subunits, Jun, Fos, ATF

Introduction

Much of our current knowledge about the characteristics of transcription factors comes from the discovery and study of activating protein 1 (AP-1). AP-1 describes an activity that controls both basal and inducible transcription of several genes containing AP-1 sites (consensus sequence 5′-TGAG/CTCA-3′), also known as TPA-responsive elements (TREs) (Angel and Karin, 1991). AP-1 collectively describes a group of structurally and functionally related members of the Jun protein family [Jun (originally described as c-Jun), JunB and JunD] and Fos protein family [Fos (originally described as c-Fos), FosB, Fra-1 and Fra-2]. Additionally, some members of the ATF (ATFa, ATF-2 and ATF-3) and JDP (JDP-1 and JDP-2) subfamilies, which share structural similarities and form heterodimeric complexes with AP-1 proteins (predominantly with Jun proteins), can bind to TRE-like sequences. Each of these proteins is differentially expressed and regulated, which means that every cell type has a complex mixture of AP-1 dimers with subtly different functions (reviewed by Wagner, 2001).

A common feature of all these proteins is the evolutionarily conserved bZIP domain, the collective term for a basic DNA-binding domain combined with a leucine zipper region (Fig. 1). The leucine zipper is responsible for dimerization, which is a prerequisite for DNA binding mediated by the basic domain. The composition of the leucine zipper is also responsible for the specificity and the stability of homo- and heterodimers formed by the various Jun and Fos proteins (for detailed information, see Wagner, 2001; Eferl and Wagner, 2003). Whereas the Jun proteins exist as homo- and heterodimers, the Fos proteins, which cannot homodimerize, form stable heterodimers with Jun proteins and thereby enhance their DNA-binding activity. In contrast to the well-characterized bZIP domain, the structural properties of the domains mediating transcriptional activation are less well understood. The individual Jun and Fos proteins have significantly different transactivation potentials. Whereas Jun, Fos and FosB are considered strong transactivators, JunB, JunD, Fra-1 and Fra-2 exhibit only weak transactivation potential. Under specific circumstances, the latter might even act as repressors of AP-1 activity by competing for binding to AP-1 sites or by forming ‘inactive’ heterodimers with Jun, Fos or FosB.

AP-1 activity is regulated in a given cell by a broad range of physiological and pathological stimuli, including cytokines, growth factors, stress signals and infections, as well as oncogenic stimuli. Regulation of net AP-1 activity can be achieved through changes in transcription of genes encoding AP-1 subunits, control of the stability of their mRNAs, post-translational processing and turnover of pre-existing or newly synthesized AP-1 subunits, and specific interactions between AP-1 proteins and other transcription factors and cofactors. The mechanism of post-translational control is most extensively documented in the case of mitogen- and cellular-stress-induced hyperphosphorylation and, in particular, activation of Jun through the Jun N-terminal kinase (JNK) cascade (reviewed by Karin et al., 1997; Wagner, 2001). JNKs are members of the mitogen-activated protein kinase (MAPK) superfamily and comprise three isoforms: JNK1, JNK2 and JNK3 (Davis, 2000). Activated by a MAPK cascade, the JNKs translocate to the nucleus, where they phosphorylate Jun within its N-terminal transactivation domain (residues Ser63 and Ser73) and thereby enhance its transactivation potential (Fig. 1). The JNKs also phosphorylate and potentiate the activity of JunD and ATF-2. By contrast, the kinases that regulate the activity of Fos are not yet clear. Potential candidates are an as-yet-ill-defined Fos-related kinase (FRK) (Deng and Karin, 1994), ribosomal S6 kinase (RSK) and ERK (Chen et al., 1993; Chen et al., 1996). However, the significance of these kinases in the control of Fos activity and function remains elusive.

Generation of genetically modified cells and mice that have

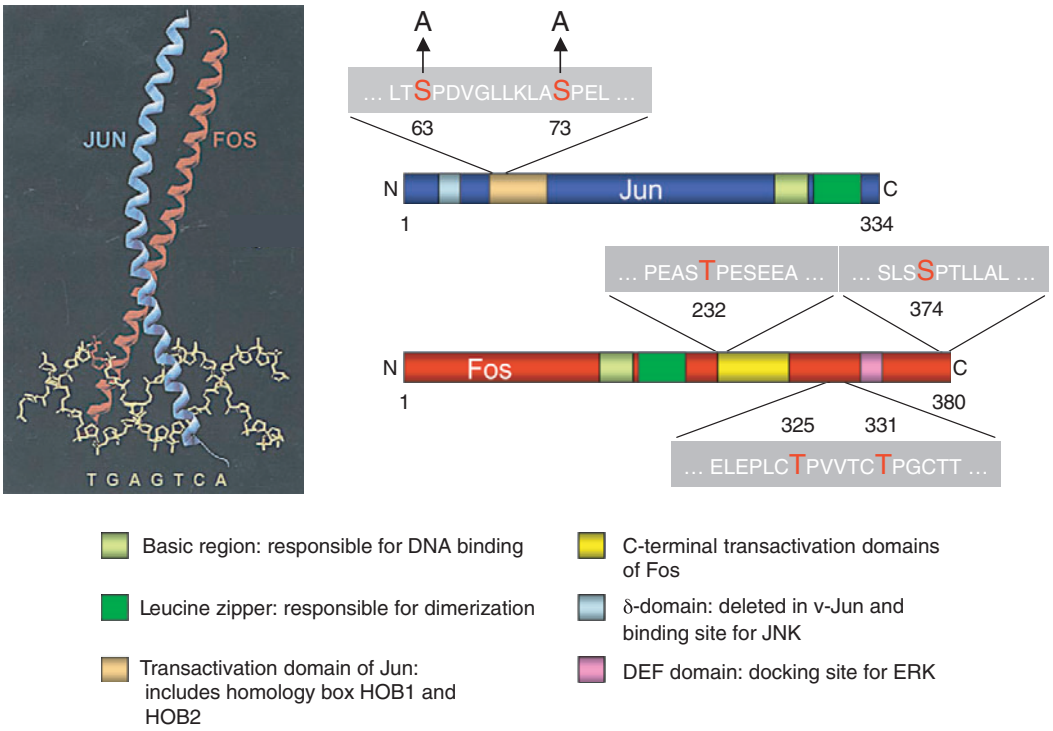

Fig. 1. The Jun-Fos heterodimer. The bZIP domains of Jun and Fos form an X-shaped α-helical structure, which binds to the palindromic AP-1 site (TGAGTCA) (Glover and Harrison, 1995). The bZIP domain of Jun is shown in blue and the bZIP domain of Fos in red. The DNA backbone is shown in yellow. The Jun and Fos proteins exhibit several domains, including the bZIP domain (leucine zipper plus basic domain), transactivation domains and docking sites for several kinases, such as JNK or ERK. These kinases phosphorylate two serine and threonine residues and thereby modulate the activity of both proteins. JNK specifically phosphorylates serine residues within the transactivation domain of Jun at position 63 and 73 and thereby regulates its transactivation activity. Mutation of serine to alanine generates a Jun mutant (Jun-AA) that cannot be activated by JNKs. Jun is also phosphorylated by casein kinase II, GSK-3β and ERK, which is not depicted in this scheme (for details, see Eferl and Wagner, 2003). ERK phosphorylates threonine residues at positions 325 and 331 and a serine residue at position 374 of Fos. Additionally, a Fos-related kinase phosphorylates a threonine residue at position 232 of Fos.

altered or ablated expression of different AP-1 members has shed light on their individual physiological functions in the control of cell proliferation and differentiation, apoptosis and neoplastic transformation (Ameyar et al., 2003; Eferl and Wagner, 2003; Jochum et al., 2001; Shaulian and Karin, 2001; Shaulian and Karin, 2002). Here, we focus on how the different Jun/Fos relatives contribute to AP-1 activity and discuss recent insights into their involvement in normal physiology and pathological conditions.

AP-1 in proliferation, differentiation and transformation

Identification of Jun and Fos as mammalian cellular homologues of retroviral oncoproteins (v-Jun and v-Fos), together with the finding that growth factors and tumour promoters induce AP-1 activity, immediately linked AP-1 to cellular growth control and neoplastic transformation. Control of cell proliferation by AP-1 seems to be mainly mediated by its ability to regulate the expression and function of cell-cycle regulators such as cyclin D1, cyclin A, cyclin E, p53, $p21^{Cip1}$, $p16^{Ink4a}$ and $p19^{ARF}$ (Fig. 2) (reviewed by Shaulian and Karin, 2001; Shaulian and Karin, 2002). Analysis of cell culture models that exhibit altered expression of individual AP-1 members revealed unique and crucial roles for each Jun protein but some functional redundancy among the Fos proteins. Below, we discuss a few examples that illustrate how studies of genetically modified mouse models have contributed to our current understanding of the role of AP-1 family members in the regulation of cell proliferation and differentiation in the context of both normal development and disease.

Proliferation of hepatocytes and keratinocytes: Jun as a central player

The tissue-specific inactivation of Jun in mice has highlighted the fact that Jun not only is essential for cell-cycle regulation in fibroblasts but also promotes proliferation of other cell types, such as liver cells and keratinocytes (Behrens et al., 2002; Li et al., 2003; Zenz et al., 2003). Perinatal, liver-specific deletion of Jun in mice ($Jun^{\Delta li}$ mice) causes reduced hepatocyte proliferation and impaired liver regeneration after partial hepatectomy (Behrens et al., 2002). In agreement with this phenotype is the finding that, in wild-type mice, Jun induction occurs in postnatal liver cells, in a phase characterized by dramatic expansion of the liver (Eferl et al., 1999). Although cyclin-dependent kinases (CDKs) and several cell-cycle regulators are affected, hepatocytes lacking Jun do not completely mirror the situation in Jun-null fibroblasts (Behrens et al., 2002). For example, p53 and $p21^{Cip1}$ protein levels are

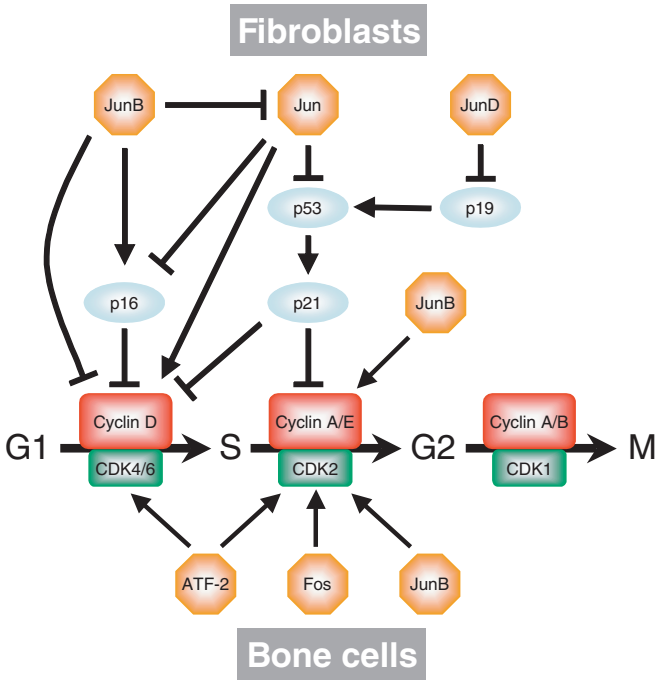

Fig. 2. Pro- and anti-proliferative activities of AP-1 subunits during cell-cycle progression. AP-1 modulates cell proliferation through its ability to regulate the expression and function of cell-cycle regulators such as cyclin D1, cyclin A, p53, $p21^{Cip1}$, $p16^{Ink4a}$ and $p19^{ARF}$. The capacity of AP-1 both to promote and inhibit cell-cycle progression is most likely due to the abundance of distinct members within a given cell, as well as the cell type and its microenvironment. JunB and ATF-2 regulate expression of cyclin A in chondrocytes and osteoblasts, whereas Fos-dependent expression of cyclin A and cyclin E has been described for chondrocytes. Additionally, ATF-2 regulates expression of cyclin D1 in chondrocytes and osteoblasts.

normal in hepatocytes lacking Jun but both proteins are significantly elevated in Jun-deficient fibroblasts (Fig. 2). Furthermore, Jun does not seem to be required for efficient induction of D-type cyclins. However, cyclin D1 and $p21^{Cip1}$ accumulate in hepatocytes lacking Jun at later time points, which could account for delayed cell-cycle progression in these cells. Similarly, impaired proliferation of mutant keratinocytes derived from $Jun^{\Delta ep}$ mice, in which Jun is conditionally inactivated in the epidermis, does not correlate with altered p53 and $p21^{Cip1}$ protein levels (Zenz et al., 2003). This suggests that different molecular mechanisms control cell proliferation in fibroblasts, hepatocytes and keratinocytes.

Reduced levels of both epidermal growth factor receptor (EGFR) and its ligand, heparin-binding EGF (HB-EGF), seem to be responsible for the severe proliferation defect in keratinocytes lacking Jun (Li et al., 2003; Zenz et al., 2003). However, impaired proliferation could be rescued not only by HB-EGF and high levels of EGF but also by other autocrine and paracrine growth factors, such as transforming growth factor α (TGF-α), keratinocyte growth factor (KGF) and granulocyte-macrophage colony-stimulating factor (GM-CSF). This indicates that AP-1 regulates keratinocyte proliferation both cell autonomously and in a paracrine manner. Interestingly, in an organotypic in vitro coculture system composed of fibroblasts and keratinocytes, which mimics features of skin re-epithelialization during wound healing, Jun and JunB antagonistically regulate the synthesis of fibroblast-derived KGF and GM-CSF RNA and protein. Thus, both are rate-limiting factors that control the balance of keratinocyte proliferation and differentiation to ensure proper formation of an epithelium (Szabowski et al., 2000).

Global gene expression analysis in wild-type, Jun-deficient and JunB-deficient fibroblasts has identified several additional AP-1-dependent target genes, including growth factors, cytokines and chemoattractants; for example, lipocalin 2, the heparin-binding factor pleiotrophin (PTN, also known as HB-GAM) and stromal-cell-derived factor 1 (SDF-1/CXCL12) (Florin et al., 2004). These factors are expressed by cells of both the epithelial and mesenchymal compartment, which supports the concept that AP-1 activity regulates complex genetic programs of cell proliferation and differentiation in skin homeostasis and repair, in a cell-autonomous manner and through the induction of paracrine effectors.

Proliferation and differentiation of lymphoid cells: dual roles for JunB and JunD

A recent study demonstrates that lymphocyte proliferation is enhanced in the absence of JunD (Meixner et al., 2004). Conversely, cells of lymphoid origin derived from transgenic mice over-expressing either transgenic JunB or JunD respond poorly to mitogenic stimuli (Meixner et al., 2004; Szremska et al., 2003), which provides in vivo evidence that JunB and JunD can act as negative regulators of cell proliferation (Bakiri et al., 2002; Passegue and Wagner, 2000; Weitzman et al., 2000). This idea is supported by the observation that lack of JunB in mice results in a transplantable myeloproliferative disease that eventually progresses to a blast crisis, characterized by a massive blast cell infiltration of nonhematopoietic organs that resembles human chronic myeloid leukaemia (CML) (Passegue et al., 2001). Impaired expression of JunB is also evident in human CML patients, and the downregulation seems to be mediated by hypermethylation of the *JunB* promoter (Yang et al., 2003).

However, replacement of Jun by JunB, using a knock-in strategy and a transgenic complementation approach, abrogates the proliferation defects and deregulated expression of cyclin D1, p53 and $p21^{Cip1}$ associated with the loss of Jun in fibroblasts (Passegue et al., 2002). These experiments suggest that, in the absence of Jun, JunB can function as a positive growth regulator and that the anti-proliferative activity of JunB requires the formation of a growth-inhibiting Jun-JunB heterodimer. JunD exhibits a similar switch from a potent growth suppressor to a growth promoter that depends on the presence of another protein (Agarwal et al., 2003). In this case, the interacting partner is not an AP-1 member but the tumour suppressor protein menin. Menin binds directly to JunD and suppresses its transcriptional activity (Agarwal et al., 1999). In fibroblasts, JunD function changes from growth suppression to growth promotion when a JunD mutant that is unable to bind to menin is expressed or a menin-deficient genetic background is present.

JunB and JunD also control the differentiation and function of T helper (Th) cells mainly by regulating specific cytokines (Hartenstein et al., 2002; Meixner et al., 2004). JunB can activate expression of interleukin 4 (IL-4) and drive Th-cell

differentiation towards the Th2 lineage, whereas JunD negatively affects IL-4 production and Th2 differentiation. This provides an example of antagonistic functions of JunB and JunD.

Proliferation and differentiation of bone cells: collaboration of several AP-1 members

Recent studies have elucidated a crucial role for AP-1 components, mainly members of the Fos family, in bone-forming cells. The first experimental evidence that AP-1 is involved in differentiation and cell-cycle regulation of bone-forming cells came from analysis of transgenic mice over-expressing Fos. In these animals, Fos promotes chondrogenic and osteogenic tumour formation as a result of the transformation of chondroblasts and osteoblasts (Grigoriadis et al., 1995; Wang et al., 1991). This implies that Fos is involved in chondrogenesis and osteoblastogenesis in vivo. Exogenous expression of Fos in developing tumours and osteoblast cell cultures accelerates S-phase entry as a result of deregulated cyclin A and cyclin E expression and altered cyclin A/E CDK2 activity (Fig. 2) (Sunters et al., 2004). Interestingly, over-expression of Fra-1, Fra-2 or ΔFosB in transgenic mice does not transform bone cells but leads either to increased bone formation and development of osteosclerosis or, in the case of Fra-2, to the development of lung and epithelial tumours (Eferl and Wagner, 2003). Therefore, it will be interesting to determine whether Fra-induced epithelial tumourigenesis is also linked to altered cell-cycle regulation. Recently, viable mice lacking Fra-1 have been generated by a conditional loss-of-function approach (Eferl et al., 2004). These mice develop osteopenia, a low-bone-mass disease, and have long bones containing reduced levels of bone matrix components produced by osteoblasts and chondrocytes.

Altered expression of cyclin A RNA and protein, and impaired proliferation of chondrocytes and osteoblasts, also occurs in mice lacking JunB or ATF-2 expression (Beier et al., 1999; Beier et al., 2000; Hess et al., 2003; Kenner et al., 2004). This suggests that several AP-1 members collaborate to activate the *cyclin A* gene (Fig. 2). Interestingly, the in vivo data for JunB are in agreement with previous studies of JunB-deficient fibroblasts, showing a growth-promoting activity of JunB through direct transcriptional activation of cyclin A (Andrecht et al., 2002).

Fos is also involved in osteoclastogenesis, since Fos-deficient animals lack osteoclasts and develop osteopetrosis, which is characterized by increased bone mass owing to reduced bone resorption (Johnson et al., 1992; Wang et al., 1992). The osteopetrotic phenotype can be partially rescued by expression of a *Fra-1* transgene and total restoration can be achieved by a knock-in strategy (Matsuo et al., 2000; Fleischmann et al., 2000). These data are in line with the identification of *Fra-1* as a crucial Fos target gene in the osteoclast lineage (Matsuo et al., 2000). *Fos* expression in osteoclasts is induced upon binding of the receptor activator of NF-κB ligand (RANKL) to its receptor RANK (Wagner, 2002). This results in the recruitment of tumour necrosis factor (TNF)-receptor-associated factor (TRAF)-family proteins, which activates MAPK pathways that include JNK. There is genetic evidence that JNK1 activation modulates osteoclastogenesis through both Jun-dependent and Jun-independent mechanisms (David et al., 2002), demonstrating that Jun proteins contribute partially to this process. JunB is another Jun member that is required for efficient osteoclast differentiation, since lack of JunB in animals results in decreased osteoclast formation (Kenner et al., 2004). However, the block in differentiation is not complete, which suggests that Jun members can partially substitute for each other during osteoclastogenesis.

AP-1 in survival and apoptosis

AP-1 is often portrayed as a general, nuclear decision-maker that determines survival or cell death in response to extracellular stimuli. However, emerging evidence demonstrates that the contribution of AP-1 to cell fate depends on the cellular context and the survival or death stimulus. Therefore, the role of AP-1 in apoptosis should be considered within the context of a complex network of signalling pathways and nuclear factors that respond simultaneously. Below, we discuss recent findings providing strong evidence that AP-1 has a dual function: it induces apoptosis in some cellular systems but is required for cell survival in others (Fig. 3).

Pro-apoptotic function of AP-1 members

Cell death induced by Fas ligand (FasL) and its cell-surface receptor Fas is a classical example of apoptosis induced by an external signal. The Fas/FasL system is important for the proper action of the immune system and immune cell homeostasis, although it is not limited to lymphoid cells (Krammer, 2000). Numerous studies have highlighted an important role for the extrinsic death receptor pathway, via JNK, Jun/AP-1 and FasL, in the control of lymphoid, fibroblast and neuronal cell fate (Eichhorst et al., 2000; Kasibhatla et al., 1998; Kolbus et al., 2000; Le-Niculescu et al., 1999; Matsui et al., 2000). JNK activated by its MAPK cascade phosphorylates Jun, which results in enhanced transcription of target genes implicated in cellular-stress-induced apoptosis. Among the pro-apoptotic targets of Jun are the genes that encode FasL and TNF-α, which both contain AP-1-binding sites.

How and when the JNK pathway is involved in the regulation of peripheral T-cell fate, which is the major role of FasL-mediated apoptosis in vivo, remains elusive and appears to depend on the type of stimulation. Indeed, studies of JNK2-deficient mice and transgenic mice expressing dominant-negative JNK reveal normal activation-induced cell death (AICD) of peripheral T cells, but the cells display a minor defect in thymocyte negative selection and the response to in vivo anti-CD3 treatment (Rincon et al., 1998; Sabapathy et al., 1999). CD3 is a component of the T-cell receptor (TCR) complex, and injection of anti-CD3 antibodies intraperitoneally results in a nearly complete depletion of $CD4^+$ $CD8^+$ thymocytes in wild-type animals. In line with this observation, Fas-mediated thymocyte apoptosis is not impaired in mutant mice expressing a Jun mutant that cannot be N-terminally phosphorylated at its JNK-targeted activation sites (Behrens et al., 2001). Despite the obvious dependence of *FasL* induction on Jun in several experimental systems, Fas/FasL signalling is clearly a complex mechanism that may be promoted by Jun only under certain conditions.

Do AP-1-family members other than Jun contribute to

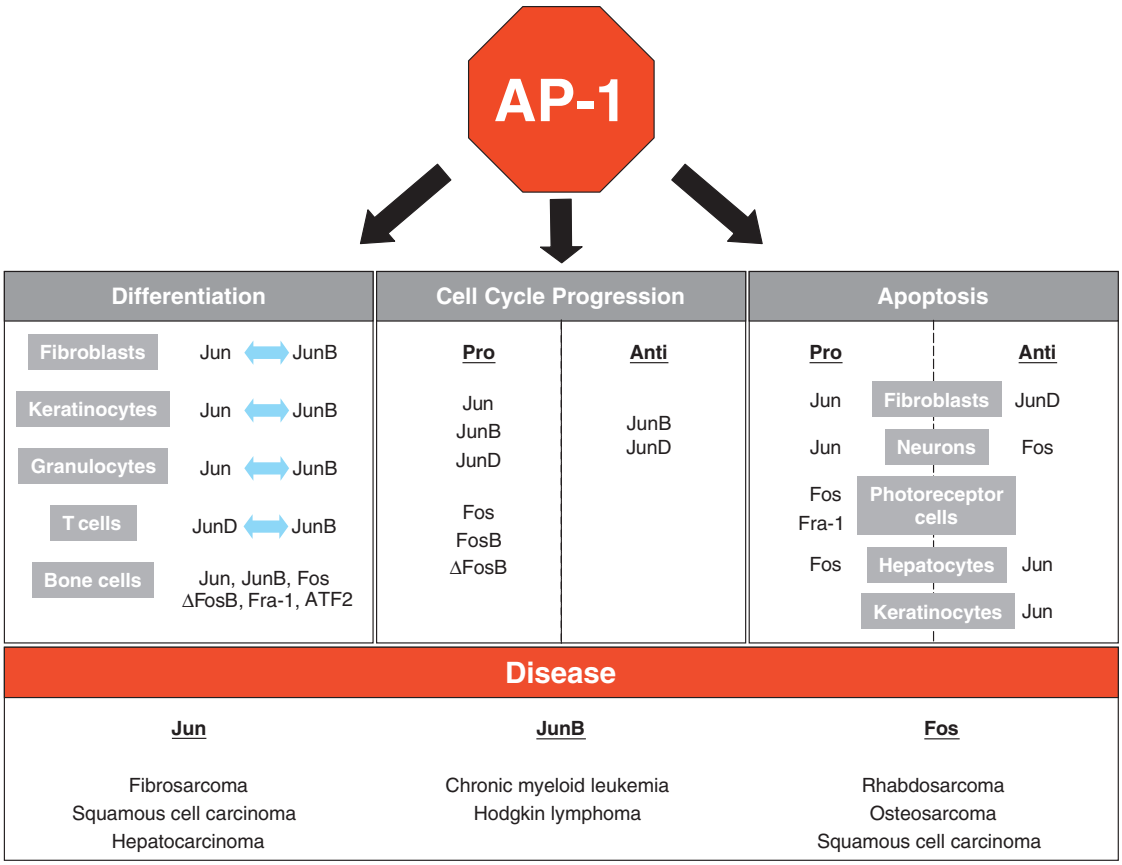

Fig. 3. Functions of AP-1 subunits in various cellular processes and disease. Antagonistic (indicated by the double-headed arrow) or common tasks for AP-1 members are observed in various cell types. Positive, negative or even dual functions of individual subunits influence pivotal cellular processes, such as differentiation, cell-cycle regulation or apoptosis. Deregulation of these processes by alterations in either single or multiple AP-1 subunits, thereby modulating AP-1 net activity, can be fatal for the cell and organism.

Fas/FasL-mediated apoptosis? Both Jun and Fos bind to the human *Fas* promoter. Jun is a potent activator of *Fas* transcription, but Fos abrogates Jun-mediated activation (Lasham et al., 2000). Fos might also negatively regulate *FasL* expression, since a transcriptional repressor element within the *FasL* promoter constitutively binds Fos but not Jun (Crist et al., 2003). In contrast to Fos, the appearance of FosB in a complex with Jun correlates with TCR/CD3-mediated induction of human *FasL* expression and AICD of human T cells (Baumann et al., 2003). Again, the in vivo relevance of these proteins in Fas/FasL-mediated apoptosis remains unclear.

Although strong evidence demonstrates a pro-apoptotic role for JNK/Jun signalling in neurons, the precise molecular mechanism is similarly elusive. Analyses of cell cultures derived from sympathetic and cerebellar granular neurons revealed a clear dependency on JNK/Jun activity for stimulation of apoptosis by withdrawal of growth factor (Harris and Johnson, 2001; Lei et al., 2002; Whitfield et al., 2001). Furthermore, expression of a dominant-negative Jun mutant reduces expression of Bim, a BH3-only member of the Bcl2 family of apoptosis regulators (Bouillet and Strasser, 2002), and inhibits mitochondrial cytochrome *c* release (Whitfield et al., 2001). These observations are consistent with previous studies establishing a fundamental role for JNK/Jun activation in the stress-induced mitochondrial (intrinsic) death pathway (Behrens et al., 1999; Lei et al., 2002; Palmada et al., 2002; Tournier et al., 2000; Yang et al., 1997; Willis et al., 2003). The importance of a putative JNK–Jun/AP-1–Bim pathway in neuronal cell death control is underscored by pathologies associated with deregulated apoptosis. Alzheimer's disease is associated with enhanced apoptosis of cerebral endothelial cells through AP-1 activation and subsequent Bim induction (Yin et al., 2002). Interestingly, the E3 ubiquitin ligase SCF (Fbw7) is an antagonist of the apoptotic Jun-dependent effector arm of JNK signalling, allowing neurons to tolerate potentially neurotoxic JNK activity, which makes the situation even more complex (Nateri et al., 2004).

In addition to functioning in neuronal apoptosis mediated by growth factor withdrawal, Jun is also induced following neuronal injury (Herdegen et al., 1997). Its precise role in response to axonal damage is controversial owing to studies that have linked Jun to both axonal regeneration and axotomy-induced cell death (Herdegen and Leah, 1998). Raivich and coworkers have observed that, in mice that do not express Jun in the nervous system, axotomized motoneurons undergo atrophy as a result of reduced cell death (Raivich et al., 2004). The motoneurons fail to re-establish a functional synapse, which correlates with impaired trophic support.

Light-induced retinal apoptosis provides another example of a pro-apoptotic function of AP-1, correlating with induction of

dimers composed of Fos, JunD and Jun. Analysis of mutant mice revealed that Fos is essential, whereas JunD and N-terminally phosphorylated Jun are dispensable for this process (Grimm et al., 2001; Hafezi et al., 1999; Rich et al., 1997). Interestingly, photoreceptors expressing Fra-1 in place of Fos are apoptosis competent despite the lack of a potent transactivation domain in Fra-1. This indicates that AP-1 members exert their pro-apoptotic function not only by positive regulation of pro-apoptotic genes but also by suppressing the function of anti-apoptotic genes in response to retinal light damage (Wenzel et al., 2002). Over-expression of Fos also inhibits cell-cycle progression and stimulates cell death in hepatocytes (Mikula et al., 2003). Additionally, Fos represses their anchorage-independent growth driven by oncogenic Ras in vitro and strongly suppresses tumour formation in vivo. These data reflect recent observations indicating that Fos is a mediator of Myc-induced cell death in hepatoma cells deprived of growth factors, an apoptosis process that does not involve p53 (Kalra and Kumar, 2004). Thus, the originally identified *Fos* oncogene could act as a potent tumour suppressor, at least in certain circumstances and cell types. $Fos^{-/-}$ $p53^{-/-}$ double-knockout mice develop highly proliferative and invasive rhabdomyosarcomas, a tumour type rarely observed in p53-null mice. Interestingly, re-expression of Fos in established tumour cell lines enhances apoptosis, which suggests that Fos can be anti-oncogenic in combination with p53 by positively regulating apoptosis-inducing genes or suppressing survival genes (Fleischmann et al., 2003).

Anti-apoptotic function of AP-1 members

Numerous experiments have demonstrated that AP-1, in addition to its pro-apoptotic function, is also critically involved in survival signalling (Fig. 3). For example, Fos expression negatively correlates with increased neuronal cell death in the hippocampus during kainic-acid-induced seizure, indicating an anti-apoptotic role for the protein in this scenario (Zhang et al., 2002). It will be interesting to elucidate the molecular mechanisms and potential target genes involved in the pro- or anti-apoptotic effects of Fos in photoreceptor or neuronal cell death. Another example is that Jun expression is needed to prevent apoptosis in foetal hepatocytes during mouse development (Behrens et al., 1999; Eferl et al., 1999; Hilberg et al., 1993), whereas other AP-1 members, such as Fos, FosB or JunD, are not. Interestingly, the precise function of Jun in hepatocytes seems to depend on their differentiation state, since foetal hepatocytes require Jun for cell survival, whereas differentiated hepatocytes require it for cell-cycle progression (Behrens et al., 2002; Eferl et al., 1999; Hilberg et al., 1993). A similar correlation between function and dependency on differentiation status occurs in the apoptosis program of PC12 cells deprived of nerve growth factor (NGF) (Leppa et al., 2001).

How does Jun distinguish the differentiation status of the cell in these instances? One possibility is that it regulates two classes of target gene that are independently required for proliferation and apoptosis. However, there is precedence for a coupling of cell proliferation and apoptosis, because failure to respond to a proliferation signal can also trigger apoptosis. In the case of Jun deficiency, hepatocytes might be unable to respond to the proliferation signals, and a strong proliferation signal could then trigger cell death in cells that fail to enter the cell cycle. In agreement with this hypothesis, enhanced apoptosis is observed in proliferation-defective keratinocytes that do not express Jun (Zenz et al., 2003).

During liver tumour formation, Jun prevents apoptosis by antagonizing p53 activity, and this might contribute to the early stage of human hepatocellular carcinogenesis (Eferl et al., 2003). Although p53 levels are not changed in Jun-deficient primary hepatocytes, Jun regulates transcription of p53 in mouse fibroblasts (Schreiber et al., 1999). In these cells, the absence of Jun results in elevated levels of p53 and increased expression of its target gene $p21^{Cip1}$, whereas over-expression of Jun represses p53 and $p21^{Cip1}$ expression. Furthermore, Jun can regulate p53 function by inhibiting its binding to the promoter of target genes such as $p21^{Cip1}$ (Shaulian et al., 2000). In this context, recent experiments demonstrate that JDP-2, a newly identified AP-1 member, protects fibroblasts against UV-induced apoptosis through repression of *p53* expression at the transcriptional level (Piu et al., 2001). This suggests that formation of Jun–JDP-2 heterodimers is critical for attenuating p53 transcription in UV-irradiated fibroblasts and possibly other cell types. Although the link between the AP-1 and the p53 pathways appears complex, it seems possible that AP-1 factors have developed multiple ways of regulating p53 action. Under some circumstances, p53 can even induce expression of AP-1 components such as Fos (Elkeles et al., 1999), which suggests that p53 activity can act upstream of AP-1 signalling and adds further complexity to the AP-1/p53 network.

Enhanced apoptosis in the absence of Jun is also observed in keratinocytes and notochordal cells (Behrens et al., 2003; Zenz et al., 2003), demonstrating a crucial role for Jun in survival signalling in a variety of cell types. In the case of keratinocytes, Jun regulates expression of EGFR and its ligand HB-EGF, which controls keratinocyte proliferation, survival and skin tumour formation. Analysis of mice expressing a Jun mutant in which the serine residues phosphorylated by JNK are replaced by alanine residues (Jun-AA) suggests that Jun N-terminal phosphorylation is not required to protect liver tumour cells from apoptosis (Eferl et al., 2003). By contrast, formation of skin and bone tumours induced by a son of sevenless (SOS) homologue transgene, which is a positive regulator of Ras proteins, or a Fos transgene, is reduced in Jun-AA-expressing mice. Thus, phosphorylated Jun appears to regulate a specific set of target genes required for transformation of keratinocytes and osteoblasts but not for survival of liver tumour cells (Behrens et al., 2000; Eferl et al., 2003). N-terminal phosphorylation of Jun is also not required for survival of foetal liver cells (Behrens et al., 1999). Moreover, foetal liver apoptosis can be rescued by a JunB knock-in allele (Passegue et al., 2002).

As discussed above, Jun together with JNK can cause cell death by activating the mitochondrial apoptosis pathway. Under certain circumstances, JNK activation can also signal cell survival (Davis, 2000; Lin, 2003). Mechanisms that account for the dual role of JNK in apoptosis are currently under intense investigation. Recently, Davis and colleagues demonstrated that JunD promotes JNK-stimulated survival after TNF treatment (Lamb et al., 2003). In this situation, the JNK/JunD pathway can collaborate with NF-κB to increase expression of anti-apoptotic genes such as the inhibitor of apoptosis IAP-2. Collaboration between JunD and NF-κB to

increase cell survival accounts for the ability of the JNK signalling pathway to cause either survival or apoptosis, depending on the cellular context. Interestingly, mice lacking JunD are viable and appear relatively healthy, but JunD deficiency renders animals sensitive to stress-induced apoptosis in the liver (Weitzman et al., 2000).

Conclusions

The examples cited above demonstrate the difficulty of drawing clear conclusions about a general physiological role for AP-1 in cellular processes. One has to re-interpret published data derived from in vitro experiments on cell cultures to take into account the more recent in vivo studies. It is quite obvious that the abundance of different AP-1 members within a given cell, as well as the cell lineage, differentiation stage, microenvironment and type of stimulus, has a large impact on how AP-1 modulates the decision of a cell to proliferate, differentiate or die by apoptosis (Figs 2, 3). Only a limited number of unambiguously AP-1-regulated target genes have been documented that can account for its function in these decisions. Thus, identification of further target genes, specifically those that are exclusively controlled by individual AP-1 members, will help to define the precise events that occur in vivo.

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