The transcription activation domains of Fos and Jun induce DNA bending through electrostatic interactions

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Introduction

The fundamental importance of DNA structural changes induced by transcription factor binding has been well established in prokaryotes (Hoover et al., 1990; Perez-Martin and Espinosa, 1993; Ansari et al., 1995). In eukaryotes, the LEF-1, YY1 and HMG-I/Y proteins have been shown to regulate transcription of the T-cell receptor (TCR) α, c-fos and interferon (IFN) β promoters respectively, most likely through mechanisms involving DNA bending (Natesan and Gilman, 1993; Falvo et al., 1995; Giese et al., 1995). However, the ability of regions outside the DNA-binding domain of many transcription factors to activate transcription when fused to heterologous DNA-binding domains has impeded general acceptance of this concept.

Structural studies of the basic-leucine zipper (bZIP) domain have revealed a simple and elegant mechanism for cooperative DNA recognition. The leucine zipper forms a coiled-coil dimerization interface and the basic region forms an α-helix that contacts base pairs within the major groove of the DNA recognition site (Oakley and Dervan, 1990; O’Neil et al., 1990; Talanian et al., 1990; O’Shea et al., 1991; Konig and Richmond, 1993; Glover and Harrison, 1995). The basic regions of Fos and Jun occupy the major groove on different sides of the DNA helix project out from the side of the DNA helix opposite to the leucine zipper. There is no high resolution structural information available for regions outside the minimal bZIP domains. The transcription activation domains of Fos and Jun are located on opposite sides of the bZIP region in the primary structure (Abate et al., 1991), and it is likely that they are located on different faces of the DNA helix in the ternary complex. Fos and Jun share a low level of sequence similarity between their transcription activation domains, and chimeras containing segments from each protein display transcriptional activity (Sutherland et al., 1992). Multiple regions in Fos and Jun have been shown to interact with several components of the general initiation complex (Metz et al., 1994; Martin et al., 1996). The roles of these interactions in transcription activation remain to be determined.

Fos and Jun were originally shown to induce DNA bending in studies that employed phasing analysis, a method that is based on the phase-dependent interaction between two closely spaced DNA bends (Kerppola and Curran, 1991a,b). Surprisingly, Fos–Jun heterodimers and Jun homodimers were found to bend DNA in opposite directions. Furthermore, whereas the bZIP domains of Fos and Jun induced small bends, the full-length proteins induced larger bends, and each subunit in the heterodimer appeared to contribute independently to DNA bending. DNA bending by Fos and Jun has been visualized directly by atomic force and electron microscopy (Griffith et al., 1994; Becker et al., 1995). The bZIP domains induce DNA bending through charge interactions that involve amino acid residues adjacent to the basic region (Leonard et al., 1997). However, X-ray crystallographic analysis of proteins encompassing the minimal bZIP domains of Fos and Jun bound to the AP-1 site did not detect significant DNA bending (Glover and Harrison, 1995). X-ray crystallographic analysis and solution studies of intrinsic DNA bending have also reached diametrically opposite conclusions (Crothers et al., 1990; Dickerson et al., 1994). Thus, alternative methods for the analysis of protein induced-changes in DNA structure are required.

Results

DNA bending by the transcription activation domains

We previously reported that truncated Fos and Jun proteins encompassing the leucine zipper dimerization and basic
**Fig. 1.** DNA bending by regions of Jun outside the dimerization and DNA-binding domains. (A) The full-length and truncated Jun proteins indicated above the lanes were incubated with the phasing analysis probes described previously (Kerppola and Curran, 1991a,b) and the complexes were analyzed on an 8% polyacrylamide gel. The diagrams above the lanes show the bZIP (++) activation (open oval) and repression (solid circle) domains of Jun mapped previously (Abate et al., 1991), with the amino-termini pointing down. Each set of lanes contained probes in which the separation between the centers of the AP-1 site and the intrinsic DNA bend was 21, 23, 26, 28 and 30 bp respectively. The differences between the mobilities of complexes bound to these probes reflect DNA bending as they result from the variation in the phasing between intrinsic and protein-induced DNA bends. The origin of electrophoresis is at the top and the free probes are at the bottom of the figure. (B) Phasing plots of the relative mobilities of homodimer complexes. To allow comparison between DNA bending by complexes with different absolute mobilities, the complex mobilities were normalized for differences in probe mobilities to an average mobility of 1, and these relative mobilities were plotted as a function of the separation between the centers of the AP-1 site and the intrinsic bend as described (Kerppola and Curran, 1991a). The best fit of the phasing function is superimposed on the data (Kerppola and Curran, 1991b). The degree of variation in the relative mobilities is a function of the magnitude of the DNA bend. Plots for complexes that induced significantly different bends based on statistical analysis of binding by all complexes (see Figure 3) are separated by heavy lines. The abscissa for each plot is 20–31 bp.

DNA-binding domains induce DNA bends that are smaller than those induced by the full-length proteins (Kerppola and Curran, 1991a,b). The majority of X-ray and nuclear magnetic resonance (NMR) studies of protein–DNA complexes have been performed using the minimal DNA-binding domains of the respective proteins. Thus, little is known about the effects of regions outside the minimal DNA-binding domains on the structures of protein–DNA complexes. To investigate whether the differences in DNA bending between full-length and truncated Fos and Jun were attributable to specific regions, we analyzed DNA bending by successive amino- and carboxy-terminal deletion derivatives of Fos and Jun. Phasing analysis of homodimers formed by Jun deletion derivatives indicated that a region located between amino acid residues 91 and 186 increased the DNA bend angle (Figure 1). A region between residues 31 and 57 reduced the DNA bend...
DNA bending by Fos–Jun activation domains

Fig. 3. Quantitation of DNA bending by heterodimers formed between all combinations of Fos and Jun deletion derivatives. The relative mobilities of complexes formed by all heterodimeric combinations between Fos and Jun deletion derivatives were plotted in the rows and columns corresponding to the subunits of each complex shown above and to the left of the matrix. The Fos and Jun deletion derivatives are shown as in Figures 1 and 2. The proteins designated JX contained a factor Xa cleavage site between the hexahistidine purification tag and the native coding region. Removal of the fusion peptide by factor Xa cleavage had no effect on DNA bending. The abscissa for each plot is 20–31 bp and the ordinate is from 0.7 to 1.3. The DNA bend angle ($\alpha$) and direction ($\beta$) derived from the phasing function (see Materials and methods) are shown in the upper left and upper right corners of each plot. For 20 complexes, a sufficient number of independent experiments were performed to derive meaningful standard deviations for the relative mobilities, which are shown as vertical bars. The DNA bend induced by each monomer was calculated based on the hypothesis that each subunit induces an independent bend (Kerppola and Curran, 1991b), and are shown to the left and above the matrix of plots. These bends were calculated by least squares minimization of the error of a model in which the bend induced by each heterodimer represented the vector sum of the bends induced by its constituent subunits. The 21 calculated subunit bends predicted the bends induced by all 110 heterodimers with an error of $H^{110}{H^2}^{21}$ in bend angle and $H^{110}{H^2}^{298}$ in bend direction (the direction of bending was predicted for complexes that induce bend angles of $>5^\circ$). Multivariate analysis of variance supported the validity of the model ($P < 0.001$) and pairwise $T^2$ tests indicated that subunits separated by heavy lines induced significantly different DNA bends.

Separation between centers of AP-1 site and intrinsic bend

and 321 had intermediate effects. This DNA-bending region also overlaps a transcription activation domain in Fos (Abate et al., 1991; Metz et al., 1994). Thus, in both Fos and Jun, specific regions outside the bZIP domains that coincide with transcription activation domains influence DNA bending.

Previously, we proposed that the individual subunits of the Fos–Jun heterodimer induce separate DNA bends, and that the overall angle and direction of DNA bending induced by the dimeric complex represents the sum of the bends induced by the two subunits (Kerppola and Curran, 1991a,b). To determine if DNA bending by complexes formed by the various Fos and Jun deletion derivatives was consistent with this model, we quantitated the DNA bends induced by all heterodimeric combinations between these proteins (Figure 3). The same regions of Fos and Jun affected DNA bending regardless of their dimerization partner. The bends induced by the individual subunits were calculated by finding the best fit of their sums to the DNA bends induced by all heterodimeric combinations. These deduced DNA bends confirm that discrete regions of Fos and Jun influence DNA bending whereas other...
regions have little or no effect. The same regions in Jun influence DNA bending in both homodimers and heterodimers. The model accurately predicted the DNA bends induced by all heterodimers and was corroborated by multivariate analysis of variance. Therefore, the individual subunits of the Fos–Jun heterodimer induce separate DNA bends.

Several of the deletions that affected DNA bending also had an effect on DNA-binding affinity. However, whereas the effects on DNA bending were observed regardless of dimerization partner or binding site, the effects on binding affinity were observed only for a subset of the complexes containing a particular protein. Thus, the region between residues 31 and 56 in Jun reduced both DNA-binding affinity and DNA bending in the context of Jun homodimers. The same region also affected DNA bending in the context of heterodimers, but had no detectable effect on their DNA-binding affinity. Conversely, the region between residues 241 and 252 of Jun increased both DNA bending and the DNA-binding affinity of Jun homodimers at an AP-1 site. This region also affected DNA bending by heterodimers, but had no detectable effect on their DNA-binding affinity. Also, whereas this region affected DNA bending at both AP-1 and CRE sites, it had no detectable effect on the affinities of either homodimers or heterodimers at the CRE site. Thus, although differences in DNA bending may influence the DNA-binding affinities of different Fos and Jun complexes, these differences in binding affinity do not influence measurement of DNA bending by these complexes using phasing analysis.

**DNA bending by chimeric proteins**

The observation that regions overlapping the transcription activation domains of Fos and Jun modulate DNA bending raised the possibility that DNA structural changes could contribute to transcription activation by these proteins. To determine if the transcription activation domains of Fos and Jun induced DNA bending when fused to a different DNA-binding domain, we examined DNA bending by chimeric proteins in which the transcription activation domains were fused to different bZIP regions (Figure 4). To determine the significance of the position of the transcription activation domain relative to the DNA-binding domain, these domains were fused on both sides of the respective bZIP regions. All of the chimeras induced DNA bends that were distinct from that induced by the minimal bZIP region peptides. Therefore, the transcription activation domains of Fos andJun induced DNA bending when fused to different DNA-binding domains.

The bends induced by complexes containing the transcription activation domains fused on different sides of the bZIP region were distinct. Complexes where the transcription activation domain was fused on the carboxy-terminal side of the bZIP region induced a larger bend than the bZIP regions alone, whereas fusion of the transcription activation domains on the amino-terminal side of the bZIP region reduced DNA bending relative to the bZIP regions alone. Thus, the position of the transcription activation domain affected the direction of DNA bending. The transcription activation domains of Fos and Jun induced qualitatively similar bends when fused to the same position in the bZIP region. Thus, the opposite directions of DNA bending induced by intact Fos and Jun are not due to an intrinsic difference in the DNA-bending properties of their respective transcription activation domains. Furthermore, whereas activation domain fusions to the carboxy-terminal ends of each of the two bZIP regions resulted in very similar bends, fusions to the amino-terminal ends resulted in different directions of DNA bending. Consequently, the transcription activation domains of Fos and Jun had qualitatively similar DNA-bending properties, and differences in the positions of these
domains relative to the DNA-binding domain resulted in dramatic differences in DNA bending.

To quantify the effects of the transcription activation domains on DNA bending, we calculated the contributions of the bZIP and transcription activation domains to DNA bending based on the hypothesis that these domains have additive effects on DNA bending (Figure 5). This hypothesis is supported by the remarkable result that the magnitudes of the DNA bends calculated based on this hypothesis were virtually identical whether the transcription activation domains were fused to the amino- or carboxy-terminal sides of the bZIP regions of Fos or Jun. In addition, multivariate analysis of variance of DNA bending by the chimeric proteins at 10 different binding sites (Rajaram and Kerppola, 1997) is consistent with independent effects of the transcription activation and DNA-binding domains on DNA bending. Thus, the transcription activation domains induce DNA bends of equal magnitude regardless of their structural context. In contrast, the direction of DNA bending was determined by the position of the transcription activation domain in the complex (Figure 5). The transcription activation domains of Fos and Jun induced bends of different magnitudes, but the directions of bending were identical for domains fused to the same position. Consequently, the extent of DNA bending induced by the transcription activation domains is independent of the bZIP domains, and the direction of bending is determined by the side of the DNA helix where the transcription activation domain is located.

The native Fos and Jun proteins induce DNA bending in opposite directions. However, when the Fos and Jun transcription activation domains were fused to the same side of the bZIP region, they induced DNA bending in the same direction (i.e. compare FD-FA:JD and FD-JA:JD, Figures 4 and 5). In contrast, when either of these transcription activation domains was fused to opposite ends of the bZIP region, it induced DNA bending in virtually opposite directions (i.e. compare FD:JD-FA and FD:FA-JD, Figures 4 and 5). The magnitudes of the DNA bends induced by the transcription activation domains when placed on either side of the bZIP region were similar to the bends induced by the same regions in the context of full-length Fos and Jun. Thus, the opposite directions of DNA bending induced by Fos and Jun are in part due to the converse arrangement of the transcription activation and DNA-binding domains in the native Fos and Jun proteins.

The DNA bends induced by Fos and Jun counteract each other, but they are not directed in diametrically opposite directions (Kerppola and Curran, 1991a,b). Similarly, the DNA bends induced by the Fos and Jun transcription activation domains fused to opposite ends of the bZIP region were oriented away from each other at an oblique angle (Figure 5C). Furthermore, the direction of DNA bending induced by the transcription activation domains when fused to the basic region of Fos was distinct from that induced when they were fused to the basic region of Jun. These DNA bends were related by a 2-fold symmetry axis, which coincided with the major groove–minor groove axis at the center of the AP-1 site. This suggests that the transcription activation domains are located in distinct, symmetrically related positions in the complex when fused...
to the basic regions of Fos and Jun. One interpretation of this result is that the Fos–Jun heterodimer binds to the AP-1 site in a preferred orientation, placing transcription activation domains fused to the Fos and Jun basic regions in rotationally symmetrical positions (Rajaram and Kerppola, 1997). Consequently, the symmetry relations inherent in the dimeric bZIP DNA-binding motif are reflected in the relative directions of DNA bending induced by transcription activation domains fused to different positions in the bZIP regions.

To examine the specificity of the effects of the Fos and Jun transcription activation domains on DNA bending, we determined the effect of the transcription activation domain from CTF-1 on DNA bending when fused to the dimerization and DNA-binding domains of Jun (Figure 6). Phasing analysis of complexes formed by the chimeric protein demonstrated that the transcription activation domain of CTF-1 did not alter DNA bending by the Jun bZIP domain in either homodimers or heterodimers. Thus, DNA bending is a property specific for a subset of transcription activation domains.

**Charge interactions contribute to DNA bending by Fos and Jun**

Since the Fos and Jun transcription activation domains have similar effects on DNA structure when placed in the same position relative to the bZIP domain, they are likely to rely on common molecular mechanisms of DNA bending. Truncation of Fos at residue 270 eliminated approximately half of the DNA-bending potential of this region (Figures 2 and 3). This truncation bisects a segment of limited sequence similarity between the Fos and Jun transcription activation domains that contains clusters of negatively charged amino acid residues (Abate et al., 1991; Sutherland et al., 1992). Conversely, the repressor domain of Jun that reduced DNA bending (Figures 1 and 3) has a net positive charge. Mutational analysis of residues adjacent to the bZIP domains of Fos and Jun indicates that DNA bending correlates with the net charge of residues adjoining the basic region (Leonard et al., 1997). The CTF-1 activation domain that did not induce DNA bending is a proline-rich transcription activation domain, and contains no clusters of charged residues. Thus, the charge of the transcription activation domains of Fos and Jun may contribute to DNA bending by these regions.

To investigate the possible role of charge interactions in DNA bending by Fos and Jun, we examined the effect of multivalent cations on DNA bending. DNA retains a shell of associated counterions in solution that partially neutralizes the charge of the phosphodiester backbone (Manning, 1978). The extent of charge neutralization varies depending on the valence of the associated counterions. Electrophoresis in the presence of 1 mM MgCl$_2$ reduced the phase-dependent variation in the mobilities of all complexes examined (Figure 7). Similar decreases in the mobility variation were observed in the presence of 1 mM spermidine and 0.1 mM hexamminecobalt. There was no significant effect of the multivalent cations on the relative electrophoretic mobilities of intrinsic DNA bend standards (Figure 7B). Thus, electrostatic interactions contribute to DNA bending by Fos and Jun.

**Discussion**

The paradigm of modular DNA binding and transcription activation domains has been a powerful influence in studies of the mechanisms of transcription activation. The independent functions of the two domains have lent support for models in which transcription activation domains act through mechanisms that do not involve changes in DNA structure. Here we show that regions outside the bZIP domain influence DNA bending by Fos and Jun. The localization of these DNA-bending regions within transcription regulatory domains (Abate et al., 1991; Metz et al., 1994) indicates that DNA structural changes may contribute to transcription regulation. The DNA-bending region in Jun also promotes T antigen-dependent DNA unwinding in the initiation of polyoma virus DNA replication (Ito et al., 1996). Activation of both transcription and replication by the same protein domain suggests that a common mechanism such as the distortion of DNA structure contributes to both processes.

Fos and Jun bend DNA in opposite directions. Nevertheless, both proteins contribute to transcription activation in cultured cells in vitro at most promoters that have been examined. In contrast, under physiological conditions, Fos and Jun perform distinct functions, as shown by the dissimilar phenotypes of mice in which one or the other gene has been deleted (Hilberg et al., 1993; Grigoriadis et al., 1994). Gene regulation in animals requires the
DNA bending by Fos–Jun activation domains

Fig. 7. Effects of multivalent cations on DNA bending by Fos and Jun bending domains. (A) Phasing analysis of DNA bending in the presence and absence of spermidine. The heterodimers indicated above the lanes were incubated with phasing analysis probes containing an AP-1 site (sequence X described in Rajaram and Kerppola, 1997). Each set of lanes contained probes in which the separations between the AP-1 site and the intrinsic bend were 26, 28, 30, 32, 34 and 36 bp respectively. DNA bending was examined by gel electrophoresis in the presence (right panel) and absence (left panel) of 1 mM spermidine in the gel and in the recirculated electrophoresis buffer. The time of electrophoresis was adjusted to equalize the average mobilities of the complexes in the presence and absence of spermidine. (B) Phasing plots of DNA bending by chimeric protein complexes in the presence of MgCl₂ and spermidine. The proteins indicated on the left were bound to phasing analysis probes containing the site used in the X-ray crystallographic analysis (Glover and Harrison, 1995; Rajaram and Kerppola, 1997, accompanying manuscript) and analyzed by electrophoresis in gels containing 1 mM MgCl₂ or 1 mM spermidine. The relative mobilities of the complexes were plotted as a function of the separation between the centers of the AP-1 site and the intrinsic DNA bend. The abscissa for each plot is 25–39 bp. Multivariate analysis of variance supported the hypothesis that the DNA bends induced in the presence of MgCl₂ and spermidine differed from the bends induced in the absence of multivalent counterions (P < 0.001). The plots at the bottom show the mobility anomalies of intrinsic DNA bend standards containing between two and nine phased A tracts prepared by NheI digestion of PCR products generated by using plasmids pJT170-2–pJT170-11 (Thompson and Landy, 1988) as templates, run on the same gels as the protein complexes. The abscissa for each plot is 0lADl–180lADl.

interdependent function of multiple regulatory elements in the promoter and can differ from that observed in cultured cells in vitro (Robertson et al., 1995). It is in the context of the topological constraints imposed by interactions among multiple proteins bound to separate promoter elements that DNA bending by the Fos and Jun transcription activation domains is likely to be most significant. Consequently, Fos and Jun regulate different target genes in the animal, and it is possible that the opposite directions of DNA bending induced by these proteins contribute to such differences in target gene selectivity. DNA bending by Fos and Jun was reduced in the presence of multivalent cations. All of the regions of Fos and Jun that affected DNA bending contain clusters of charged residues. Thus, DNA bending by Fos and Jun is mediated at least in part by charge interactions. The converse effects of sequences flanking the AP-1 site on DNA bending in opposite directions (Rajaram and Kerppola, 1997) suggest that these charge effects include interactions with the phosphates of base pairs flanking the AP-1 site. However, a high density of charged residues is not sufficient to induce DNA bending since other charged regions in Fos and Jun have no effect on DNA bending. Thus, the structural context of the charged residues is important for their influence on DNA bending. The transcription activation domains of Fos and Jun may share a common structural fold that is required for DNA bending and transcription activation.

The X-ray crystallographic analysis of the minimal
bZIP regions of Fos and Jun was performed in the presence of high concentrations of salt, including multivalent cations (Glover and Harrison, 1995). Thus, the charge interactions that induce DNA bending may have been partially shielded in the crystal. Multivalent cations do not completely neutralize the charge of the phosphodiester backbone, nor do they eliminate DNA bending by Fos and Jun. Analysis of DNA bending at different MgCl₂ concentrations indicated no concentration dependence of DNA bending, consistent with the near-constant charge of DNA under these conditions predicted by polyelectrolyte theory (Manning, 1978). Thus, moderate concentrations of multivalent cations, such as those encountered in the cell, do not preclude DNA bending by charge interactions with the phosphodiester backbone. Additional factors including crystal packing forces (DiGabriele et al., 1989) and agents used to promote crystallization (Sprous et al., 1995) may influence the conformation of DNA in the crystal.

A simplified Coulombic analysis indicated that the electrostatic potential energies of the transcription activation domains exceed the energy required for DNA bending. Long range electrostatic interactions have been reported previously between the active sites on proteins and distant charged residues (Thomas et al., 1995; Gao et al., 1996). More detailed calculation of the electrostatic interactions between the transcription activation domains and DNA is not possible because of the lack of structural information about the Fos and Jun transcription activation domains. However, studies of other structurally defined complexes using linearized Poisson–Boltzmann equations predict free energies for long-range charge interactions that are of magnitudes comparable with that required for DNA bending (Zacharias et al., 1992; Misra et al., 1994; Phillips and Phillips, 1994).

Four different mechanisms of protein-induced DNA bending have been proposed. The mechanism observed most commonly in X-ray crystal and NMR structures is mediated by side chain intercalation between the base pairs in the DNA (J.L.Kim et al., 1993; Y.Kim et al., 1993; Love et al., 1995). A second mechanism is mediated by the arrangement of DNA contact residues on a curved surface, generally in a dimeric protein complex (Brennan et al., 1990; Schultz et al., 1991; Jin et al., 1995; Li et al., 1995). DNA bending can also be induced indirectly through interactions between proteins that bind to separate sites on DNA and thereby constrain the intervening DNA in a loop (Lobell and Schleif, 1990; Mandal et al., 1990). Finally, it has been shown that neutralization of phosphates on one side of the DNA helix by chemical modification can result in DNA bending (Strauss and Maher, 1994; Strauss et al., 1996). Such asymmetric charge neutralization has been proposed to mediate wrapping of DNA around the nucleosome core (Mirzabekov and Rich, 1979).

Fos and Jun induce DNA bending through a mechanism that also involves asymmetric charge interactions. However, in the case of DNA bending by the Fos and Jun transcription activation domains, this asymmetric charge interaction does not alter the local charge distribution of the DNA helix, but imposes a directional electrostatic force on DNA (Figure 8). Mutational analysis of the bZIP domains indicates that DNA bending is directly proportional to the charge of amino acid residues adjoining the basic region, demonstrating that DNA bending by the bZIP domains is also caused by electrostatic interactions (Leonard et al., 1997). Such electrostatic forces provide a potentially general mechanism for DNA bending by charged domains that does not require direct contact between the bending domain and DNA.

The observation that charge interactions between transcription activation domains and the phosphodiester backbone can induce DNA bending independently of the DNA-binding domain has important implications for eukaryotic transcription regulation. A large number of transcription activation domains contain a high proportion of negatively charged residues. These domains may, in addition to contacting several components of the transcription initiation machinery (reviewed in Goodrich et al., 1996), also alter DNA structure. Many transcription fac-
Materials and methods

Plasmid construction and protein purification. The phasing analysis plasmids pTK401-21, -23, -26, -28 and -30 have been described (Kerppola and Curran, 1991a). Phasing analysis plasmids pNR421-28, -30, -32, -34, -36 and -38, containing the sequence used for X-ray crystallography, were constructed as described (Rajaram and Kerppola, 1997).

Phasing analysis

Probes were prepared for phasing analysis by amplification of fragments between 349 and 366 bp in length containing the AP-1 site (encoding RS) at the carboxy-terminus, and an amino-terminal BglII site (encoding 18° per A100–198, JA) containing a bZIP domain to ensure quantitative heterodimerization. Homodimers were constructed by amplification of fragments from the initiation and from ALSAC.

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