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invited review

Role and regulation of activator protein-1 in toxicant-induced responses of the lung

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Reddy, Sekhar P. M., and Brooke T. Mossman. Role and regulation of activator protein-1 in toxicant-induced responses of the lung. *Am J Physiol Lung Cell Mol Physiol* 283: L1161–L1178, 2002; 10.1152/ajplung.00140.2002.—Aberrant cell proliferation and differentiation after toxic injury to airway epithelium can lead to the development of various lung diseases including cancer. The activator protein-1 (AP-1) transcription factor, composed of mainly Jun-Jun and Jun-Fos protein dimers, acts as an environmental biosensor to various external toxic stimuli and regulates gene expression involved in various biological processes. Gene disruption studies indicate that the AP-1 family members c-*jun*, *jun*B, and *fra*1 are essential for embryonic development, whereas *jun*D, c-*fos,* and *fos*B are required for normal postnatal growth. However, broad or target-specific transgenic overexpression of the some of these proteins gives very distinct phenotype(s), including tumor formation. This implies that, although they are required for normal cellular processes, their abnormal activation after toxic injury can lead to the pathogenesis of the lung disease. Consistent with this view, various environmental toxicants and carcinogens differentially regulate Jun and Fos expression in cells of the lung both in vivo and in vitro. Moreover, Jun and Fos proteins distinctly bind to the promoter regions of a wide variety of genes to differentially regulate their expression in epithelial injury, repair, and differentiation. Importantly, lung tumors induced by various carcinogens display a sustained expression of certain AP-1 family members. Therefore a better understanding of the mechanisms of regulation and functional role(s), as well as identification of target genes of members of the AP-1 family in airway epithelial cells, will provide additional insight into toxicant-induced lung diseases. These studies might offer a unique opportunity to use AP-1 family members and transactivation as potential diagnostic markers or drug targets for early detection and/or prevention of various lung diseases.

asbestos; tobacco smoke; silica; gene expression; carcinogenesis

AIRWAY EPITHELIUM is the primary target of various mixtures of environmental (habitual, chemical, and biological) toxicants and/or carcinogens. Some of these include tobacco or cigarette smoke (TS), asbestos, silica, ozone, particulates, cytotoxicants, viruses, and bacteria. It has been widely reported that exposure of airway epithelium to these toxins either alone and/or in combination can lead to the development of various respiratory diseases including lung cancer. For instance, exposure to TS causes chronic obstructive lung disease, bronchitis, emphysema, and cardiovascular disease as well as lung cancer (37, 123). Occupational exposure to asbestos has been linked to the development of pulmonary fibrosis, bronchogenic carcinoma, and mesothelioma (95). Exposure of animals to asbestos also causes lung inflammation and injury, which may play a role in asbestos-induced lung diseases (95). Similar to asbes-

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tos, exposure to silica also causes lung inflammation and fibrosis, as well as carcinogenesis, in rodents (28). Environmental toxicants may also act additively or synergistically. For example, TS exposure increases the incidence of asbestos-associated lung carcinogenesis in human populations (94). Prior exposure of mice to TS also impairs naphthalene (a cytotoxicant)-induced bronchiolar epithelial repair with the persistence of squamous cells in terminal bronchioles (141). Although there is considerable evidence documenting the effects of various environmental agents on lung injury and inflammation as well as their causative role in the development of respiratory pathogenesis, the cellular and molecular mechanisms governing these processes remain unclear.

Considerable experimental evidence generated in both tissue culture and animal models indicates that after toxic injury, airway epithelial cells, in a protective response, rapidly undergo changes in their structure and function to repair epithelium. This phenomenon is a very dynamic and multistep process by which epithelial cells rapidly migrate to the injured area, proliferate, and finally differentiate into a normal phenotype to restore regular airway functions (70, 113). However, aberrant cell proliferation and differentiation during this process can result in altered phenotype and tissue dysfunction. Although the molecular responses of the lung to toxic injury remain enigmatic, it has been documented that various toxicants or carcinogens, after interacting with epithelial cells, initiate a cascade of both cellular and molecular reactions that activates various transcription factors. This is accompanied by the induction of a plethora of proinflammatory cytokines, growth factors and their receptors, antioxidant enzymes, proliferation and differentiation markers, etc. (70, 113). However, abnormal expression and/or activation of these transcription factor(s) can deregulate the expression of their downstream target genes, thereby altering normal injury and repair processes, which may lead to the development of pathogenesis. Thus a better understanding of the mechanisms of regulation and role of the transcription factors governing toxicant-induced injury and the repair process is critical in order to develop an effective strategy to modulate respiratory pathogenesis.

It is widely documented that both cellular signaling mechanisms and activation of transcription factors play a pivotal role in regulation of gene expression. Among the transcription factors, activator protein (AP)-1, comprising Jun (c-Jun, JunB, and JunD) and Fos (c-Fos, FosB, Fra1, and Fra2) family members, plays a central role in regulating gene transcription in various biological processes (125). AP-1 family members, also referred to as "immediate-early genes" and "early response proto-oncogenes," directly couple intracellular signals initiated by various external mitogenic and toxic stimuli to regulate gene expression involved in cell proliferation and differentiation, transformation, apoptosis, pulmonary defense, inflammation, immune responses, etc. (Fig. 1) (5). The mitogen-activated protein kinase (MAPK) signal transduction pathway

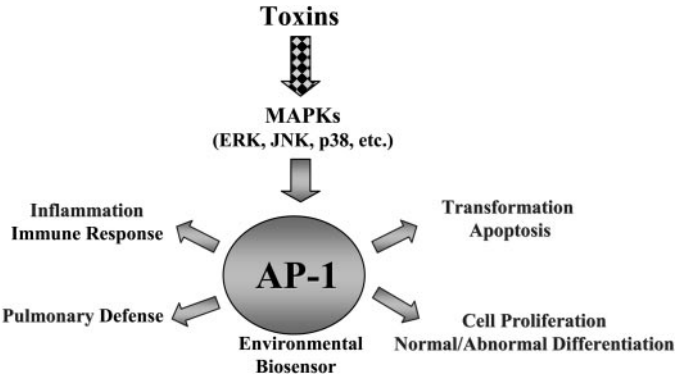

Fig. 1. Activation of activator protein-1 (AP-1) by toxins. Various toxins, after interacting with cells, activate different mitogen-activated protein kinase (MAPK) signaling pathways, which in turn activate the AP-1 transcription factor. Upon activation, AP-1 binds to its target sites located in promoter regions to regulate expression of a wide variety of genes involved in various biological processes. ERK, extracellular signal-regulated kinase; JNK, c-Jun NH_2-terminal kinase.

uses AP-1 as a converging point not only to regulate expression of various genes but also to autoregulate AP-1 gene transcription, thereby increasing their abundance to amplify the signals to various external stimuli. Most importantly, several genes, which play very important roles in injury, repair, and differentiation, contain a bona fide binding site(s) of AP-1 in their promoter and/or enhancer regions (5). Some of the genes include extracellular matrix metalloproteinases (MMPs), antioxidant enzymes, surfactant proteins, growth factors and their receptors, differentiation markers, cytokines, chemokines, other transcription factors, etc. (125).

Several studies performed in both tissue culture and mouse models indicate an essential role for AP-1 proteins in normal cell growth and development (65). Paradoxically, overexpression of some of these proteins results in an overt phenotype(s), including tumor/cellular transformation both in vivo and in vitro, respectively (65). Thus altered AP-1 protein expression and/or activation by toxicants can deregulate gene expression, resulting in aberrant cell proliferation and differentiation, which may lead to the development of various diseases. Although much is known about their involvement in other systems, the mechanisms of activation and individual contribution of AP-1 family members both in lung homeostasis and in the development of toxicant-induced respiratory pathogenesis are unclear. The objective of this article is to provide a brief review on the biology and role of the members of the AP-1 family as well as their mechanisms of activation. In addition, their involvement in the regulation of gene expression involved in lung injury, repair, and transformation is briefly discussed.

BIOLOGY OF AP-1 PROTEINS

AP-1 is a homo- or heterodimer mainly composed of Jun-Jun and Jun-Fos transcription factors that belong to the basic region-leucine zipper (bZIP) group of DNA

binding proteins. The basic region or DNA binding domain (DBD) of bZIP proteins contains positively charged amino acid residues required for DNA binding activity. The leucine-zipper domain (LZD), located immediately downstream of DBD, contains a heptad repeat of leucine residues (Fig. 2*A*). LZD mediates the dimerization of proteins, bringing two DBDs into juxtaposition, thereby facilitating the interaction of protein dimers with DNA (Fig. 2*B*). Unlike Jun proteins, Fos proteins cannot homodimerize among themselves because of a subtle difference in amino acid composition within their LZDs (65). However, they can partner with Jun proteins to form Jun-Fos heterodimers, which are more stable and therefore possess a higher DNA binding activity than the Jun-Jun homodimers. Although LZD and DBD are highly conserved among all AP-1 proteins, their amino (NH_2)- and carboxy (COOH)-terminal regions are quite divergent. The Jun proteins contain the transactivation domain (TAD) at their NH_2-terminal region, whereas Fos members, except Fra1 and Fra2, possess TADs at their NH_2- and COOH-terminal regions (125). Both homo- and heterodimers of AP-1 can bind to the 7-bp palindromic DNA sequence 5′-TGAGTCA-3′, also known as the 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-responsive element (TRE or AP-1 site), in the promoter and/or enhancer regions of variety of genes to regulate their transcription (5). AP-1 can also bind to the variant TREs, such as 5′-TTAGTCA-3′ and 5′-TGATTCA-3′, which somewhat deviate from the perfect recognition sequence by one or two bases (20). However, the AP-1 binding at these sites appears to be mostly influenced by the flanking DNA sequences and cognate binding proteins. In addition, AP-1 proteins can dimerize with other bZIP family of proteins, such as activating transcription factors/cyclic AMP response element binding proteins (ATFs/CREBs). These AP-1 heterodimers preferentially bind to the 8-bp DNA sequence 5′-TGACGTCA-3′, also known as cyclic AMP-responsive element (CRE) (Fig. 2*B*). The CRE has an extra base insertion into the AP-1 consensus recognition sequence TRE. Unlike TRE, the Jun-Jun and Jun-Fos dimers have a lower affinity to the CRE. Although both c-Jun and JunB contain a well-conserved LZD and DBD, unlike the former, the homodimers of the latter bind less efficiently to the promoters/enhancers that have a single TRE compared with the promoters/enhancers that contain multiple TREs in tandem or in close proximity (102). Therefore, it is envisioned that, by virtue of both a selective dimerization and diverse binding specificities, AP-1 proteins distinctly regulate both cell type- and stimulus-specific gene expression involved in various biological processes.

FUNCTIONAL ROLE OF AP-1 PROTEINS

Cell culture studies. AP-1 proteins display a distinct expression pattern during cell cycle progression (71, 74). Upon mitogenic stimulation, the AP-1 complex is mainly composed of Jun-c-Fos heterodimers, whereas Jun-Fra1 and Jun-Fra2 dimers are predominant during exponential cell growth. Moreover, AP-1 proteins differentially regulate the expression of several key players of cell cycle machinery such as cyclin D1, p16, and p53 (91, 125). For example, c-Jun upregulates the promoter activity of cyclin D1, whose expression correlates well with cycle progression, whereas JunB has an opposite effect (7). Importantly, intracellular injection

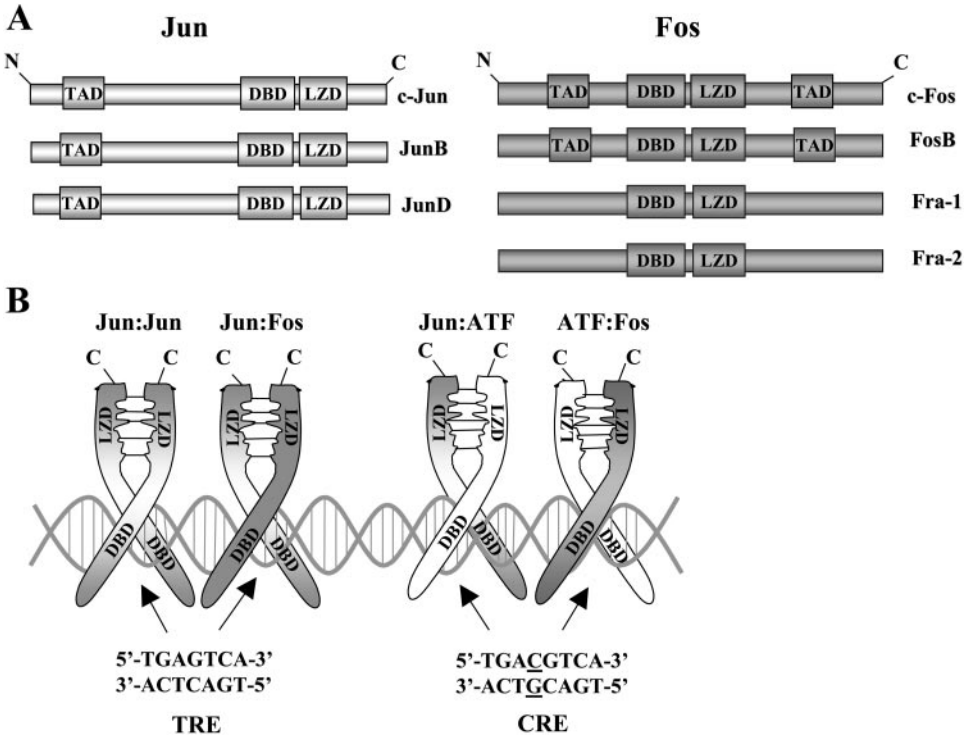

Fig. 2. Schematic diagram showing the modular structures (*A*) and dimerization and DNA binding properties (*B*) of Jun and Fos proteins. *A*: the location of various modules is indicated. TAD, transcription-activating domain; LZD, leucine-zipper domain; DBD, DNA binding domain; N, amino terminus; C, carboxyl terminus. *B*: LZD mediates the dimerization of proteins bringing two DBDs into juxtaposition, thereby facilitating the interaction of protein dimers with DNA. ATF, activation transcription factor; TRE, 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-responsive element; CRE, cyclic AMP-responsive element. Note that CRE has an extra base (underlined) compared with TRE.

of neutralizing antibodies to AP-1 proteins alters cell cycle progression (71, 74), indicating their role in cell proliferation. Consistent with this view, mouse embryonic fibroblasts (MEFs) devoid of AP-1 protein(s) exhibit defects in proliferation and undergo senescence prematurely. Together, these studies underscore a pivotal role for AP-1 family members in cell proliferation.

Knockout studies. Gene ablation (knockout or disruption) approaches have been used most widely to examine the functional role of many protein factors involved in cell growth and development and inflammation as well as transformation. c-*jun* null (−/−) embryos die at ~13 days postcoital (dpc) from multiple defects in neural crest, heart, and liver development (31). Similarly, embryos lacking *jun*B (120) die between 8.5 and 10 dpc due to vascular defects in various extraembryonic tissues. In contrast to c-*jun*$^{-/-}$ and *jun*B$^{-/-}$, *jun*D$^{-/-}$ mice are viable and appear healthy. Although male mice exhibit impaired spermotogenenesis with an altered sperm structure, no fertility effects are noticeable in female mice (133).

Deletion of Fos family members c-*fos,* *fos*B, *fra*1, and *fra*2 results in a distinct phenotype(s). Although c-*fos*$^{-/-}$ mice grow normally, they display impaired gametogenesis, neural development, and oesteogenesis (144). *Fos*B$^{-/-}$ mice have a nurturing defect, but they develop normally without any obvious phenotype (41). Interestingly, similar to *jun*B, mice lacking *fra*1 die at 10 dpc because of defects in placenta and yolk sac (122), indicating some overlapping functions between JunB and Fra1. The deletion studies of *fra*2 await further investigation to determine its phenotypic effect(s) in vivo. Although JunD, c-Fos, and FosB are required for proper postnatal development, mice lacking these proteins apparently display no abnormal phenotypes in the lung (65). On the other hand, the role of c-Jun, JunB, and Fra1 in lung growth and development is not clearly established because of an early embryonic lethality of these knockouts.

"Knockin" and transgenic complementation studies. Intriguingly, deletion of specific AP-1 members does not result in compensatory upregulation of other members, indicating some functional redundancy among Jun and Fos proteins (65). To address this issue, investigators have used knockin and/or transgenic complementation approaches in which another gene is replaced or ectopically overexpressed in mice lacking a particular AP-1 gene. The knockin of *jun*B cDNA in the c-*jun* locus rescues both liver and cardiac defects observed in c-*jun*$^{-/-}$ mice during development (102). However, mice do not survive postnatally because of certain defects in cardiac outflow. Interestingly, c-*jun*$^{-/-}$ mice overexpressing the *jun*B transgene develop normally and can survive up to 7 mo, indicating that JunB, if present in sufficient amounts, can rescue main defects that were noticed in the absence of c-Jun (102). Although c-*fos*$^{-/-}$ mice develop osteopetrosis, substitution of *fra*1 in the c-*fos* locus restores the phenotypic defect. Consistent with this, ectopic expression of *fra*1 also restores an abnormal phenotype in c-*fos*$^{-/-}$ mice (64). These results indicate an overlapping function between JunB and c-Jun, and Fra1 and c-Fos. It is not clear why the presence of *jun*B in c-*jun*$^{-/-}$ mice and vice versa cannot restore each other's functions during development. One possible explanation for this paradox, as suggested by Passegue et al. (102), is either the lack of sufficient amounts of respective protein or incomplete activation of their target genes to restore normal functions. For example, c-Jun and JunB differ significantly in both their DNA binding and transactivation potential as well as their target gene regulation (26).

Transgenic overexpression studies. Overexpression of the individual members of Jun and Fos in transgenic mice under the control of a ubiquitous and/or targeted promoter displays specific phenotypes. Broad overexpression of c-*jun* (39) and *jun*B (119) does not result in an abnormal phenotype, whereas *jun*D overexpression studies have not been reported yet. Intriguingly, targeted expression of *jun*B using a CD4 promoter interferes with T helper (Th) cell differentiation (78). In these studies, Th1 cells overexpressing JunB display a higher level of Th2 cytokines, such as IL-4 and IL-5. Consistent with this, *jun*B expression levels are selectively induced in Th2, but not Th1, cells during differentiation. Moreover, JunB activates IL-4 transcription. Together, these observations strongly support a critical role for JunB in T cell differentiation.

The ectopic expression of c-*fos* results in mainly osteosarcomas, malignant bone tumors, due to transformation of osteoblasts (39). However, *fos*B overexpression has no effects (39). Overexpression of Δ*fos*B, a naturally occurring truncated form of FosB that lacks the TAD, results in osteosclerosis, an increase in bone formation (116). Similarly, targeted specific expression of Δ*fos*B in thymocytes using a T cell receptor promoter disrupts normal T cell differentiation (15). Transgenic mice overexpressing *fra*1 under the control of a ubiquitous promoter mainly display osteosclerosis. Intriguingly, authors have also noted the presence of a few lung tumors and liver cirrhosis in these mice (64). In contrast to *fra*1, the ectopic expression of *fra*2 causes corneal abnormalities; however, no overt phenotypes or tumor formation are noticed in other organs (89).

Collectively, these genetic models, summarized in Table 1, strongly suggest that abnormal expression and/or activation of specific AP-1 proteins can lead to the development of distinct phenotypes. Intriguingly, the above studies also indicate some functional redundancy among AP-1 family members. This fact and the fact that the regulatory (promoter) regions of AP-1 components are not well conserved (see TRANSCRIPTIONAL REGULATION OF AP-1 FAMILY MEMBERS) underscore a potential role for both cell type- and toxicant-specific mechanisms in regulating AP-1 expression in lung injury, repair, differentiation, and transformation.

AP-1 FAMILY MEMBER EXPRESSION IN LUNG CELLS DURING DEVELOPMENT AND NEOPLASIA

The basal level of expression of members of the AP-1 family is quite different in various tissues and cell types during embryonic and postnatal development

Table 1. *Summary of biological functions of activator protein-1 as analyzed by genetic models*

	Knockout Phenotype			Transgenic Phenotype				Knock in and/or Complementation	
Gene	Other tissues	Lung	Ref.	Promoter	Other tissues	Lung	Ref.	Functional redundancy	Ref.
c-Jun	Embryonic death liver and heart defects	?	31	H2K^b	None	None	39	JunB	102
JunB	Embryonic death (defects in extraembryonic tissues)	?	120	Ubiquitin C	None	None	119		
				CD4	Enhanced Th2 maturation		78		
JunD	Male sterility	none	133	*Not reported*	*Not reported*	?			
c-Fos	Osteopetrosis	none	144	H2K^b	Osteosarcoma	None	39	Fra1	64
FosB	Nurturing defect	none	41	H2K^b	None	None	39		
ΔFosB		?		TCRb		Impaired T cell differentiation	15		
				NSE	Osteosclerosis	None	116		
Fra1	Embryonic death (defects in extraembryonic tissues)	?	122	H2K^b	Osteosclerosis, liver cirrhosis	Few lung tumors	64	JunB	120
Fra2	Not reported	?		CMV	Occular malformations	None	89		

H2K, histocompatibility complex class I antigen H2-K; TCR, T cell receptor; NSE, neuronally specific enolase; CMV, cytomegalovirus.

and adulthood. In general, the mRNA levels of c-*jun*, *jun*B, and *jun*D are high compared with *fos* family members in various tissues including the lung. c-*jun* transcripts are detectable during mouse lung development at 15.5–17.5 dpc (146), whereas expression in postnatal and adult mouse lungs is quite abundant (102). In contrast to *jun* family members, the expression of *fos* family members, with the exception of *fra*2, is very low during development and in adult lungs (97). During development, c-*fos* expression can be noticeable in developing bronchioles but not in mesenchyme of 14-dpc embryos. However, its expression decreases in 17-dpc bronchioles but is sporadically present in mesenchyme (96, 146). Immunohistochemical analysis of 17.5-dpc embryos reveals the localization of FosB in bronchial epithelial cells and endothelial cells of the mouse lung (41). However, its expression, with the exception of bone, is very low or undetectable in various adult mouse tissues including lung (154). Similar to *fos*B, the expression of *fra*1 is very low but can be detectable by RT-PCR in various adult mouse tissues, including the lung (13, 23, 64). The expression of *fra*2 is very low around midgestation but can be detected in the epithelial cells of the trachea and bronchi of 16.5-dpc embryos. In adult tissues, *fra*2 expression is somewhat similar to that of *jun* members with abundant localization in various differentiated epithelia, including the lung (13). It is noteworthy that the levels of JunB and JunD, but not c-Fos and c-Jun, are higher in the lungs of neonates (~12 h old) compared with adult rats, indicating certain differences in the expression pattern of Jun and Fos proteins during lung development (149). In another study, it was shown that at 14 dpc the expression of c-*fos* is low, whereas at birth its expression significantly higher compared with 14-dpc rat lungs (21). Conversely, compared with 17-dpc lung, c-*fos* expression in 14-dpc and neonatal hypoplastic lungs is very low (21). In summary, differential expression of *jun* and *fos* proto-oncogenes occurs during embryonic and postnatal development as well as in adult tissues.

Jun and *fos* expression is also variable between different normal and malignant cell lines. Consistent with in vivo observations, RNase protection analysis reveals high-level expression of c-*jun*, *jun*B, *jun*D, and *fra*2 in the nontransformed mouse type II alveolar epithelial cell line C10, whereas the expression of c-*fos*, *fos*B, and *fra*1 is very low or undetectable (126). However, detectable amounts of c-*fos* and *fra*1, in addition to c-*jun*, *jun*B, and *jun*D, were noticed in normal human bronchial epithelial (HBE) cells (76). Intriguingly, malignant HBE cells variably express AP-1 proto-oncogene expression. One study demonstrates significantly lower levels of *jun*B, c-*fos*, and *fra*1 mRNA in malignant HBE cells compared with normal cells (11). In contrast, a different study shows a high but a variable expression of c-*jun*, *jun*B, *jun*D, and c-*fos* message levels in various human lung cancer cell lines (130). Immunohistochemical analysis of various neoplastic human lung tissues reveals a high level of expression of *c-jun* antigen in atypical, hyperplastic, and metaplastic epithelium, whereas its expression in surrounding normal bronchial and alveolar epithelia is very low or undetectable (130).

TRANSCRIPTIONAL REGULATION OF AP-1 FAMILY MEMBERS

In response to various toxic and mitogenic stimuli, *jun* and *fos* mRNA expression is rapidly induced severalfold above the basal level in a wide variety of tissues and/or cell types. In general, the mRNA levels of c-*jun*, *jun*B, *jun*D, c-*fos*, and *fos*B peak within 15–30 min of stimulation and return to basal level within 1–2 h (5, 48). The induction of *fra*1 and *fra*2 mRNA expression mainly occurs between 30 and 60 min, peaking at 90–180 min. However, the mRNA expression remains elevated above basal level for 2–24 h, depending upon the stimuli (48).

Intriguingly, although all AP-1 members are rapidly induced, albeit with somewhat different kinetics, the 5′-flanking regions of AP-1 family members are not

well conserved. They contain both common and distinct regulatory *cis*-acting elements, including their own target sites (5, 48, 145). The proximal promoter of the c-*jun* gene is highly conserved among species and contains a TATA-like sequence, CAAT box, Sp1 site, and three CREs (Fig. 3*A*). Genomic footprinting analysis in living cells reveals that these sites are constitutively occupied in vivo (115) and respond rapidly to various stimuli, such as TPA, insulin, and epidermal growth factor (EGF), to induce c-*jun* transcription. Such induction is mediated mainly by the c-Jun-ATF-2 heterodimer binding to the CRE (68). This indicates that c-Jun positively regulates its own transcription. In addition to CRE, the protein binding at the myocyte binding site located at −59 also regulates c-*jun* transcription (44). Similar to c-*jun*, the *jun*B promoter is also well conserved and contains a TATA box, CAAT box, Sp1 site, and a CRE (91). In addition, it contains signal transducer and activator of transcription (STAT) binding sites, multiple Ets transcription factor binding sites (EBSs), and a serum-responsive element (SRE). The SRE mediates the growth factor- and serum-inducible *jun*B transcription through recruitment of ternary complex factor (TCF). Recent studies show that, in addition to the proximal CCAAT box (32), Smad binding elements located at −2,980 to −2,611 regulate transforming growth factor (TGF)-β-induced *jun*B transcription (66). The proximal promoter of mouse *jun*D also contains a TATA box, CAAT box, Sp1 site, and two CREs (91). Unlike c-*jun* and *jun*B, the transcription of *jun*D does not appear to be inducible by various stimuli, including TPA and growth factors (91). This is mainly attributed to the presence of a functional octomer motif. This motif is uniquely present in *jun*D promoter and constitutively occupied by a ubiquitous protein, Oct-1 (91).

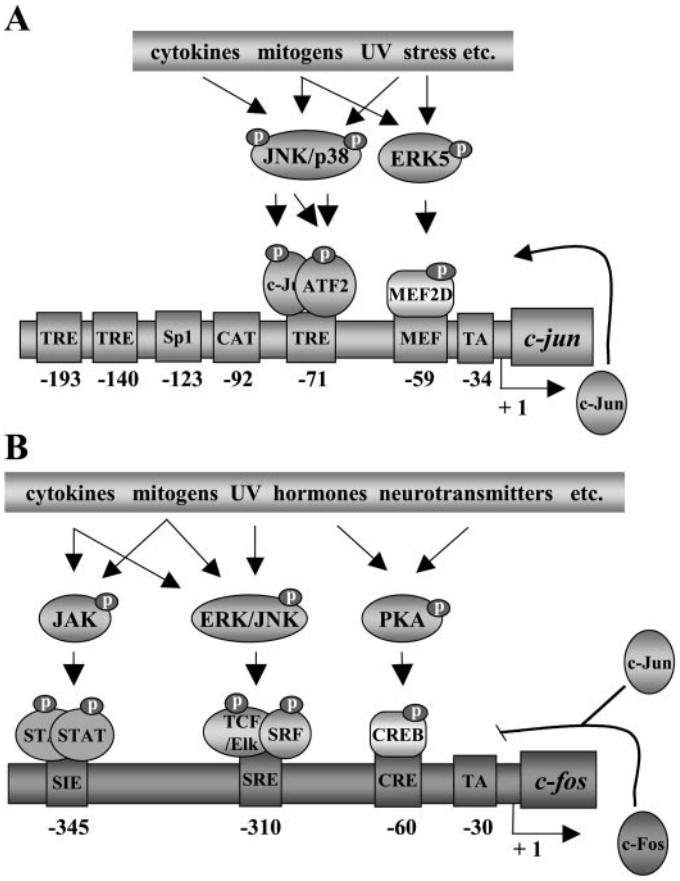

Fig. 3. Transcriptional regulation of members of the AP-1 family. Various extracellular signals initiating signaling pathways that activate c-*jun* (*A*) and c-*fos* (*B*) transcription are shown. The major *cis*-elements located with relative to the transcriptional start site (+1) are depicted. These include TA, TATAA box; CAT, CCAAT protein binding site; CRE, cyclic AMP-responsive element; MEF, myocyte enhancer factor binding site; SIE, sis-inducible element/enhancer; SRE, serum response element; Sp1, Sp1 binding site; TRE/CREB, CRE-binding protein; SRF, serum response factor; STAT, signal transducer and activator of transcription. The external stimuli distinctly activate various MAPKs, such as ERK, JNK, Janus kinase (JAK), and protein kinase A (PKA), which then translocate into the nuclei and phosphorylate specific transcription factors leading to the stimulation of target gene transcription. P, phosphorylation site. Arrows indicate the positive activation of the response; blunt line indicates an inhibition of the response.

Among the Fos family members, the transcriptional stimulation of c-*fos* is most extensively studied. The promoter of c-*fos* contains several *cis*-elements. The most notable ones include a CRE, SRE, and the sis-inducible element/enhancer (SIE) (24) (Fig. 3*B*). The SRE is generally bound by the serum response factor (SRF). SRF plays a major role in the recruitment of TCF after serum and growth factor stimulation. Upon activation by various MAPKs, Elk1 and SAP-1, which belong to the family of Ets transcription factors, are recruited at an SRE site to form TCF with the bound SRF, thereby inducing c-*fos* transcription (5, 24, 145). The SIE located distally to SRE also plays a role in cytokine- and growth factor-inducible c-*fos* transcription. In response to various cytokine stimuli, STAT1 and -3 bind at the SIE and act in concert with SRE to stimulate c-*fos* transcription. The CRE located at −70 to −50 mediates cAMP and Ca^{2+}-inducible c-*fos* transcription (145). The most notable difference between c-*jun* and c-*fos* transcriptional regulation is that the former is positively autoregulated by its own product, whereas the expression of the latter is suppressed by Jun-Fos dimers (5, 24, 145). The proximal promoter of *fos*B contains a TATA box, SRE, and TRE, which are located at nearly identical positions and in the same order as the c-*fos* promoter. Similar to c-*fos*, *fos*B transcription is also suppressed by its own protein and c-Fos (75).

The proximal promoter of human and mouse *fra*1, although moderately conserved with that of c-*fos* (70%), does not contain a TATA box and a consensus DNA sequence of SRE (138). However, its expression can be strongly inducible after serum stimulation in airway epithelial cells (S. P. M. Reddy, unpublished data). Unlike c-*fos* and *fos*B, *fra*1 transcription can be upregulated by most of the AP-1 family members, including its own product (9, 121). Moreover, some studies have shown that a 50-bp fragment of the first intron containing a perfect TRE and two variant TREs can mediate the transcriptional regulation of the mouse and rat *fra*1 gene (9, 121). Although this is

unique among *fos* family members, examination of the human genomic sequence of *fra*1 did not reveal a similar sequence in its first intron. However, results from our laboratory indicate that TPA-, EGF-, and serum-inducible *fra*1 transcription in A549 cells is mainly mediated by the −379- to −283-bp DNA fragment containing multiple motifs such as AP-1, EBS, and Sp1 (3). Unlike *fra*1, the mouse and rat *fra*2 promoter contains a TATA box, two TREs, CRE, SIE, and SRE (128). The TREs located immediately downstream of the start site are required for serum-inducible *fra*2 transcription (128). In unstimulated cells, the TREs are weakly occupied by the c-Jun-Fra2 dimers. Upon stimulation with serum, c-Jun-c-Fos dimers form a strong complex that is replaced by c-Jun-Fra2 dimers at later time periods (128). Similar to *fra*1, *fra*2 transcription is positively autoregulated by AP-1 proteins.

It is noteworthy that although Jun and Fos mRNA levels are rapidly induced, this is not reflected at the protein level (5, 145). Moreover, treatment of cells with cycloheximide also enhances AP-1 family member expression. Thus, in addition to transcription, both post-transcriptional and posttranslational modifications also regulate AP-1 expression (5, 145).

In summary, the presence of both common and distinct regulatory elements in the 5′-flanking regions of AP-1 family members, the fact that their transcription is differentially (positively or negatively) regulated by themselves and other proteins, and the existence of some functional redundancy among AP-1 proteins as demonstrated by in vivo genetic models strongly suggest that both spatial expression and regulation of AP-1 family members may play a central role in toxicant-induced injury, repair, and/or cellular transformation.

AP-1 ACTIVATION BY MAPKS

It is well documented that different signaling cascades initiated by various extracellular stimuli converge at AP-1, which then regulates its own transcription as well as the transcription of other genes required for various biological processes (27). Among various MAPKs, the c-Jun NH_2-terminal kinases (JNKs) and extracellular signal-regulated kinase (ERKs) mainly phosphorylate Jun and Fos proteins, respectively (27). Although Jun and Fos proteins are rapidly induced by various stimuli, most cells possess a certain amount of preexisting Jun and Fos proteins that are initial targets for JNK and ERK MAPKs (145). Upon their activation, ERKs and JNKs phosphorylate both preexisting and newly synthesized AP-1 proteins. The activated JNKs bind to the docking site located in the NH_2-terminal region of c-Jun and phosphorylate Ser63 and -73 located within its transactivation domain (145). Due to its higher affinity to the docking site, JNK2 phosphorylates c-Jun more efficiently than JNK1 (42). JunB also contains the JNK docking site but lacks NH_2-terminal acceptor serine residues and therefore appears to be poorly activated by JNKs. However, a recent study shows phosphorylation of Thr102 and -104 of JunB by JNKs in COS-7 cells and a B-lymphoma cell line, M12 (78). Although it contains a putative serine residue, JunD cannot be phosphorylated by JNKs due to the lack of a functional JNK docking site within its NH_2-terminal region. However, after dimerization with c-Jun, JunD can be phosphorylated on its serine residue by the JNK (67). Because of these differences, although they contain similar DNA recognition properties and binding activities, Jun proteins apparently have different transactivating characteristics (42, 145).

In contrast to Jun proteins, which are mainly phosphorylated by JNKs within their NH_2-terminal region, Fos proteins are mainly phosphorylated by ERKs on serine and/or threonine residues located within their COOH-terminal domain. Upon activation, ERK MAPKs translocate into the nuclei and phosphorylate c-Fos on Ser374 (19), whereas FosB appears to be phosphorylated on several serine residues: 284, 297, 299, 302, and 303 (127). In addition, Fos-regulating kinase and ribosomal S-6 kinase phosphorylate c-Fos on Ser133 and Thr232 (25) and Ser362 (19), respectively. ERK1 also phosphorylates Fra1 both in vitro and in vivo (40). Upon mitogenic stimulation, Fra1 displays different band sizes, as analyzed by Western analysis, indicating the existence of multiple phosphorylation sites. A recent study indicated the requirement of ERK-dependent phosphorylation of Thr231 of Fra1 for mitogen-activated epidermal transformation (150). Similar to Fra1, Fra2 contains putative serines and threonines (three each) that are extensively phosphorylated by the mitogen-activated protein/extracellular signal regulated-kinase (MEK)/ERK pathway both in vivo and in vitro (99).

Although p38 MAPKs do not activate AP-1 proteins directly, they can regulate *jun* and *fos* transcription by phosphorylating ATF-2, Elk1, SAP-1, and CCAAT enhancer binding proteins (C/EBPs), which then bind to the promoter elements of *jun* and *fos* and regulate their transcription. Regulation of c-*jun* transcription by ERK5 (also known as big mitogen-activated kinase 1) has been demonstrated (86). ERK5 plays a regulatory role in proliferation and differentiation and regulates c-*jun* expression through the activation of MEF-2D (86), which binds at the c-*jun* promoter to stimulate its transcription (44). In addition to various MAPKs, AP-1 protein phosphorylation by other kinases, including protein kinase (PK) C, PKA, casein kinase II, and Cdc2, has been documented, suggesting their potential roles in the activation of AP-1 proteins (145).

REGULATION OF AP-1 GENE EXPRESSION AND/OR ACTIVATION BY TOXICANTS

TS contains a wide variety of compounds that are both volatile and nonvolatile. Some of the major volatile components include acrolein, napthalene, aldehydes, and hydrogen peroxide (H_2O_2), whereas the nonvolatile fraction contains tumor promoters such as phorbol ester analogs and carcinogens such as aflatoxin B1, benzopyrene, and 4-(methyl-*N*-nitrosamino)-

1-(3-pyridyl)-1-butanone (NNK) (52). A variety of exposure protocols are being used to study the effects of smoke on the cells of the lung both in tissue culture and animal models. Some of these protocols include exposure of cells or animals directly to mainstream TS (MTS), TS bubbled through phosphate buffer solution [called TS extract (TSE)], or aged and diluted sidestream TS [referred to as environmental TS (ETS)]. Exposure of rats to MTS (1 h/day, 5 day/wk for 8 wk) enhanced c-Fos, but not c-Jun, expression in the terminal bronchioles. This was associated with the increased levels of MEK1 and ERK2 but no effect on MEK kinase 1 and p38 protein levels (18). However, a different study showed elevated levels of both c-Jun and c-Fos in the lung tissues of MTS-exposed (6 mo) ferrets (82). Moreover, elevated levels of c-Jun and c-Fos were positively correlated with the expression of proliferating cell nuclear antigen and squamous metaplasia in bronchial epithelium (82). TSE also enhanced c-*fos* mRNA levels in fibroblasts (98). Likewise, the exposure of A549 cells to TSE significantly enhanced AP-1 binding activity (110). The latter studies show that supplementation of cells with antioxidants or superoxide scavengers before TSE exposure attenuated c-*fos* induction (98) and AP-1 DNA binding activity (110), indicating a role of reactive oxygen species (ROS) in AP-1 induction. In contrast to A549 cells, TSE had no effect on AP-1 DNA binding activity in a human premonocytic cell line, U397 (34), indicating the existence of cell type-specific responses to TS.

Although studies above examined the effects of TS mainly on c-*jun* and c-*fos* expression, little is known with regard to other members of AP-1 family. Recently, we showed that Fra1 and Fra2 distinctly regulate transcription of the squamous differentiation marker *SPRR1B* in HBE cells (103). Because inhalation of TS causes squamous metaplasia in airways, the effects of MTS and ETS on AP-1 gene expression in HBE cells were investigated in tissue culture and animal models, respectively. Exposure of primary cultures of HBE or A549 cells to MTS markedly enhanced *fra*1 mRNA levels at 5 h, which remained elevated through 14 h, while *fra*2 message levels were unchanged and/or slightly reduced (112). Exposure of mice to ETS (6 h/day, 5 days) considerably induced *fra*1 mRNA levels in the lungs compared with the filtered air-exposed control group. In contrast, TS showed no effect on *fra*1 expression in the liver. Moreover, immunohistochemical analysis revealed an elevated level of Fra1 in both bronchial and alveolar type II epithelial cells of ETS-exposed mouse lungs, further corroborating mRNA levels. ETS did not alter *jun*B or c-*fos* mRNA levels, whereas a slight reduction ($< 30\%$) in c-*jun* message levels was noticeable after ETS exposure (112). Together, these results indicate that TS differentially regulates the induction of members of the AP-1 family in HBE cells. However, the discrepancy observed in the expression pattern of *jun* and *fos* members in lung cells after exposure to TS in the above studies may be related to differences in the exposure protocols or in the animal models as well as the detection methods. The individual contribution of AP-1 family members in smoke-induced injury and in the pathogenesis of the lung diseases, as well as downstream target genes, needs more thorough investigation.

Asbestos is a fibrogenic dust and potent inducer of pulmonary fibrosis as well as carcinogenesis. Asbestos fibers differentially upregulate *jun* and *fos* mRNA expression in the cells of the lung both in vivo and in vitro (85). Most notably, asbestos fibers cause a sustained induction of c-*jun* and c-*fos* mRNA expression as well as AP-1 DNA binding activity in pulmonary epithelial cells and mesothelial cells (53, 60, 135). Moreover, the increase in c-*jun* transcription positively correlates with cell proliferation and transformation of asbestos-exposed tracheal epithelial cells both in vivo and in vitro (108, 136). Recent studies have shown a protracted induction of *fra*1 mRNA both in asbestos-induced rat lung tumors (117) and in asbestos-exposed rat mesothelial cells (117), as well as in mouse pulmonary epithelial cells (126). The latter study revealed the mRNA expression of c-*jun*, *jun*B, and c-*fos* to be somewhat less protracted than that of *fra*1, whereas no change in the expression of *fra*2 and *jun*D was detected after the asbestos exposure (126). Studies performed by the same laboratory have also shown that the induction of *jun* and *fos* by asbestos is oxidant dependent (63) and mainly mediated through the EGF receptor (EGFR), a tyrosine receptor kinase, which activates the ERK/MAPK pathway (152, 153). In support of this notion, pretreatment of cells with EGFR antibodies, GSH, antioxidants, and/or pharmacological inhibitors of the MEK1/2 pathway significantly attenuates asbestos-inducible *jun* and *fos* expression (85). In contrast to the above studies, cDNA microarray analysis reveals an inducible (fourfold) expression of c-*fos,* but not c-*jun and fra*1, in asbestos-induced tumorigenic human bronchial cells, indicating certain species-specific differences in responses to asbestos (155). In a transgenic mouse model that expresses the luciferase reporter gene under the control of AP-1 binding sites or TREs, a strong AP-1 transactivation by asbestos was demonstrated in the lung and in bronchiolar and alveolar type II epithelial cells (29, 93). Together, these studies indicate that a protracted induction of certain AP-1 family members, such as Fra1, in epithelial cells of the lung after asbestos exposure can lead to deregulation of cell proliferation and differentiation and the development of pulmonary disease.

Similar to asbestos, silica selectively upregulates the expression of c-*jun*, *jun*B, c-*fos,* and *fra*1 message levels in pulmonary epithelial cells, whereas the expression of *jun*D and *fra*2 remained unchanged (126). Most notably, the *jun*B and *fra*1 message levels persist over 24 h, indicating a potential role for these proteins in silica-induced pathogenesis. Furthermore, the induction of *jun* and *fos* expression is accompanied by an increase in AP-1 DNA binding activity and AP-1 transactivation associated with alterations in cell cycle progression (126). Indeed, the expression of *fra*1 correlates with cells entering into S phase (126). The increase in AP-1 expression and DNA binding activity correlates

with sustained phosphorylation and activation of JNK but not p38 and ERK MAPKs (126). Pretreatment of cells with intracellular scavengers suppresses both silica-induced JNK activity and *fra*1 mRNA levels, suggesting a role for oxidative stress in this process (126). Silica-inducible AP-1 activation was also confirmed in a mouse model. Intratracheal instillation of freshly prepared crystalline silica to transgenic mice carrying a reporter luciferase gene under the control of TREs markedly elevated AP-1 transactivation in the lung (30). Although patterns of asbestos- and silica-induced AP-1 proto-oncogene expression are nearly identical and mediated by oxidative stress, how they activate upstream MAPK signaling cascades and their downstream target genes is unclear.

A growing body of evidence suggests an association between exposure to airborne particulate matter (PM) and increases in respiratory morbidity and mortality (2). Links to the development of lung cancers and increases in asthma, chronic bronchitis, and pneumonia in predisposed individuals suggest that injury to respiratory epithelium is an initiating factor in the development of these diseases. The composition of PM is complex, consisting of soluble agents, insoluble particles, metals, and contaminants such as endotoxin. In alveolar type II epithelial cells, endotoxin-free PM samples show a modest but significant transcriptional activation of AP-1-dependent genes compared with the known pathogenic fiber, asbestos, and earlier, transient increases (1 h) in JNK1 activity and phosphorylated Jun protein (134). These increases occur in the presence of increased incorporation of 5′-bromodeoxyuridine by epithelial cells, which later proved to be indicative of cell proliferation (137). In these recent studies, the development of dose-related proliferation and apoptosis occurs with unique patterns of *jun* and *fos* family member expression. For example, more protracted increases (24 h) in c-*jun, jun*B*, fra*1*,* and *fra*2 are seen at lower, proliferative concentrations of PM, whereas transient induction (2 h) of all *jun* and *fos* family members occurs at apoptotic concentrations of PM. The ultrafine particulate component of PM appears to be important in eliciting both proliferation and increases in expression of AP-1 family members, whereas fine titanium dioxide and glass beads (nontoxic particles) have no effect.

ROS and reactive nitrogen species (RNS), such as peroxynitrite and nitric oxide (NO), are also components of air pollution and cause acute damage to respiratory epithelial cells. In rat alveolar epithelial cells, different species of RNS or ROS cause AP-1 transactivation, which correlates with their ability to induce membrane permeability or apoptosis (61). For example, NO generation or exposure to H_2O_2 causes increased c-*jun* and c-*fos* mRNA/protein levels and AP-1 DNA binding, leading to increased membrane permeability and apoptosis. In contrast, exogenously administered peroxynitrite does not induce toxicity or induction of early response genes despite increased nitration of tyrosine, a signature of exposure (61).

In conclusion, the results above suggest that increased *jun* and *fos* family member expression and AP-1 transactivation may be sensors for environmental stresses in the pulmonary epithelium, most importantly those that cause injury to and proliferation of epithelial cells. The lack of effect of nontoxic particles or inactive analogs in these models is especially encouraging.

REDOX REGULATION OF AP-1 ACTIVATION

The redox state of the cysteine moieties of various proteins, including transcription factors such as NF-κB and AP-1, plays a regulatory role in various biological processes, including gene regulation (72). The AP-1 proteins Jun and Fos contain cysteine residues both in the DBD and in the flanking NH_2- and COOH-terminal regions (Fig. 4). Human c-Jun contains three cysteine residues, located in the DBD, downstream of the LZD, and in the NH_2-terminal region. Oxidation of the cysteine residue located in the DBD leads to intermolecular disulfide formation or *S*-glutathionylation of c-Jun, thereby inhibiting its DNA binding activity (69, 72). Similar to c-Jun, the oxidation of Cys154 on human c-Fos modulates its DNA binding activity (1). Supplementation of cells with antioxidants such as GSH, thioredoxin (TRX), and redox factor-1 (Ref-1), which promote a reducing environment, enhances AP-1 DNA binding activity (55); conversely, the oxidized form of GSH, GSSG, has an opposite effect (69, 72). Whether redox regulation modulates DNA binding activities of other AP-1 proteins is unknown. However, all of them contain several cysteine residues located both within the DBD and downstream of the LZD (Fig. 4). The well-conserved nature of the two cysteine residues in both Jun and Fos families of proteins suggests a potential a role for redox regulation in the modulation of AP-1 DNA binding activity in various cell types, including the lung.

Paradoxically, exposure of cells of the lung to agents such as H_20_2, TNF-α, TS, asbestos, and silica, which are potent inducers of oxidative stress, stimulates both the DNA binding activity as well as the transcription of AP-1 proto-oncogenes. For example, TSE, which causes depletion of intracellular GSH levels, increases AP-1 DNA binding activity, which correlates with the transcription of γ-glutamylcysteine synthetase (γ-GCS), a regulator of GSH synthesis, in A549 cells (110). Similar to GSH and *N*-acetyl-L-cysteine (NAC), TRX acts as an antioxidant. TRX is a ubiquitously expressed protein and contains two functional redox-active cysteine residues that play an important role in redox-dependent gene expression in various cell types. The expression level of TRX is high in airway epithelia (46). After translocation into the nucleus, TRX activates Ref-1, which in turn reduces the cysteine residues of the Jun and Fos proteins thereby stimulating AP-1 DNA binding activity (55). Recently, it was shown that Ref-1 modulates granulocyte-machophage colony stimulation factor-induced AP-1 DNA binding in human alveolar macrophages (35). Recent studies also indicate

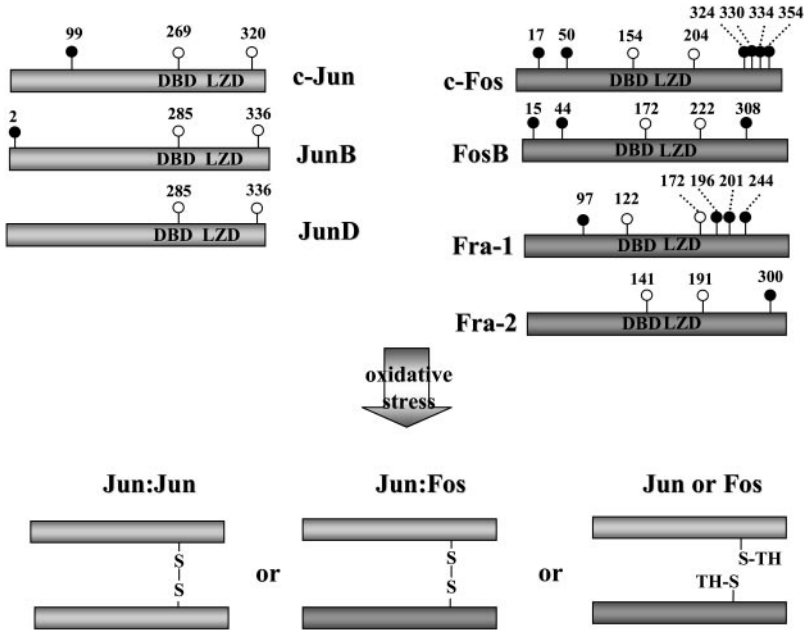

Fig. 4. The redox regulation of AP-1 proteins by oxidative stress. The diagram represents the modular structures of human Jun and Fos proteins. Circles with numbers indicate the cysteine residues that might participate in the redox regulation. Open circles represent the cysteine residues located in DBD and LZD of AP-1 proteins. Oxidation of dimeric proteins can lead to either a disulphide bond (S-S) or thionylation (S-TH) formation, thereby inhibiting their biological functions.

that TRX, NAC, and GSH exhibit differential effects on the activation of redox-dependent transcriptional activity. For instance, TRX inhibits NF-κB activation in the cytoplasm (56, 131), whereas in the nucleus it stimulates NF-κB transcriptional activity (46). Thus it appears that compartmentalization and close proximity, as well as the availability of the GSH, TRX, Ref-1, and other reducing agents to oxidized cysteine residues probably contribute the ultimate physiological response, depending on the toxic stimuli and cellular context (69, 109).

REGULATION OF GENE EXPRESSION BY AP-1 IN LUNG CELLS

After toxic injury, airway epithelial cells dedifferentiate, flatten, rapidly migrate, and proliferate to repair the injured area. During this process, extracellular matrix (ECM) protein deposition and degradation also play a key role in restoring normal cellular structures (70, 113). A plethora of genes coding for proinflammatory cytokines, growth factors and their receptors, antioxidant enzymes, and proliferation and differentiation markers, which participate during the injury and repair process, contain functional AP-1 binding site(s) or TREs in their promoter regions (5, 6). Although various studies demonstrate a correlation between the candidate gene expression and AP-1 DNA binding, relatively few studies have examined the role of individual members of the AP-1 family in detail. Here we discuss genes whose expression and regulation by AP-1 proteins have been investigated after toxicant-associated injury, repair, and cellular transformation.

Differentiation markers. Upon exposure to toxicants and/or carcinogens, proximal and distal alveolar epithelial cells can lose their normal secretory functions and express squamous and keratinizing properties. Surfactant proteins (SP-A to -D) and Clara cell secretory protein (CCSP) play important roles in pulmonary function, host defense, and innate immunity of the lung. Although Nkx2.1 (or TTF-1) and HNF-3 (Fox) family members mainly regulate their expression, AP-1 proteins also distinctly regulate *SP-A, -B, -D,* and *CCSP* transcription in alveolar epithelial cells. The suppression of *SP-A* and *-B* transcription by PMA and TNF-α, potent inducers of AP-1 activation, is attributed in part to the cytoplasmic trapping of Nkx2.1 as well as to differentially enhanced binding of AP-1 proteins (104, 107). The suppression of *SP-A* transcription by TPA correlates with an enhanced Jun binding to the functional TRE located in the intron region (57). Intriguingly, overexpression of *jun*D upregulates mouse *SP-B* promoter activation, whereas c-*jun* and *jun*B have opposite effects (124). Likewise, c-Jun, ATF-2, and CREB binding to −81 CRE located adjacent to HNF-3 and Nkx2.1 sites suppresses rabbit *SP-B* transcription (10). Unlike *SP-C* transcription, which apparently is not regulated by AP-1 (81), mutation of −109 TRE totally abolishes human *SP-D* promoter activity (51). Moreover, JunB, JunD, and Fra1 bind to the TRE, and the overexpression of latter two proteins upregulates *SP-D* promoter activity, whereas c-Jun and c-Fos suppress it (51). The binding of JunB and Fra1 to a composite element, admixed with TRE, HNF-3, and octomer motifs, correlates with rat *CCSP* expression in bronchiolar cells (118). The fact that TRE/CRE is either embedded or located in close proximity to other functional motifs, such as Nkx2.1, HNF-3, and NF-1, strongly suggests that the distinct regulation of *SPs* and *CCSP* transcription is probably mediated by complex, cooperative, and/or mutually exclusive interactions between AP-1 proteins. Cell type and/or ubiquitous factors may also be important.

TPA and TS, which mainly suppress normal epithelial secretory cell functions, strongly upregulate the

expression of genes involved in airway squamous cell differentiation (143). Among them, small proline-rich protein 1B (*SPRR1B*) transcription is mainly mediated through AP-1 binding to the two TREs located at −140 and −109 (111). We have shown that ectopic expression of c-Jun, JunB, JunD, c-Fos, and Fra1 upregulates *SPRR1B* transcription in both BEAS-2B and H441 cells (103). Intriguingly, Fra2 suppresses basal, TPA-, and c-Jun-enhanced *SPRR1B* transcription (103). Although PMA upregulates both mRNA expression and DNA binding activity of Fra2 to the consensus TRE in both cell types, electrophoretic mobility shift analysis reveals a lack of Fra2 binding to the TREs of *SPRR1B* promoter (103). Consistent with this, c-Jun, JunB, c-Fos, Fra1, and CREB1 proteins bind to the functional −480 CRE of the promoter of transglutaminase 1 (*TG-1*), which cross-links cornified envelope proteins such as *SPRR1B* in differentiated rabbit bronchial epithelial cells (92). Thus, similar to *SPs* and *CCSP,* AP-1 dimers distinctly regulate gene expression involved in airway squamous cell differentiation.

Antioxidant enzymes. Exposure of pulmonary epithelial cells to TS, asbestos, silica, and other toxicants induces the cellular stress arising from the generation of ROS. The antioxidant enzymes (AOEs) play a pivotal role in the detoxification of various ROS and are rapidly induced after toxic injury. Among the *AOEs*, the regulation of γ-GCS, NAD(P)H:quinone oxidoreductase 1 (NQO1), metallothionein-I (MT-I), heme oxygenase 1 (HO-1), and glutathione *S*-transferases, which play a key role in detoxification process, has been extensively investigated. Because toxicant-induced AP-1 proto-oncogene expression appears to precede *AOE* expression, and the regulatory regions of several *AOE* contain functional TRE(s), a potential role for AP-1 is implicated in antioxidant responses.

ECM proteins and growth factors. During the injury and repair process, deposition and degradation of ECM proteins (e.g., collagens, fibronectin, integrins) play a key role in restoration of normal cellular structures. AP-1 regulates the expression of these proteins, MMPs, tissue inhibitor of metalloproteinases, and growth factors (e.g., EGF) and their receptors (e.g., EGFR). Many of the above-mentioned genes also contain AP-1 binding sites in their promoter region. For example, recently, it was shown that downregulation of elastin gene expression by basic fibroblast growth factor-2 is probably mediated by a protracted induction of *fra*1 (14). TS exposure induces the expression of MMP-12, which degrades other ECM proteins in the mouse lungs (47), and also decreases the lung elastin content (17, 47). MMP-12 expression is also regulated by AP-1, and Fra1 is one of the AP-1 components that bind to its functional AP-1 site (148).

INTERACTION OF AP-1 PROTEINS WITH OTHER TRANSCRIPTION FACTORS

In addition to self homo- or heterodimerization, Jun and Fos proteins selectively partner with other related bZIP families of proteins (Fig. 5), including ATFs, C/EBPs, Nrf/Mafs, and helix-loop-helix ZIP proteins, such as upstream stimulatory factors (USFs) (20). Furthermore, they can physically interact with non-ZIP proteins, such as nuclear factor of activated T cells, Ets, NF-κB, glucocorticoid receptor, CREB-binding

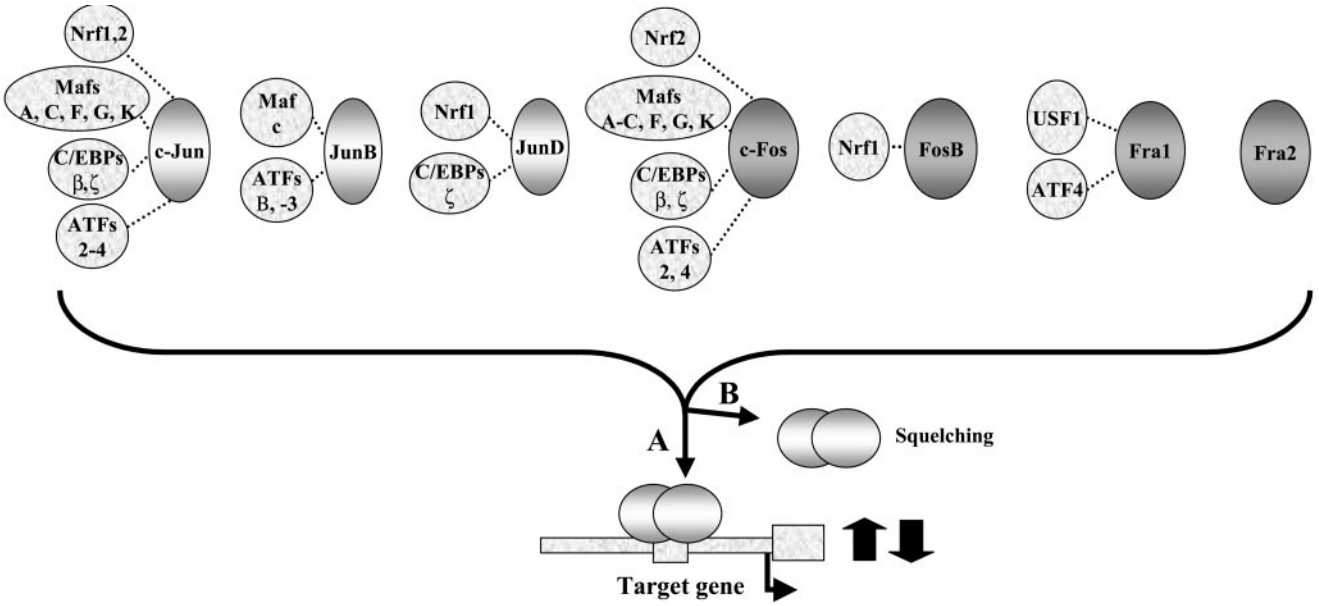

Fig. 5. The interactions of AP-1 proteins other related family proteins. The dimerization of AP-1 proteins with themselves or with other related basic region-leucine zipper (bZIP) family proteins facilitates the binding of bZIP proteins to DNA. This results in either positive (up) or negative (down) regulation of target gene transcription (*A*). Alternatively, dimerization could sequester or squelch the transcription factor(s) away from their target sites (*B*). It can also prevent their association with other factors or cofactors, leading to differential gene transcription. C/EBP, CCAAT enhancer binding protein; USF, upstream stimulating factor; Nrf, nuclear factor erythroid 2 (NF-E2)-like factor; NRL, neural retina leucine zipper.

protein, and TATA-binding protein (20). These interactions not only increase the complexity and repertoire of the protein factors present in a given cell type but also result in differential DNA binding and transactivation activities, thereby tightly controlling AP-1-dependent gene expression in various biological processes (20). In addition, the involvement of a variety of MAPK modules that differentially activate various partners can also integrate signals elicited by diverse stimuli. The interactions between AP-1 proteins and non-ZIP proteins and their role in inflammatory and immune responses have been discussed elsewhere (33, 36, 54, 83). In this review, we briefly discuss some of the potential interactions between AP-1 and other bZIP proteins and their role in regulation of gene expression in cells of the lung.

Interactions with bZIP proteins. Jun and Fos proteins can distinctly heterodimerize with ATF family members, B-ATF, ATF-α, ATFs 1–4 and -6, and CREB. These heterodimers more efficiently bind to CRE compared with TRE (20). The specific interactions of AP-1 include c-Jun with ATF-2, -3, and -4; JunB with ATF-3 and B-ATF; c-Fos with ATF-4; and Fra1 with ATF-4 (20). Compared with c-Jun and c-Fos, Fra1 binds to ATF-4 with a stronger affinity (43). Several genes expressed in the lung contain functional CRE in their regulatory regions and are regulated by Jun-Fos-ATF heterodimers. Some of them include c-*jun*, *SP-B*, *TG-1*, *HO-1*, and *interferon-α*. Mice devoid of ATF-2 display severe respiratory distress and die shortly after birth, suggesting a critical role for it in respiratory functions (84). As described above, ATF-2-c-Jun binding at CRE located at the proximal promoter plays a prominent role in regulation of c-*jun* transcription (125). However, c-*jun* transcription can be inducible in the cells that are devoid of ATF-2, suggesting other factors might compensate for its absence. A suppressive role for ATF-Jun dimers exists in regulation of *SP-B* transcription (10), whereas the binding of Jun and CREB positively correlates with *TG-1* transcription (92). Disruption of *ATF-4* results in severe fetal anemia in mice (87). Because ATF-4, a ubiquitous protein, positively upregulates Nrf2-induced *HO-1* expression in other cell types (49) and because Nrf2 and HO-1 also play a role in pulmonary defense mechanisms (22, 100), it is likely that the interactions between ATF-4 and AP-1 proteins have differential effects on gene expression in cells of the lung.

The members of the C/EBP family, C/EBP-α, -β (also called NF-IL6), -γ, -δ, -ϵ, and -ζ (CHOP or GADD153), regulate gene expression involved in cell proliferation, differentiation, and stress, as well as inflammation and immune responses (77, 106). C/EBP-α, -β, and -δ are expressed in the cells of the lung. C/EBP-α and -δ mainly regulate expression of differentiation markers, such as *SP*s (50, 79, 129) and *CCSP* (16), whereas C/EBP-β activates the transcription of inflammatory cytokines, including *IL-4* and *IL-8* (106). Mice devoid of C/EBP-α die immediately after birth from abnormalities in alveolar structure (129), whereas C/EBP-β null mice are more susceptible to inflammation when challenged (132). c-Jun and c-Fos, through their ZIP domain, can interact with C/EBP-β to suppress its transcriptional activation (58). The protein-protein interactions between C/EBP-ζ and c-Jun, JunD, or c-Fos also regulate target gene expression (139). Overexpression of C/EBP-α and -β upregulates the transcription of *SPRR1B*, whereas C/EBP-ζ suppresses both TPA- and c-Jun-enhanced *SPRR1B* transcription in airway epithelial cells (S. P. M. Reddy, unpublished data). Although the downstream target genes regulated by AP-1-C/EBP dimers remain enigmatic, it appears that such interactions could play a potential role in gene transcription in cells of the lung.

Fos and Jun proteins also form heterodimers with the family of cap 'n' collar bZIP proteins, such as the NF-E2 family members Nrf1 and Nrf2; Maf family members c-Maf, MafB, MafF, MafG, and MafK (also called p18); and neural retina leucine zipper; thereby expanding the range of AP-1 protein target sites (Fig. 5) (20, 101). These mixed family heterodimers of AP-1 preferentially interact with palindromic TGAC and TGCtgaC half-sites, whereas the dimers of NF-E2 and Maf proteins bind mainly at the DNA sequence a/gTGACNNNGC, referred to as antioxidant response element (ARE) (20, 101). Because TRE is often embedded with ARE, it is likely that cooperative and/or mutually exclusive interactions between Jun and Jun, Jun and Fos, Jun/Fos and Nrf1/2, and Jun/Fos and Maf proteins play a role in the regulation of gene expression involved in pulmonary defense and injury-repair mechanisms.

It is noteworthy that among the AP-1 proteins, Fra1 specifically interacts with USF-1, but not with USF-2. USF-1 and -2 belong to the helix-loop-helix bZIP family, which binds at E-box elements (CACGTG) as a homo- and heterodimer and regulates gene expression (105). For example, USF-1 activates cadmium-inducible expression of the MT-I (80) and *SP-A* transcription in alveolar type II cells (38). The downstream targets of the Fra1-USF-1 dimer that regulates airway epithelial specific gene expression remain enigmatic.

In conclusion, selective dimerization of bZIP family members, their interactions with other transcription factors, as well as their exclusive and/or inclusive interactions at composite regulatory elements can modify the regulatory specificities of Fos and Jun proteins. These patterns may vary in specific cell types and permit increased stability of complexes, sequence specificity, and regulatory selectivity (20). Such interactions may also allow the cooperative recruitment of other transcription factors, coactivators, and chromatin remodeling factors to promoter and enhancer regions, yielding cell- and stimulus-specific transcriptional activities (20).

ROLE OF AP-1 FAMILY MEMBERS IN LUNG TUMORIGENESIS

Cells of the respiratory tract epithelium, a direct target of inhaled toxicants/carcinogens, are considered as the progenitor cell types for the majority of various

lung tumors. Experimental evidence suggests that lung cancer development is a multistep process characterized by multiple but sequential morphological, molecular, and genetic changes involved in cell growth and differentiation (12, 45). In addition to deregulation of tumor suppressor gene expression (e.g., p53, Rb), the activation and/or overexpression of oncogenes (e.g., K-ras, c-myc) and growth factors and their receptors (e.g., EGFR) also play a role in cellular transformation (12, 45). Because various oncogenic and mitogenic signaling pathways converge at AP-1, which in turn regulates the expression of cell cycle machinery, it is likely that abnormal activation of members of the AP-1 family by toxicants amplifies the intracellular signals, resulting in unregulated epithelial cell growth and cellular transformation. Mutations or deletions in coding and/or noncoding regions of several genes have been correlated with the progression of several diseases including cancer. Intriguingly, none of these modifications have been documented for AP-1 family genes, alternatively suggesting that modulation of their abundance and/or activity by toxicants plays a more significant role in cellular transformation (65). In support of this notion, as discussed earlier, cells and mice overexpressing individual AP-1 components demonstrate cellular transformation and tumors, respectively (65). Although previous studies have mainly focused on the role of c-Jun and c-Fos in lung epithelial cell transformation (5, 11), relatively little is known about the involvement of other AP-1 components. Surprisingly, although thought to play a role in transformation in vitro, ectopic expression of either c-Jun or c-Fos does not result in an overt phenotype in the lung or any other organ, with the exception of bone tumors in the case of the latter (39). Recent studies also rule out a role for JunB (78), FosB (39), or Fra2 (89) in cellular transformation, as their overexpression produces no phenotype in mouse lungs. Although a transgenic overexpression of JunD has not been reported, it is unlikely to have a profound phenotypic effect in the lung, as, similar to c-Jun, JunB, and Fra2, the endogenous expression of JunD is very high in various tissues, including the lung, and is not significantly altered after mitogenic or toxic stimuli (133).

Does then Fra1 play a role in cellular transformation? As discussed earlier, several lines of recent evidence generated by various laboratories indicate a potential role for Fra1 in abnormal differentiation and transformation of lung cells. First, most importantly, the broad overexpression of *fra*1*,* but not other AP-1 components, induces some lung tumors in mice (64). Second, various toxicants and known carcinogens, such as TS (112), silica (126), and asbestos (53, 117, 135), persistently activate *fra*1 expression in lung cells both in vitro and in vivo. This appears to be selective, as exposure of bronchial epithelial cells to TS upregulates *fra*1, but not *fra*2*,* expression both in vitro and in vivo (112). Third, Fra1 positively upregulates gene expression associated with squamous cell metaplasia, a preneoplastic lesion (103, 143). Fourth, the transition from small cell to nonsmall cell lung cancer phenotype induced by H-Ras/c-Myc is associated with the specific induction of *fra*1 but not other AP-1 family members (114). Last, Fra1 is a predominant component of the AP-1 complex in asbestos-induced mesothelioma and proliferating rat mesothelioma cells, and overexpression of the dominant-negative Fra1 mutant inhibits growth of these cells in soft agar (110a). *Fra*1 mRNA is also highly induced in NNK-induced lung tumors compared with control lung (112). Together, these observations highlight a potential role for Fra1 in lung tumorigenesis.

In support of this hypothesis, a causal role for Fra1 in cellular transformation has been documented in other systems. For example, *fra*1 expression is high in stomach (88) and esophageal (59) squamous cell carcinomas, as well as in breast tumor cells (151). Ectopic expression of *fra*1 increases the motility and metastatic behavior of invasive mammary adenocarcinoma cells (73). A higher level of *fra*1 expression is essential for v-*mos*-induced transformation of thyroid cells (90). Consistent with this observation, human thyroid tumors also express a high levels of *fra*1 (140). Fra1 is also the predominant component of AP-1 complex formation induced by activated Ras, which promotes fibroblast transformation (8). Overexpression of *fra*1 in fibroblasts results in an anchorage-independent cell growth in vitro and tumor formation in nude mice (9, 147). Most recently, the requirement of Fra1, but not Fra2, for TPA- and EGF-induced neoplastic transformation of epidermal cells has been documented (150).

Somewhat puzzling is the mechanism by which Fra1 induces cellular transformation. Unlike c-Fos and FosB, Fra1 apparently lacks a transcriptional activation domain and therefore is thought to suppress or limit the transcriptional activity of AP-1 by forming stable heterodimers with Jun proteins (65). Although deletion of *fra*1 causes early embryonic lethality, and its expression is high during late G1 and asynchronous cell growth, MEFs devoid of *fra*1 surprisingly have no defects in cell proliferation (65). There are several possibilities to explain why Fra1 expression may not be required for MEF proliferation, including compensation of c-Fos, FosB, or Fra2 for Fra1. For example, MEFs devoid of both c-Fos and FosB, but not each individually, show proliferative defects, thus suggesting that Fos proteins indeed complement each other (65). This phenomenon is also confirmed by another study that showed that microinjection of antibodies specific for individual Fos proteins did not block cell proliferation, whereas their combined use did (65). If compensatory mechanisms exist, an unexplained question is why the disruption of *fra*1 causes embryonic lethality, whereas disruption of c-*fos* and *fos*B shows no effects. This paradox suggests that Fra1 might have functions distinct from those of other Fos proteins. Surprisingly, contrary to the previous belief that Fra1 lacks a transactivation domain, a recent study demonstrates that Fra1 possesses transactivation potential required for TPA- and EGF-induced neoplastic transformation of epidermal cells. This is mediated by the mitogen-activated ERK-dependent phosphoryla-

tion of Thr231 located in the COOH-terminal region of Fra1 (150).

Alternatively, it is possible that a protracted induction of Fra1 by mitogens and/or toxicants alters the dynamics of AP-1 by changing dimer composition (Fig. 5). This might either positively or negatively influence the transcriptional activation of target genes. In addition, Fra1 can distinctly interact with other proteins, such as USF-1 (105) and ATF-4 (43), thereby playing a regulatory role in gene expression involved in pulmonary defense mechanisms. For instance, USF-1 (105) regulates gene expression of MT-I (80) and *SP-A* transcription in alveolar type II cells (38), whereas ATF-4 interacts with Nrf-2 (4, 49) and positively regulates gene expression of NQO1, γ-*GCS,* and HO-1, which are involved in the detoxification of ROS (100). However, Fra1 suppresses Nrf-2-inducible *NQO1* (142) and possibly γ-*GCS* expression (62). Thus one could speculate that the interaction of Fra1 with USF-1 and/or ATF-4 probably modulates gene expression that plays a role in pulmonary defense mechanisms.

On the basis of the above observations, it is quite reasonable to assume that a protracted induction of *fra*1 in cells of the lung, which endogenously is very low or undetectable, could modulate downstream target gene expression by toxicants and carcinogens. This in turn may compromise normal cell growth and differentiation, thereby altering the injury and repair processes, and culminate in cellular transformation. The fact that Fra1 cannot bind to DNA by itself suggests that interactions of Fra1 with other transcription factors, as well as their posttranslational modifications, may play a central role in the pathogenesis.

SUMMARY AND FUTURE DIRECTIONS

As the downstream transcription effector of a variety of signaling pathways activated by toxic stimuli, AP-1 can act as a "master switch" to regulate gene expression involved in lung injury, repair, and transformation (Fig. 1). Through heterodimerization of AP-1 family members and interactions with other transcription factors (Figs. 2 and 5), AP-1 proteins provide different layers of both multiplicity and diversity to control the regulation of gene expression tightly and distinctly. The lack of a well-conserved promoter structure, their distinct regulation by various stimuli (Fig. 3), and the existence of some functional redundancy (Table 1) strongly suggest that both spatial expression and regulation of AP-1 family members are important in various cellular responses. Intriguingly, the presence of TREs, which either overlap with or exist adjacently and/or in close proximity with other motifs recognized by cell type (e.g., Nkx2.1, HNF-3) and/or ubiquitous (e.g., Nrf2, Sp1, and Ets) transcription factors, indicates that activation of AP-1 family members by toxic agents may tilt the dynamics of transcription. These interactions may deregulate the expression of a particular set of target genes(s), which may lead to abnormal injury and repair process, culminating in lung disease. A daunting task, based on the fact that lung is a complex tissue consisting at least 40 different cell types, is the design of studies to gain a better understanding of the distinct regulatory mechanisms of individual AP-1 subunits in various lung cells. These investigations should provide additional insight into the molecular changes involved in abnormal differentiation and cellular transformation. The generation of in vitro and animal models that overexpress wild-type or mutant protein(s) in an "on/off" manner in the specific cell types may help identify the role of AP-1 family members in normal and abnormal lung biology. In addition, cDNA microarray and proteomic analysis will enable us to identify the critical players and the sequence of interactions of AP-1-inducible (both early- and late-responsive) gene expression. This information may offer unique opportunities to use AP-1 family members or target genes as potential diagnostic markers or drug targets for early detection and/or prevention of various lung diseases.

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