kbar (*8*), which is 5 kbar lower than the likely pressure of cumulate crystallization. If the layered cumulate were a precipitate from the parent magma of the pyroxenites (that is, HV parent magmas), then it should have had a higher content of clinopyroxene and certainly not be devoid of this phase. Therefore, we consider it unlikely that the parental magma of the xenolith was related to the rejuvenated stage alkalic HV lavas. On the other hand, the magma from which the xenolith crystallized could represent the ultimate parent of the Koolau tholeiites because post-shield alkalic magmas did not erupt on Koolau. These magmas may have been generated at a minimum depth of about 90 km and underwent ponding and fractionation in deep magma chambers before ascending and fractionating further in shallow crustal chambers and then finally erupting through the shield volcano.

Seismic studies of the currently active Kilauea volcano on Hawaii suggest that earthquakes record the ascent of magma through the lithosphere (*4*). The foci of the majority of the deep earthquakes are about 60 km, which has been suggested to be the depth at which magma accumulates before its explosive ascent (*4*). The xenolith that we have described, however, indicates that a large magma chamber was present at significantly greater depth beneath Koolau volcano. Data from Nd, Sr, and He isotopes indicate that in general, Hawaiian tholeiites carry a chemical signature of a deep plume in the mantle beneath Hawaii (*8, 15*). Thus, Hawaiian shield-building magmas may originate at deeper levels and pond at about 90 km. The general absence of earthquakes below 60 km beneath Kilauea indicates that the upper mantle below that depth behaves in a ductile manner and that magma rises diapirically rather than by fracturing (*16*). As the magma reaches 60 km depth, however, it enters a more brittle lithosphere and continues its ascent by way of fractures. The occurrence of veined xenoliths, which represent magma-filled fractures, from 75 to 80 km (*9*) and of the cumulate xenolith in the Honolulu Volcanics of Oahu suggests that about 1.2 million years after the Koolau tholeiitic magmatism stopped and the volcano moved away from the hot plume, the brittle-ductile transition beneath the Koolau volcano descended to about 90 km depth.

Another important implication of this xenolith is concerned with the generation of the post-shield Honolulu Volcanics in which this xenolith occurs as an inclusion. On the basis of Nd and Sr isotopic similarity (that is, depleted character) between HV and mid-ocean ridge lavas (which are generally believed to have been extracted from the lithospheric mantle), Chen and Frey suggested that HV magmas were generated by partial fusion of the lithosphere (*15*). However, in order for a HV magma to entrain this xenolith, at least some of the magma must have formed at or near the base of the lithosphere or within the asthenosphere.

A petrologic model of the Hawaiian lithosphere was presented by Sen (7) on the basis of mantle xenoliths from Oahu; the inferences made above necessitate a revision of this model (Fig. 3). As the lithosphere moves over the hot plume, shield-building tholeiite magmas separating from the plume (and surrounding asthenosphere) initially accumulate and fractionate in chambers near the base of the lithosphere. Fractionation of olivine, spinel, and garnet from these magmas leads to residual basaltic magmas with higher absolute abundances of rare earth elements (REE) and relatively light REE-enriched (chondrite-normalized) character. These differentiated magmas then leave the chambers and rise first through a ductile region, and then above ~60 km through fractures in the brittle lithosphere. In the rejuvenated (or HV type) stage, descent of the brittle-ductile transition to about 90 km beneath Oahu allows post-shield magmas, generated from the asthenosphere, to carry xenoliths from this depth.

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13. Abbreviations used: ol, olivine; fo, forsterite; di, diopside; gt, garnet; sp, spinel; l, liquid; py, pyrope; gr, grossular.
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Redox Regulation of Fos and Jun DNA-Binding Activity in Vitro

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The proto-oncogenes c-*fos* and c-*jun* function cooperatively as inducible transcription factors in signal transduction processes. Their protein products, Fos and Jun, form a heterodimeric complex that interacts with the DNA regulatory element known as the activator protein–1 (AP-1) binding site. Dimerization occurs via interaction between leucine zipper domains and serves to bring into proper juxtaposition a region in each protein that is rich in basic amino acids and that forms a DNA-binding domain. DNA binding of the Fos-Jun heterodimer was modulated by reduction-oxidation (redox) of a single conserved cysteine residue in the DNA-binding domains of the two proteins. Furthermore, a nuclear protein was identified that reduced Fos and Jun and stimulated DNA-binding activity in vitro. These results suggest that transcriptional activity mediated by AP-1 binding factors may be regulated by a redox mechanism.

A VARIETY OF SYSTEMS HAS EVOLVED in prokaryotic and eukaryotic cells for coupling environmental signals to the selective regulation of gene expression. These have in common the ultimate formation of specific protein-DNA complexes (*1*). A class of genes, including c-*fos* and c-*jun*, encodes inducible transcription factors that function as intermediary transcriptional regulators in signal transduction

processes (*2*). Expression of c-*fos* and c-*jun* is induced transiently by a broad range of extracellular stimuli (*3*). Their protein products form homodimeric and heterodimeric complexes through a leucine zipper domain (*4–6*). Dimerization results in the juxtaposition of conserved basic regions of each protein, which forms a bipartite DNA-binding domain (*6*). The dimeric protein complexes interact with DNA regulatory elements known as AP-1 binding sites and cyclic adenosine monophosphate (cyclic AMP) responsive elements (CRE) (*2–4, 7, 8*). These elements are present in the regulatory regions of several genes that are responsive to a variety of extracellular stimuli (*2*). Jun, but not Fos, forms homodimers that bind to DNA (*4–6*); however, Fos-Jun heterodimers bind to AP-1 sites with a higher apparent affinity than do Jun homodimers, due to an increased stability of the protein-DNA complex (*4*). Jun also forms heterodimers with a CRE-binding protein, CREBP-1, which bind more readily to CRE sites than to AP-1 sites (*9*).

Previously, we identified a nuclear factor that stimulates the DNA-binding activity of Fos and Jun in vitro (*10*). This factor does not bind to the Fos-Jun complex or to the AP-1 site (*11*), suggesting that it regulates DNA-binding activity indirectly. The stimulatory factor is inhibited by sulfhydryl-modifying reagents and can be partially compensated for by high concentrations of reducing agents (*10*). We hypothesized that the nuclear factor reduces a critical cysteine residue in Fos and Jun that is required for DNA-binding activity. To address this possibility, we have examined the effects of sulfhydryl-modifying agents and mutations of specific cysteine residues on the dimerization and DNA-binding activities of Fos and Jun.

The leucine zipper and adjacent basic region of Fos and Jun are required for DNA binding (*4–6, 8, 10*) (Fig. 1). Two cysteine residues in this region are highly conserved among members of the *fos* and *jun* gene families (*12*). One of these cysteines (C1) is located in the basic region and the other (C2) is located COOH-terminal to the leucine zipper (Fig. 1). To investigate the participation of these cysteine residues in DNA binding, truncated Fos and Jun polypeptides that contained the leucine zipper and DNA-binding domain (Fos 116–211 and Jun 224–334) (Fig. 1) were expressed in and purified from *Escherichia coli* (*10*). DNA binding of Jun (J) and Fos plus Jun (F + J) were inhibited by the sulfhydryl-specific modifying agent, *N*-ethylmaleimide (NEM) (Fig. 2A). Treatment of Fos or Jun with NEM prior to heterodimer formation inhibited the DNA-binding activity of the complexes, indicating that cysteine residues in both proteins contributed to DNA binding (Fig. 2A). However, incubation of the proteins with an oligonucleotide that contained an AP-1 site, prior to NEM treatment, protected the complexes from inactivation. This suggests that the cysteine residue or residues that are required for DNA binding are associated with DNA. Although the Fos (F) and Jun (J) proteins migrated as monomers on nonreducing SDS-polyacrylamide gels, treatment of these proteins with diazenedicarboxylic acid *bis*[*N*,*N*-dimethylamide] (diamide), a sulfhydryl oxidizing agent, resulted in their conversion to slower-migrating forms, likely representing disulfide crosslinked dimers (Fig. 2B). Incubation of Fos and Jun together (F + J) prior to diamide treatment resulted in oxidation to a covalently cross-linked heterodimeric complex (Fig. 2B). Oxidation with diamide inhibited the DNA-binding activities of Jun homodimers (J) and Fos-Jun heterodimers (F + J) (Fig. 2C). Taken together, these data suggest that cysteine residue or residues in Fos and Jun are important for DNA binding and that reduction is required for association with DNA.

We investigated the contribution of individual cysteine residues to DNA binding using mutated Fos and Jun polypeptides that contained serines in place of C1, C2, or both cysteine residues (Fig. 1). The Fos-Jun heterodimers that contained serine substitutions in the basic region [F(C1-S) + J(C1-S) and F(C1-S,C2-S) + J(C1-S,C2-S)] (Fig. 1) exhibited high amounts of DNA-binding activity, even in the absence of the nuclear factor or reducing agents (Fig. 3A). These proteins were also active in deoxyribonuclease I (DNase I) footprinting assays without dithiothreitol (DTT), unlike Fos and Jun proteins that contained cysteine residues in the basic region (*13*). Enhancement of DNA-binding activity was specific for substitution of C1, as Fos-Jun heterodimers that contained a serine substitution at C2 [F(C2-S) + J(C2-S)] did not bind DNA in the absence of the nuclear factor or reducing agents (Fig. 3A). DNA-binding activities of J(C1-S) and F(C1-S) + J(C1-S) were not inhibited by alkylation with NEM (Fig. 2A). Oxidation of J(C1-S) and F(C1-S) + J(C1-S) with diamide converted the monomeric proteins to covalently cross-linked homodimeric and heterodimeric forms on nonreducing gels (Fig. 2B); however, these complexes exhibited high amounts of DNA-binding activity (Fig. 2C). Conversely, oxidation of C1 in J(C2-S) and F(C2-S) inhibited DNA binding (*13*). Thus, the redox regulation of DNA binding in vitro was mediated by a specific cysteine residue in close proximity to DNA.

The mutated Fos and Jun proteins that contained serine in the C1 position exhibited increased DNA-binding activity, as compared to wild-type proteins, in the presence of reducing agents or the nuclear factor (Fig. 2, A and C). Thus, the C1-S substitutions represented gain-of-function mutations, which suggests that the cysteine residue may serve a regulatory function in vivo. Although F + J(C1-S) and F(C1-S) + J complexes bound to DNA in the absence of the nuclear extract or reducing agents, F(C1-S) + J(C1-S) bound to DNA more efficiently under these conditions (Fig. 3B). Thus, residues at the C1 position of both Fos and Jun participated in DNA binding.

Substitution of C1 with serine did not alter the DNA-binding specificity as determined by DNase I footprinting (Fig. 3C).

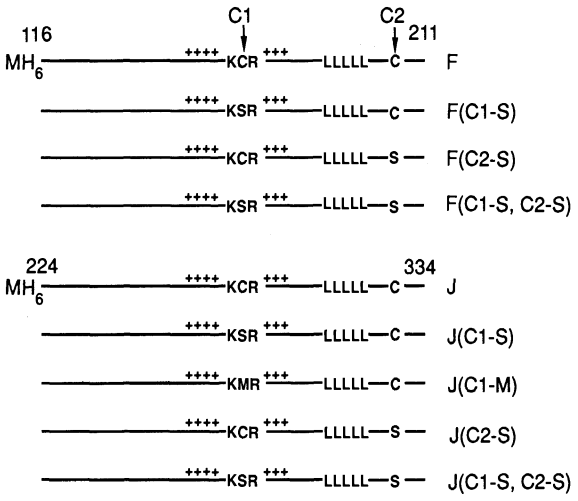

Fig. 1. Schematic diagram illustrating the location of cysteine residues in the leucine zipper and basic DNA-binding domains of Fos and Jun. The regions of Fos (F) and Jun (J) that contain the leucine zipper (LLLLL) and basic regions (+++) are contained within Fos amino acids 116 to 211 and Jun amino acids 224 to 334. The truncated Fos and Jun polypeptides were expressed in *E. coli* as histidine fusion proteins (H_6) and were purified by nickel-chelate chromatography (*10*). Point mutations were introduced into *fos* and *jun* by a polymerase chain reaction strategy (*27*) and the structure of the mutated genes was verified by dideoxynucleotide sequencing. The mutated Fos and Jun polypeptides contained substitutions in the cysteine residues (C) to serine (S) or methionine (M) as indicated in parentheses (*28*).

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Both J(C1-S) and F(C1-S) + J(C1-S) protected the same region of DNA as did wild-type Jun and Fos + Jun (Fig. 3C). Furthermore, stability of the association of F(C1-S) + J(C1-S) with DNA was similar to that of the wild-type Fos-Jun complex (*13*). Thus, while substitution of C1 in Fos and Jun dramatically enhanced DNA-binding activity in the absence of reducing agents or the nuclear factor, it did not alter the nature of the protein-DNA interaction.

Unlike with serine, substitution of C1 with methionine decreased DNA-binding activity. Jun homodimers that contained a methionine substitution, J(C1-M), did not bind DNA under any conditions (Fig. 3, B and C). It is possible that the side chain of methionine, which is larger than that of cysteine, inhibited DNA binding by steric hindrance. Alternatively, methionine residues, unlike cysteine, may not permit formation of specific hydrogen bonds that are required for protein-DNA interaction. These findings are consistent with the results of the alkylation experiments (Fig. 2A), which suggested that C1 was in close proximity to DNA. Interestingly, in the scissors grip model (*14*), the equivalent position in the CCAAT/enhancer binding protein (C/EBP) is in close apposition with DNA.

The factor in nuclear extracts that stimulates Fos-Jun DNA-binding activity is protease-sensitive and inactivated by heat treatment (*11*). The present findings suggest that the stimulatory factor may modify C1 in Fos and Jun. To test this possibility, we determined whether the factor was active at low temperature (Fig. 4A). The Fos + Jun complex exhibited DNA binding after incubation for 2 min with liver nuclear extract at 37°C, and DNA-binding activity was maximal by 15 min of incubation (Fig. 4A). However, the activity of the nuclear factor was greatly reduced at 25°C, and no stimulatory activity was apparent after incubation at 4°C (Fig. 4A). Thus, similar to a majority of modifying enzymes, the stimulatory activity exhibited a sharp temperature dependence. In contrast, high concentrations of DTT stimulated DNA-binding activity fully at 25°C and partially at 4°C (*13*). A relatively high concentration of liver nuclear extract protein (2 μg) was required to stimulate DNA-binding activity of Fos + Jun, and activity decreased markedly as the extract concentration was reduced (Fig. 4B). The activity in the nuclear extract was stimulated by thioredoxin, an enzyme that catalyzes the reduction of cysteine residues (Fig. 4C). Thioredoxin was most effective in the presence of its regenerating system, thioredoxin reductase and NADPH. Under these conditions, much lower concentrations of nuclear extract protein (0.1 μg and 0.05 μg) were effective in promoting the DNA-binding activity of Fos-Jun heterodimers (Fig. 4C). Thioredoxin affected activity of the nuclear factor rather than Fos and Jun, as it did not activate DNA binding of Fos + Jun in the absence of nuclear extract (Fig. 4C). Therefore, a free sulfhydryl residue was required for function of the nuclear factor, suggesting that the stimulatory factor enhances DNA binding of Fos and Jun by a reduction mechanism.

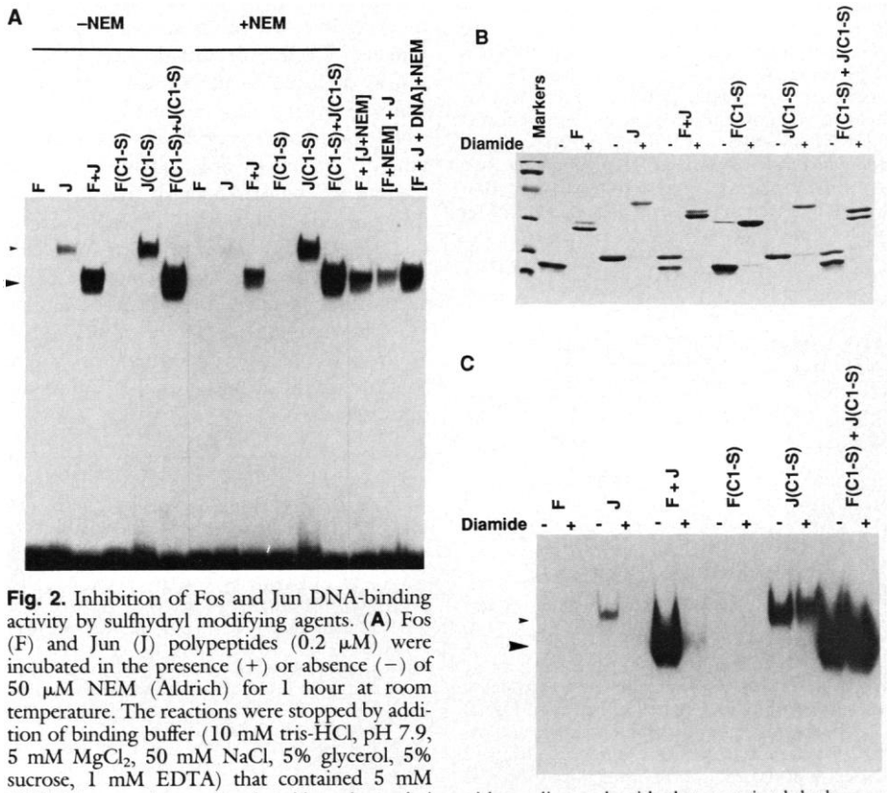

Fig. 2. Inhibition of Fos and Jun DNA-binding activity by sulfhydryl modifying agents. (**A**) Fos (F) and Jun (J) polypeptides (0.2 μM) were incubated in the presence (+) or absence (−) of 50 μM NEM (Aldrich) for 1 hour at room temperature. The reactions were stopped by addition of binding buffer (10 mM tris-HCl, pH 7.9, 5 mM $MgCl_2$, 50 mM NaCl, 5% glycerol, 5% sucrose, 1 mM EDTA) that contained 5 mM DTT. The proteins were analyzed by gel retardation with an oligonucleotide that contained the human metallothionein IIA ($HMTIIA$) AP-1 site (*4, 10*). Protein-DNA complexes were resolved from unbound oligonucleotide on a 6.5% nondenaturing polyacrylamide gel that contained 0.5× tris-borate-EDTA (0.045 M tris-borate, pH 8.3, 0.012 M EDTA) and were visualized by autoradiography. The small arrowhead indicates the position of Jun homodimeric complexes and the large arrowhead indicates the position of Fos-Jun heterodimeric complexes. (**B** and **C**) Fos and Jun polypeptides (1 mg/ml) were incubated in the presence (+) and absence (−) of 70 μM diamide (Sigma) for 1 hour at 37°C. In (B), treated (+) and untreated (−) proteins (5 μg) were resolved on a 13.5% nonreducing SDS-polyacrylamide gel and visualized by staining with Coomassie brilliant blue. The marker lane contains molecular size standards (*29*). In (C), Fos and Jun proteins (0.2 μM) were analyzed by gel retardation assay. The small arrowhead indicates the position of Jun homodimeric complexes and the large arrowhead indicates Fos-Jun heterodimeric complexes.

Regulation in response to environmental cues by a redox mechanism was proposed almost 40 years ago (*15*). Our findings suggest that modification of the redox state of Fos and Jun may contribute to the formation of specific protein-DNA complexes. The bacterial transcriptional regulatory protein, OxyR, which regulates gene expression in response to oxidative stress, changes DNA-binding specificity depending on the redox state (*16*). Thus, regulation by reduction-oxidation may be a general mechanism of control for transcription factors. Indeed, several transcriptional regulatory proteins require free sulfhydryl residues for DNA binding or transcription activation (*17*). Furthermore, transcriptionally active nucleosomes preferentially contain proteins with free sulfhydryl residues (*18*). A redox mechanism also contributes to regulation of hormone-receptor interactions (*19*), bacteriophage DNA replication (*20*), transduction of light to metabolic events (*21*), and translational regulation (*22*). Results in the present report and elsewhere (*16*) implicate redox regulation in protein-DNA interactions.

The target cysteine residue in Fos and Jun is conserved among members of the Fos and Jun gene families (*12*), as well as in several proteins of the CRE/adenovirus transcription factor family (*23*). However, v-*jun*, the oncogenic homolog of c-*jun*, contains a point mutation that converts the conserved cysteine residue (C1) to serine (*24*). Because substitution of C1 to serine stimulates DNA binding of Fos and Jun, the transforming

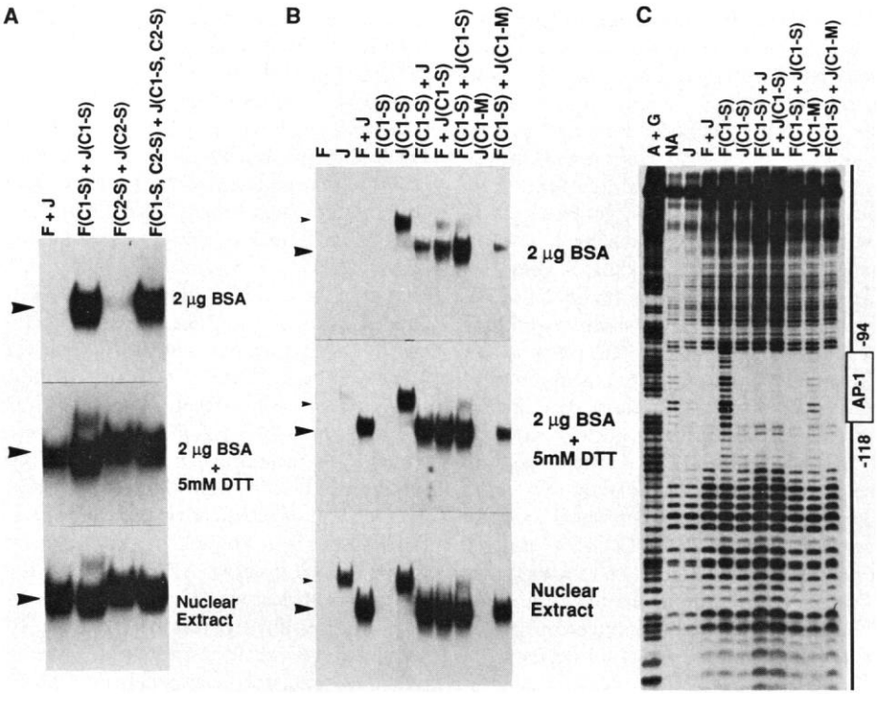

Fig. 3. DNA-binding activities of Fos and Jun polypeptides that contained substitutions of the cysteine residues. (**A** and **B**) Fos (F) and Jun (J) or mutated Fos and Jun polypeptides (described in Fig. 1) (0.2 μM) were incubated for 15 min at 37°C in binding buffer that contained 2 μg of BSA, 2 μg of BSA plus 5 mM DTT, or 2 μg of liver nuclear extract (*30*). The protein-DNA complexes were analyzed by a gel retardation assay. The small arrowhead indicates the position of Jun homodimeric complexes and the large arrowhead indicates Fos-Jun heterodimeric complexes. (**C**) Fos and Jun proteins were incubated for 15 min at 37°C in binding buffer that contained 0.1% NP-40 and 5 mM DTT. A ^{32}P-labeled DNA fragment of the $HMTII_A$ promoter (−154 to −54) was added, and the protein-DNA complexes were digested with DNase I as described (*10*). The digested DNA fragments were resolved on a 10% acrylamide/7 M urea gel. Lane A + G is an adenine + guanine nucleotide sequence ladder. Sequencing reactions were performed as per the manufacturer (NEN). NA, no addition.

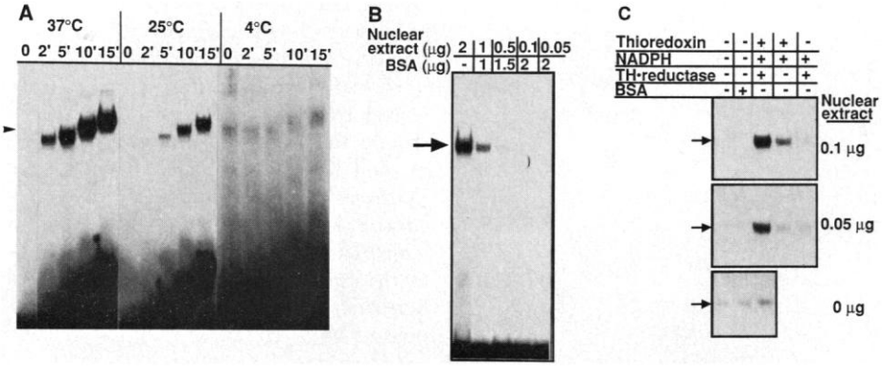

Fig. 4. Characterization of a stimulatory factor from liver nuclear extracts. (**A**) Temperature dependence of the stimulatory activity. Fos and Jun (0.05 μM) were incubated in binding buffer (legend to Fig. 2) at 37°C for 15 min to allow heterodimer formation. A ^{32}P-labeled oligonucleotide was added and the samples were incubated further at room temperature for 10 min. Subsequently, liver nuclear extract (2 μg of protein) was added, and the mixture was incubated at 37°C, 25°C, or 4°C for 0 to 15 min as indicated. The protein-DNA complexes were resolved by polyacrylamide gel electrophoresis (indicated by the arrowhead). The variable mobilities of the complexes result from the delayed loading at the indicated times. (**B**) Dependence of stimulation of DNA binding on the amount of liver nuclear extract added. Fos and Jun (0.05 μM) were incubated with the indicated amounts of liver nuclear extract (micrograms of protein) in the presence of BSA, for a final protein concentration of 2 μg, and the samples were incubated at 37°C for 15 min. The arrow indicates the position of specific protein-DNA complexes. (**C**) Stimulation of the nuclear factor by thioredoxin. Nuclear extract (0 to 0.1 μg of protein) was incubated with Fos and Jun (0.05 μM) in the presence (+) or absence (−) or thioredoxin (10 units), NADPH (0.2 mM), thioredoxin reductase (1.4 μg), and BSA (2 μg) in various combinations as indicated in the figure. Samples were incubated at 37°C for 15 min prior to the addition of ^{32}P-labeled DNA. The protein-DNA complexes were analyzed by a gel retardation assay. The arrows indicate the position of the specific protein-DNA complexes.

potential of v-*jun* may be due, in part, to a deregulation of DNA binding conferred by loss of a regulatory cysteine residue. The C1 cysteine is located in a highly conserved triamino acid sequence (Lys-Cys-Arg) that is invariant among the *fos* and *jun* gene families (*12*). This particular local environment enhances the reactivity of cysteine residues by several orders of magnitude (*25*). It is possible that the reactive nature of the cysteine residue, promoted by the local basic environment, makes it readily susceptible to oxidation, and thus, it is an attractive candidate for regulation by a nuclear redox system. Similar properties have recently been described for the E2 transcription factor of papilloma virus. This protein requires DTT for DNA-binding activity (*26*). The requirement for a single cysteine residue and the sensitivity of Fos and Jun to NEM excludes the possibility that oxidation of C1 involves intra- and intermolecular disulfide bond formation. In the case of OxyR, it was suggested that conversion of single essential cysteine to sulfenic acid regulates DNA-binding specificity (*16*). Conversion of cysteine to reversible oxidation products such as sulfenic (RSOH) or sulfinic (RSO_2H) acids, as opposed to the usually irreversibly oxidized form, sulfonic acid (RSO_3H), could contribute to regulation of the DNA-binding activity of Fos and Jun. We have identified a nuclear activity that catalyzed the reduction of Fos and Jun. It is possible that there are other enzymes in localized areas of the nucleus that would act in an antagonistic manner. This would be an economical means of regulating protein-DNA interactions in specific situations where members of the *fos* and *jun* gene families are expressed.

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Surface-Induced X-Ray Reflection Visualization of Membrane Orientation and Fusion into Multibilayers

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The fusion of lipid membranes at the air-water interface has been detected with the use of x-ray reflection as a high-resolution, surface-sensitive technique. The rate of this fusion for dimyristoylphosphatidylcholine (DMPC) bilayers is the highest at 29°C, which coincides with the chain-melting phase-transition temperature for the top membrane layers. After 6 hours of incubation a stack of at least ten surface-ordered membrane bilayers in equilibrium with the bulk vesicle suspension is formed. Such fusion is thus surface-catalyzed but not restricted to the first surface layer. The process involves partial membrane dehydration near the solution surface which decreases toward the bulk.

Molecular function and reorganization in the biological systems often involve interfaces. Membrane fusion provides good examples for this. To clarify the detailed physical principles that control the structural reorganization near the biological surfaces—and the role of interfaces in general—we have monitored the ordering and fusion of lipid vesicles into multibilayers near the air-water interface. We have used x-ray reflection as a high-resolution, surface-sensitive technique for this purpose (*1*). Our results indicate that both membrane ordering and membrane fusion depend on surface phenomena but are not always restricted to the top layer in close contact to the air. The rate of the observed structural reoganization is temperature-dependent. It changes abruptly a few degrees above the membrane chain-melting phase-transition temperature. The precise value of such a characteristic temperature is surface-sensitive because of the partial surface-induced membrane dehydration and concomitant isothermal phase transitions and fusion. In our case, this temperature coincides with the value that has previously been reported to give rise to the spontaneous formation of single bilayers under different conditions (*2*). We believe that this is a manifestation of a general principle of the surface catalysis. The relatively short range of the observed surface effects, which is probably less than 80 nm, provides a means for the preparation and structural investigation of the surface-supported, well-oriented membrane specimen.

Dimyristoylphosphatidylcholine (DMPC; Boehringer Mannheim) and water (>18 MΩ cm) of the highest available purity (>99.9%) were used for all experiments. In independent measurements, the order-disorder, chain-melting phase transition of the lipid suspensions was confirmed to be 23.5° ± 0.2°C for a 1-day-old bulk sample; the corresponding value for a sample aged for 4 weeks in humid air above pure water in a closed compartment at ambient temperature was measured by differential scanning calorimetry to be 29° ± 1°C, suggesting a partial sample dehydration. The transition enthalpies in both cases were not significantly different (26 ± 1.5 kJ mol^{-1}).

X-rays ($\lambda = 0.138$ nm) were directed nearly parallel to the surface of the lipid suspension at a series of incident angles ($1 \leq \theta \leq 60$ mrad). The resulting initial reflectivity of the vesicle suspension surface was found to be similar to that characteristic of the lipid monolayers deposited at the air-water interface. For a specimen kept below 26°C such a picture persists for at least one-half day (and probably longer). Conversely, the reflectivity measured at 29°C after less than 1 hour is already different, especially in the low-angle region. After a few hours a series of sharp, asymmetric but equidistant peaks evolves from the initial state (Fig. 1). To clarify the nature of these peaks we have also calculated the reflectivity of a series of layers (consisting of an electron-dense headgroup region and two adjacent, electronically less dense hydrocarbon and water layer zones) under the assumptions that such layers normally form bilayers and that the overall contrast decreases exponentially toward the bulk. From the detailed comparison of the measured and calculated data for various combinations of the layer structure parameters we deduce that the observed peaks reflect the formation of multilamellar, stacked membranes. The calculated number of coplanar layers pertaining to Fig. 1 is 11 to 12, the repeat distance of these membranes being 6.2 nm at 29°C; this value is slightly less than the characteristic intermembrane distance in the bulk in excess water, 6.25 nm. Once created, the peaks keep growing for hours, even at temperatures below 29°C.

The shape of the maxima in the reflectivity curves is temperature-independent within the investigated range (20° to 32°C). The

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