Fos and Jun : Oncogenic Transcription Factors

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CURRAN, T. *Fos and Jun : Oncogenic Transcription Factors*. Tohoku J. Exp. Med., 1992, **168** (2), 169-174 — The *fos* and *jun* proto-oncogenes are members of the set of genes known as cellular immediate-early genes. Their expression is induced transiently by a great variety of extracellular stimuli associated with mitogenesis, differentiation processes or depolarization of neurons. They encode DNA binding proteins that form dimeric complexes through a leucine zipper structure that function as transcription factors. Continuous overexpression of *fos* or *jun* causes transformation of fibroblasts. Because of their ubiquitous expression it is believed that the target genes regulated by Fos and Jun are different in the many circumstances in which they are expressed. Thus, their functional specificity is likely to be regulated at several levels. We have uncovered several potential mechanisms that could contribute to their regulation. These include formation of a large number of heterodimeric complexes, post-translational modification by phosphorylation and a novel reduction/oxidation (redox) mechanism, presence of both positive and negative transcriptional domains and the ability of Fos and Jun to induce distinct bends in DNA structure. ——— *fos* ; heterodimeric complex ; *jun* ; phosphorylation ; reduction/oxidation

The oncogenes *fos* and *jun* were first described as the genetic information responsible for the induction of tumors by the FBJ murine sarcoma virus and avian sarcoma virus 17 (for review see Curran 1988). They were derived from normal cellular genes (*c-fos* and *c-jun*) that function as regulators of gene transcription (for review see Curran and Vogt 1992). Thus, oncogenesis by the *fos* and *jun* oncogenes is a consequence of a defect in gene regulation processes. By studying the properties of the normal cellular *fos* and *jun* genes we hope to gain insight into the mechanisms responsible for tumor induction.

In the majority of cell types, though not all, *fos* and *jun* are expressed at relatively low basal levels. However, they can be induced rapidly and transiently by several extracellular stimuli (Fig. 1). Their protein products, Fos and Jun, respectively, are thought to function in coupling short-term stimuli elicited by cell surface stimuli to long-term alterations in cellular phenotype by regulating expression of target genes. Thus, Fos and Jun can be considered as nuclear “third messenger” components of a signal transduction cascade.

Fig. 1. Role of Fos and Jun in signal transduction in the nucleus.

Stimulation of cell surface receptors or ion channels, or direct activation of second messenger systems generates a signal that triggers transcription of immediate-early genes in the nucleus. Transcription of *c-fos* and *c-jun* occurs transiently and mRNA is transported to the cytoplasm. Fos and Jun are translated and transported to the nucleus where they form dimers. Fos-Jun dimers bind to AP-1- and CRE-related sequences and regulate gene expression.

Although they were both isolated independently, the *fos* and *jun* oncogenes function cooperatively in the regulation of gene expression (reviewed in Curran and Franza 1988). They do so by forming a heterodimeric complex through a leucine zipper structure (Fig. 1). The leucine zipper serves to juxtapose regions of Fos and Jun, rich in basic amino acids, that form a bipartite DNA-binding domain (Gentz et al. 1989). Protein dimers bind to DNA sequences related to the Activator Protein-1 (AP-1) and cAMP responsive element (CRE) motifs. The zipper structure is not exclusive to Fos and Jun; it was first identified in the sequence of the C/EBP transcription factor (Landschulz et al. 1988). However, there is a high degree of specificity involved in leucine zipper interactions and only certain subsets of proteins can form heterodimers. Both *fos* and *jun* are members of gene families that share the leucine zipper motif. There are 3 *fos*-related genes (*fra-1*, *fra-2* and FosB) and two *jun*-related genes (*junB* and *junD*) that are highly related in the leucine zipper and basic regions (Kerppola and Curran 1991a). Unlike the Jun-related proteins, the Fos family members do

not form stable homodimers, although they are all capable of forming heterodimers with each of the Jun-related proteins. The Jun-related proteins can form homodimers and heterodimers with each other as well as with the Fos-related proteins. Furthermore, Fos and Jun-related proteins are also capable of forming cross-family dimers with certain members of the CREB/ATF family of transcription factors (Hai and Curran 1991). Thus, the leucine zipper serves as a mechanism to generate diversity. A large number of transcription complexes can be generated from a relatively small number of components. The different components have distinct affinities and specificities for AP-1 and CRE binding sites. In addition, they appear to have different transcriptional properties. This raises the question of how is specificity controlled if there are so many protein complexes and so many potential DNA target sequences?

RESULTS AND DISCUSSION

A summary of some of the regions and potential mechanisms that could regulate the specificity of Fos and Jun is indicated in Fig. 2. Although the *fos*- and *jun*-related genes are all considered immediate-early genes, they are not all induced at the same time or expressed in the same situations. For example, although *fra-1* is highly inducible by serum stimulation of fibroblasts and by treatment of PC12 with nerve growth factor, unlike *c-fos*, it is not induced in neurons *in vivo* following seizure (Cohen and Curran 1988). Thus, different subsets of leucine zipper proteins may be available to participate in heterodimeric complexes in different cell types. Furthermore, there are several cell types in which certain immediate-early genes are expressed continuously. *c-fos* is expressed at high basal levels in terminally differentiated skin and hair cells (Smeyne et al. 1992). This implies that there is a complex balance among inducible and resident leucine zipper proteins that determines the array of heterodimeric complexes expressed in any cell type at a given time.

As discussed above, each of the dimeric leucine zipper complexes may have unique DNA-binding and transcriptional properties. We have undertaken a molecular dissection of these functions in Fos and Jun. Fig. 2 summarizes the

Fig. 2. Functional map of Fos-jun heterodimer.

several domains we have mapped using in vitro DNA-binding assays and in vitro transcription assays. The primary dimerization domain is the leucine zipper structure. However, it should be noted that the dimerization region extends beyond the leucines to include a histidine residue 7 amino acids C-terminal of the last leucine (Fig. 2) (Cohen and Curran 1990). Amino acids located between the leucine confer specificity on zipper interactions. The region rich in basic amino acids N-terminal of the zipper is the primary DNA-binding domain. However, amino acids outside of this region also influence DNA binding (Fig. 2) (Abate et al. 1991). An ancillary DNA-binding domain is required for high affinity binding of Jun homodimers to DNA. In addition, other regions influence the ability of Fos and Jun to induce DNA bending (see below). Thus, the dimerization and DNA-binding properties of Fos and Jun involve regions of the proteins that are not as well conserved as the zipper and basic regions. Therefore, different homodimeric and heterodimeric leucine zipper complexes may have subtle differences in their dimerization and DNA-binding properties. Several domains of Fos and Jun were found to have both positive and negative effects in in vitro transcription assays. The overall transcriptional activity of the dimer resulted from a combination of the effects of the positive and negative domains delineated in Fig. 2. This implies that each protein dimer may have a unique transcriptional profile.

The domains of Fos and Jun that affect dimerization, DNA binding and transcriptional regulation are illustrated diagrammatically in Fig. 2. The bars indicate the leucine zipper region. (+ +) is the DNA binding domain. The position of an ancillary DNA binding in Jun is indicated. (H) indicates the location of the histidine residue that affects dimerization. (C) represents the cysteine residue involved in redox regulation of DNA binding. A white bar indicates the region of *jun* deleted in *v-jun*. The white boxes represent activation domains and the black boxes represent regions that affect transcription negatively in vitro. (P) are sites of serine and threonine phosphorylation. (NH) is the N-terminus and COOH is the C-terminus.

The viral *fos* oncogene (*v-fos*) causes a very specific osteogenic neoplasm in mice (Finkel et al. 1966). It seems that *v-fos* transforms a pre-bone cell that retains the capacity for both osteodi and chondroid differentiation. There may be a particular target(s) of Fos in this cell type, deregulation of which results in aberrant cell growth. By analyzing the factors that specify target gene selection, we may gain insight into the molecular mechanisms responsible for tumor induction. One correlation that we noticed previously was that the *v-Fos* protein was only partially phosphorylated (Curran et al. 1984). Indeed, even in cells transformed by the *c-fos* gene expressed continuously we also observed that the *c-fos* protein is less modified than in serum-stimulated fibroblasts (Lee et al. 1989). Therefore, we analyzed the sites and enzymes responsible for phosphorylation of Fos and Jun. Using purified Fos synthesized in *E. coli* as a substrate several

protein kinases were found to use Fos as a substrate. These included protein kinase C, p34^{cdc2}, MAP kinase, DNA-dependent protein kinase and an unknown nuclear kinase, but not casein kinase II (Abate et al. 1992). The approximate sites of phosphorylation are indicated in Fig. 2. Interestingly, they all lie within the regions shown to affect transcription negatively in vitro. In serum stimulated cells, all of these sites in Fos appear to be phosphorylated. Thus, it is possible that phosphorylation could be a mechanism for overcoming inhibitory constraints on transcription.

Jun is also phosphorylated by several kinases including protein kinase C, p34^{cdc2}, MAP kinase, DNA-dependent protein kinase, EGF receptor kinase, casein kinase II and an unknown nuclear kinase, but not protein kinase A. Phosphorylation occurs in the DNA-binding domain and in an N-terminal domain that has a negative effect of transcription. Interestingly, removal of the region deleted in *v-jun* blocks phosphorylation of the N-terminal sites (Fig. 2). Phosphorylation is also dramatically affected by dimerization and DNA binding. Several enzymes are inhibited by DNA binding and heterodimer formation. Thus, phosphorylation could be used as a mechanism for distinguishing monomers from dimers and DNA-bound from non DNA-bound complexes.

Previously, we demonstrated that DNA binding by Fos and Jun was regulated by a novel redox mechanism involving a conserved cysteine residue in the DNA-binding domain (Abate et al. 1990). This regulation does not involve disulfide bond formation, instead an undetermined oxidized state of cysteine forms which inhibits DNA binding. High levels of reducing agents or a cellular factor are required to stimulate DNA binding. We have now purified and cloned the cellular redox factor, termed Ref-1 (Xanthoudakis and Curran 1992). It appears to be identical to enzymes involved in DNA repair processes. The exact relationships among DNA binding by Fos and Jun, redox and DNA repair are not yet clear. However, redox control of DNA binding may provide an additional mechanism whereby individual leucine zipper complexes can be selected to function in gene regulation.

A further complexity was encountered when we examined the ability of Fos and Jun to induce DNA bending. Like many DNA-binding proteins, Fos and Jun induce bends in the DNA helix. However, surprisingly, Fos-Jun heterodimers bend DNA in the opposite direction from Jun homodimers (Kerppola and Curran 1991a, b). Thus, different leucine zipper dimers cause very different topologies of protein-DNA complexes even though they may bind to the same DNA sequences. This could have dramatic consequences for interactions between factors bound to adjacent DNA sequences or proteins that function as co-activators and basal transcription factors. It is likely that several of the above mechanisms operate in concert to achieve the high degree of specificity required for the interaction of immediate-early leucine zipper proteins with their proscribed target genes.

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