A Novel, Transformation-Relevant Activation Domain in Fos Proteins

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We have previously demonstrated that transformation by Fos is critically dependent on an intact DNA-binding domain (bZip) and a functional N-terminal transactivation motif (N-TM). We now show that a novel motif (C-terminal transactivation motif [C-TM]) near the C terminus also plays an important role in both transformation and the activation of AP1-dependent transcription and that the hydrophobic amino acids in the C-TM are functionally essential. The C-TM is the most crucial element in the C-terminal transactivation domain in Fos, as indicated by its relative strength and context-independent function. The C-TM is clearly different from the previously identified HOB1 domain, located N terminally to the C-TM, and the C-terminally positioned TATA-binding protein-binding domain. We also show that the C-terminal transactivation domain strongly synergizes with the HOBI-like N-TM, even when both domains are present on different proteins within a dimeric complex, and that the C-TM plays a crucial role in this cooperation. These observations can be corroborated in a model in which multiple contacts with the basal machinery are established either to stabilize the transcription complex or to facilitate its sequential assembly.

The fos oncogene has been detected in three different RNA tumor viruses, the Finkel-Biskis-Jinkins mouse osteosarcoma virus (FBJ-MuSV), the Finkel-Biskis-Reilly mouse osteosarcoma virus (FBR-MuSV), and the chicken retrovirus NK24 (14, 15, 34). Both murine viruses cause chondro-osseous sarcomas in newborn mice and induce morphological transformation in murine fibroblasts and other connective tissue cells. The FBR-MuSV fos oncogene is, however, more potent in that it induces higher numbers of foci after a shorter latency period and can trigger the establishment of low-passage-number transformed cultures (9, 10, 18). The two viral fos oncogene products show a considerable degree of structural divergence. While the FBJ-MuSV-encoded protein, p55v-Fos, closely resembles the c-Fos protein except for a frameshift mutation at its C terminus, the FBR-MuSV gene product, p75v-Fos-Fox, has undergone N-terminal and C-terminal truncations, fusion with heterologous sequences at both ends, internal deletions, and several single-amino-acid changes (46, 47). A single-point mutation immediately adjacent to the DNA-binding site (Glu-138→Val) has been shown to be responsible for the immortalizing potential of the FBR-MuSV fos gene product (19). Analysis of structurally altered Fos proteins in focus assays has shown that the most crucial region of Fos for the induction of transformation lies between amino acids (aa) 111 and 220 (19, 33, 40). This region harbors the bZip motif, consisting of the leucine zipper, mediating complex formation with Jun, and the basic region identified as the DNA-binding site (16, 22, 32, 40, 45). The region between aa 40 and 111 is also required, since in the absence of these sequences, transformation is visible only after selection (19). It was recently shown that this region represents an N-terminal transactivation (N-TA) domain harboring a novel type of transactivation motif (N-terminal transactivation motif [N-TM]) which functions in a context-independent fashion (20). Interestingly, the N-TM resembles a HOBI motif and has therefore also been referred to as the HOBI-N motif (7). This finding indicates that the deregulation of Fos target genes, presumably via AP1 recognition sites (12-O-tetradecanoylphorbol-13-acetate [TPA] response elements [TREs]), may play a crucial role in the induction of transformation by v-Fos. This hypothesis is supported by the observation that transfected fos and jun genes cooperate in the induction of transformation (33).

To analyze the relationship between transformation and transactivation in greater detail, we have performed a comprehensive analysis of the Fos C terminus in both processes. We find that the efficient induction of transformation by Fos also requires the C terminus of the protein, in particular when expression is driven by a weaker promoter. These C-terminal sequences have previously been shown to contain an autonomous transactivation domain (20, 42), again pointing to a correlation between transformation and transactivation. This region of Fos has been shown to contain three motifs involved in transcriptional activation. These are the HOBI1 and HOBI2 motifs (42), also present in other Fos and Jun family members, and a domain (TATA-binding protein [TBP]-binding domain [TBD]) that interacts with TBP and is also present in FosB but not in Fra-1 (27, 28). However, the HOBI1 motif is not present in the potent FBR-MuSV Fos oncoprotein, and the TBD is deleted in both the FBJ-MuSV and FBR-MuSV Fos gene products, indicating that these elements are not important for transformation. We now show that the functionally crucial region in the c-Fos C terminus is a transactivation domain. A short motif within this domain, the C-terminal activation motif (C-TM), is shown to activate transactivation in a context-independent fashion. We also show by using an intergenic complementation assay that the C-TM cooperates with the N-terminal HOBI1-like activation motif N-TM, even when both activation domains are present on different proteins within an AP1 complex.
FIG. 1. (A) Effects of C-terminal truncations on the transforming (focus formation) potential of E300 and E316 v-Fos proteins in 208F rat fibroblasts. E316 is a viral Fos protein based on FBJ-MuSV (47) that terminates at aa 316 and contains the FBR-MuSV-specific point mutations at positions 64 and 138 (46). E300 (20, 40) is similar to E316 except that it contains the FBR-MuSV-specific internal deletions at its C terminus terminating the HOB1 domain. C-terminal deletion variants of E316 are named by the endpoints of their deletions (E292 includes aa 1 to 292 of E319). Values represent the means of four independent experiments with standard deviations of less than 15%. Values were calculated relative to that for E316. (B) Immunoassay analysis of Fos expression in cells stably transfected with pRAX (FBR-MuSV LTR; left) (19) and pMSE (SV40 early promoter/enhancer; right) (39) expression vectors carrying full-length E300 or the C-terminally truncated variant E220 (truncated at aa 220).

MATERIALS AND METHODS

Cell culture, F9, 208F, and NIH 3T3 cells were cultured in Dulbecco-Vogt modified Eagle’s minimum essential medium supplemented with 10% fetal calf serum, 0.5% glucose, penicillin (100 U/ml), and streptomycin (100 μg/ml).

Focus assay. Focus assays were carried out essentially as described previously (20). After trypsinization, 7 × 106 cells were seeded per dish (6-cm diameter) and transfected 24 h later with 3 μg of fos expression plasmid plus 8 μg of 208F carrier DNA. For cotransfections, 6 μg of carrier DNA and 3 μg of each fos and Yfos expression plasmid were transfected. Ten hours later, the cells were rinsed and cultures were split into three dishes, one of which was selected with G418 (500 μg ml−1). The medium was changed every 3 to 4 days. Formation of G418-resistant colonies and foci of morphologically transformed cells was determined 14 to 21 days postransfection.

Luciferase assay. Transient transfections were performed as described previously (20). Either 105 F9, 3 × 105 NIH 3T3, or 7 × 105 208F cells were seeded per 6-cm-diameter plate. Cells were transfected with 4 μg of each expression plasmid and 4 μg of reporter construct, usually 12 h later. After 10 h, the cells were rinsed; approximately 30 h postransfection. Yeast protein extracts were prepared as described previously (49). Immunostaining for p53 in strain Y1GHC. The p53 protein expression vector based on FBJ-MuSV (47) that terminates at aa 316 and contains the FBR-MuSV-specific point mutations at positions 64 and 138 (46). E300 is similar to E316 but includes the two FBR-MuSV-specific internal deletions near the C terminus. The p53E300 gene contains the c-Jun leucine zipper instead of the v-Fos zipper. The different PCR-generated fragments were then cloned as BamHI/XhoI fragments into the expression vector PMSE (39) into which a synthetic XbaI-Sall linker had been introduced, creating pMSEKJ (20). The Jun NTP expression vector contains the DNA-binding region and the leucine zipper of the c-Jun protein (aa 228 to 334). The PCR-generated Jun NTP fragment (mouse c-jun DNA positions 1066 to 1460) was cloned as an EcoRI/BamHI fragment into the p36/7 expression vector (37) (kindly provided by U. Rüther) into which a synthetic MunBglII linker had been introduced, creating p67/8BP. All nucleotide sequences were verified by DNA sequencing. The following PCR primers were used: 5′ primer, 5′-GAGAATTCATGCCGGGAGAGACGGG-3′; and 3′ primer, 5′-CTAGATTCCATACCTCCCGCGCC-3′.

The pTREEluc reporter construct was generated by cloning five tandem copies of the collagenase TRE (2) into the SalI/XhoI sites 5′ to the herpes simplex virus thymidine kinase (tk) promoter of pT1Luc (35).

Immunoprecipitation, immunoblotting and immunofluorescence. Immunoprecipitations were performed as described previously (49). Immunostaining for Fos protein was carried out by indirect immunofluorescence as described previously (21). Immunoblot analysis of yeast cells was done with a polyclonal serum from rabbit raised against a glutathione S-transferase–LexA fusion protein (Pharmacia LKB). Yeast protein extracts were prepared as described previously (17) and used for immunoblot analysis (3).

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The CYC1/lacZ reporter construct with one TRE element from the human collagenase gene promoter was integrated between the NcoI and PvuII sites of the URA3 gene, which was used to replace the YAPI gene. Strains were grown on selective medium containing 2% glucose (3). β-Galactosidase assays (3) were performed in all cases with four independent transformants.

**RESULTS**

**Role of Fos C-terminal sequences in transformation.** We have previously shown that the region C terminal to the leucine zipper in v-Fos is not required for the induction of transformation, although it contributes to the oncogenic potential of the protein (19, 26). Since these studies used an expression vector (pRΔX) based on the very strong FBR-MuSV long terminal repeat (LTR), we reasoned that a role of the C terminus might become more readily detectable, and thus amenable to a functional analysis, under conditions of lower expression. This was confirmed by the analysis shown in Fig. 1A, using a simian virus 40 (SV40)-based vector for the expression of v-Fos proteins, which gives rise to a three- to five-fold lower level of expression (Fig. 1B and data not shown). Efficient focus formation was seen with the E300 protein with both vectors, although the transforming potential was 2.7-fold higher with the pRΔX vector. E300 is an FBJ-FBR-MuSV hybrid protein which, owing to its strong transforming potential, has been used in all our previous studies (20, 33, 40). E300 contains an intact N terminus but harbors the FBR-MuSV-specific internal deletions removing the HOBI motif. In contrast to the results obtained with E300, the C-terminally truncated E220 protein induced a high number of foci only when expressed from the stronger pRΔX vector (23-fold higher relative to the pMSE vector; Fig 1A). Since this observation clearly suggests a crucial role for the C terminus in the induction of transformation, we performed our subsequent studies with a protein (E316) not altered in the HOBI and HOB2 domains but otherwise similar to E300. Interestingly, E316 shows slightly less transforming potential than E300 (Fig. 1A), pointing to a negative regulatory role for HOBI (see also below).

The 316-aa v-Fos E316 protein (see the legend to Fig. 1 for details) was also found to be a potent inducer of transformation. Surprisingly, the truncation of just 24 C-terminal amino acids (in E292) diminished the transforming potential of the protein approximately 16-fold, to 6.5% of the E316 level. This truncation does not affect the integrity of the HOB domains (represented schematically in Fig. 2A). A similar value (8.6%) was obtained upon removal of the HOB2 domain (E260), while the additional truncation of HOBI (in E220) led to a modest but reproducible increase (13%). The truncation of a further 15 aa (in E205) led to another drop in the transforming potential, to 6.5%. The marked differences in transforming potential seen with E316 and the C-terminally truncated proteins were not due to effects on protein expression or synthesis, as shown by immunostaining and immunoprecipitation of stably transfected cells (Fig. 3). These results led to the conclusion that the region between aa 292 and 316 is crucial for transformation under the conditions of the assay and that the region harboring the HOBI motif may have a negative effect.

**Effect of C-terminal deletions on transactivation.** We next investigated the transactivation capacity of the C terminus present in the five constructs described above. To facilitate the interpretation of the results, we performed this analysis with constructs lacking the N-TA domain (amino acids 1 to 109 deleted) (Fig. 1A). Constructs truncated to various degrees at the C terminus were analyzed in transient transfection assays in F9 cells cotransfected with a 5×TRE- tk-luciferase gene (lac) reporter plasmid. Since F9 cells contain practically noJun protein, an expression vector containing the c-Jun bZip domain was cotransfected. In addition, the same Fos and c-Jun bZip constructs were coexpressed in yeast and analyzed for transactivation of a 1×TRE-lacZ reporter. Both experiments clearly demonstrated that the region shown above to play a crucial role in transformation is also essential for efficient transactivation. Thus, truncation of aa 292 to 316 led to a ∼6-fold drop in F9 cells and an ∼8-fold decrease in yeast. We will subsequently refer to this region of the protein as the C-TM. Further truncations led to another ∼5-fold drop in activity in yeast, suggesting some transactivation potential of the HOB region in yeast. In F9 cells, however, further truncations seemed to lead to a slightly increased activation, but the significance of this observation is unclear.

**The C-TM plays a central role in the C terminus of c-Fos.** In contrast to the v-Fos proteins, the c-Fos gene product harbors a TBD C terminally to the C-TM. It was therefore of interest to investigate the relative contributions of the C-TM and the TBD to the transactivation potential of the c-Fos C terminus. This analysis was performed by testing LexA fusion proteins containing the entire c-Fos C terminus (aa 200 to 380) or different subdomains of the C terminus in NIH 3T3 cells (Fig. 2B). From this analysis, we conclude that (i) a region of 36 aa from positions 280 to 316 harboring the C-TM is able to transactivate in a context-independent manner (40% activity relative to the entire C terminus); (ii) the TBD (aa 317 to 380) also showed context-independent transactivation, but its activation
potential was not as strong as that of the C-TM; and (iii) constructs containing only the HOB1 and HOB2 motifs (aa 200 to 292) were not able to transactivate to any significant extent (0.05%). The LexA–C-TM construct (aa 280 to 316) also showed a clear transactivation in *S. cerevisiae* (8.6% of the entire C terminus starting at aa 200). Since the *S. cerevisiae* systems allow for much more precise measurements, further analyses were performed with yeast cells.

We next investigated whether any of the HOB domains, and if so which one, cooperated with the C-TM by using constructs with double-point mutations in either the HOB1 or HOB2 motif (kindly provided by T. Kouzarides) (Fig. 4A). These mutations have previously been shown to impair transactivation by GAL4 fusion proteins in IBR cells (42). As shown in Fig. 4B, both mutations in the HOB domains had no detectable effect on the expression of the proteins. Analysis of the respective LexA fusion proteins in yeast showed, however, that the HOB2 mutation had a negative effect on transactivation (30% of wild-type activity [Fig. 4A]). In contrast, mutations in HOB1 resulted in a significantly higher activity than the wild-type protein, suggesting a negative modulatory role. Such an attenuator function of HOB1 would be in agreement with its deletion in the FBR-MuSV Fos oncoprotein.

**Identification of a conserved structural motif in the C-TM.**

We next performed a high-resolution deletion analysis (Fig. 5) mapping the C-TM core sequence to aa 298 to 306 in Fos: FYAADWEPIL (hydrophobic amino acids are boldfaced) (Fig. 6A). In addition, the data in Fig. 5 point to some role of the adjacent amino acids, especially in the region from aa 292 to 297, VDLSGS. This sequence is very similar in human, mouse, rat, and chicken c-Fos, in murine and avian Fos, and in FosB (Fig. 6A). The highlighted hydrophobic amino acids are also conserved in the viral VP16 protein, where especially the phe-

![FIG. 3. Synthesis and steady-state levels of Fos proteins in murine and *S. cerevisiae* cells. (A) Detection of E316 and mutant Fos proteins in stably transfected 208F cells by indirect immunofluorescence (left) and staining for nuclear DNA with Hoechst 33258 (right). Vector control, cells transfected with the empty expression vector. (B) Synthesis of E316Δ110 and E292Δ110 proteins in stably transfected 208F cells. G418-resistant mass cultures were metabolically labelled with [35S]methionine, and Fos proteins were immunoprecipitated with Fos-specific antibodies (bands around 50 kDa). Vector, cells transfected with the empty expression vector. Positions of molecular mass markers (lane kD) are shown on the left in kilodaltons.

![FIG. 4. C-terminal subdomains cooperating with the C-TM. (A) Effects of point mutations in either HOB1 or HOB2 on C-TM-dependent transactivation. Shown are the amino acid sequences of HOB1 and HOB2. Amino acids in boldface are conserved in the HOB motifs of c-Jun. The relative transactivation of the mutants with the indicated substitutions to alanine was determined as described for Fig. 2A. (B) Immunoblot detection of LexA fusion proteins in *S. cerevisiae* cells expressing a wild-type (wt) Fos fusion protein (aa 210 to 306; two independent transformants: lane 1 and 2), a double-point mutation in HOB1 (TS, lanes 3 and 4), and a double-point mutation in HOB2 (FD; lanes 5 and 6).](http://mcb.asm.org/)
Nylalanine is essential for transactivation (8). We therefore investigated whether L-294, F-298, and W-303 are also important for the function of the C-TM. Single substitutions of these residues with alanine resulted in a significant (35 to 46%) decrease of transactivation. A triple mutant showed a residual activity of only 15%, which is similar to the activity of a deletion variant that lacks the C-TM (Fig. 6B, constructs 2, 3, and 5). When the same mutant was tested in NIH 3T3 cells, a similar decrease, to 16% of the wild-type activity, was observed, which most likely corresponds to the activity of the TBD.

The C-TM synergizes with the N-TM of Fos. The N-terminal (aa 1 to 205) and C-terminal (aa 110 to 316) parts of Fos clearly harbor autonomous activation domains but on their own give rise to relatively low levels of transactivation (Fig. 7A). We therefore examined whether the N- and C-terminal activation domains of Fos cooperate making use of an intergenic complementation assay. For this purpose, the bZip domain of the N-terminal Fos construct was replaced with the c-Jun zipper to enable this construct to form a DNA-binding heterodimer with the C-terminal Fos construct harboring the natural Fos bZip region. As shown in Fig. 7A, coexpression of these two constructs restored the transactivation capacity, yielding a transactivation value that was even higher than that of the wild-type protein. This intermolecular complementation depends on the presence of both an intact N-TM motif and a functional C-TM motif, since mutations in either domain abolish the cooperative effect (Fig. 7B).

**DISCUSSION**

Transformation by v-Fos requires a functional DNA contact site (bZip) and an intact N-NTA domain, suggesting that transactivation plays a crucial role (20, 33, 40). Under conditions of high expression (i.e., in an FBR-MuSV LTR-based expression vector), the C terminus of Fos is not crucial for transformation (19). In the present study, we have identified a novel motif (C-TM) in the C terminus which is required for efficient transformation under conditions of lower expression, as is the case with the SV40 early promoter. This region represents a context-independent activation domain, thus providing further ev-

![FIG. 5. Fine mapping of the C-terminal activation domain. (A) Transactivation by N- or C-terminal deletions fused to LexA. Transactivation in S. cerevisiae by LexA fusion proteins was determined by using a lexA-oplacZ reporter construct. Values represent the mean of results obtained with four independent transformants. The standard deviation was 5% for all values. Transactivation (b-galactosidase activities) was calculated relative to that of LexAF280-380 (N-terminal deletion) or LexAF200-316 (C-terminal deletion). Fusion proteins were expressed in yeast, and transactivation of a LexA-oplacZ reporter gene was determined as for Fig. 3. (B) Detection of LexA fusion proteins by immunoblotting in S. cerevisiae. Start points (N) and endpoints (C) of the deletions are indicated above the lanes. See the legend to Fig. 3 for details.](http://mcb.asm.org/)

![FIG. 6. Effects of point mutations in the C-TM. (A) Structural motifs conserved in the transactivation domains (in the C-TM) of different vertebrate Fos and FosB proteins (29, 34, 47, 48, 50). Conserved hydrophobic amino acids are shown in boldface; hum, human; mus, mouse. (B) Effects of single double- and triple-point mutations on C-TM-dependent transactivation. LexA fusion proteins were expressed in yeast, and transactivation of a LexA-oplacZ reporter gene was determined as for Fig. 3. (C) Detection of LexA fusion proteins in S. cerevisiae by immunoblotting. Lanes are numbered according to the system in panel B. The occurrence of protein forms with a lower mobility (particularly in lanes 5 to 9) is presumably due to posttranslational modifications. See the legend to Fig. 3 for further details.](http://mcb.asm.org/)

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Synergistic interactions in the establishment of transcriptional activation. The C-TM is required for efficient TRE-dependent transactivation of the C terminus in F9 cells, as well as for the reconstituted TRE-dependent transactivation in yeast, and can transactivate as a LexA fusion in both NIH 3T3 cells and yeast, i.e., in a context-independent fashion. In contrast, the HOB domains alone are not able to drive transactivation to any significant extent. Autonomous transactivation by the HOB domains apparently is seen only when they are duplicated as in the context of the GAL4 fusion used by Sutherland et al. (42).

In the experiments described in this report, aa 280 to 380 of c-Fos transactivate efficiently even in the absence of HOB1 and HOB2. In contrast, it has been reported that a region from aa 276 to 380 (including the C-TM) is not able to transactivate as a GAL4 fusion in 1BR cells (27). We attribute this discrepancy to cell-specific properties rather than to the presence of the four additional amino acids in the latter construct. It is possible that in 1BR cells, the C-TM requires a positive modulatory interaction, or the release from a negative modulatory constraint, mediated by HOB1 or HOB2.

Our experiments with the yeast system show that only the HOB2 domain contributes to C-TM-dependent transactivation, while a mutant in HOB1, which abolishes transactivation by a GAL4 fusion protein in 1BR cells, shows a positive stimulatory effect. Interestingly, the deletion of HOB1 as found in FBR-MuSV also results in an increased transactivation and transformation capacity of the oncprotein (19, 26). A similar effect is observed for LexA fusion proteins carrying the C terminus of either E316 or E300 (data not shown). This observation could be explained similarly to the discrepancy discussed above, namely, by a negative regulatory function exerted by HOB1. The mutation introduced into HOB1 destroys the target sequence of the FRK kinase (12). Thus, the repressing function by the HOB1 domain could be either alleviated by phosphorylation or abolished by its deletion (as in FBR-MuSV). This mechanism would resemble the one found with the Δ region of c-Jun, thought to bind a repressor (4–6). The Δ region is the target for the direct binding and phosphorylation by stress-activated protein kinase/JNK and is deleted in the constitutively active v-Jun (4, 11).

Coexpression of the Fos N and C termini clearly demonstrated that the N- and C-terminal activation domains cooperate in a synergistic fashion. We found that this synergism is dependent on the presence of a functional N-TA domain and the C-TM, again pointing to a central role for the C-TM in transactivation. This finding that a cooperation of the N and C termini was seen even when both domains were present on different polypeptides within a heterodimeric complex strongly suggests that both domains act as individual, separable modules rather than forming a larger multipartite domain.

The role of the C-TM in transactivation. We pinpointed the functional region of the C-TM to a region between aa 292 and 306, which are highly conserved among the various vertebrate c-Fos proteins and the FBJ-MuSV and NK24-AvRV oncproteins, and to a considerable degree also in FosB. The C-TM of FosB also seems to play a role in transactivation in concert with a domain binding TBP. The deletion of a region (aa 243 to 282) encompassing a proline-rich sequence and half of the C-TM impairs transactivation by FosB, even more severely than deletion of the TBD (28).

The degree of conservation and the results obtained with C-TM point mutants clearly show that the large hydrophobic amino acids in the C-TM are critical for its function. In this respect, some similarity with the TFIIIB-binding transactivation domain of VP16 exists (24, 25). Also in this case, the bulky, hydrophobic amino acids, and in particular a central phenylalanine, are required for transactivation (8). The yeast AP1

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FIG. 7. (A) Synergistic cooperation of the Fos N and C termini of E300 v-Fos. Transactivation of the coexpressed Fos N and C termini in F9 cells was analyzed. Dimerization of the Fos C and N termini was achieved by replacement of the Fos zipper by the Jun zipper in one of the partners. These constructs are able to form dimers with other Fos variants carrying a Fos zipper. Transactivation was determined as for Fig. 2. (B) Domains required for the synergistic cooperation of the Fos N and C termini. Shown are the relative transactivation by a mutated E300 v-Fos (s4) in which serines in the C-TM at positions 295, 297, 308, and 310 (47) were substituted by alanine and the transactivation properties of the coexpressed Fos N and C termini carrying either the s4 mutation in the C-TM or the m2 mutation in the N-TA domain (20).
homolog GCN4 also carries two transactivation domains which are characterized by a set of bulky hydrophobic amino acids (13). The region of the protein harboring these domains is also required for the GCN4-dependent transactivation in mammalian cells (36). For each of these cases, it has been shown that multiple hydrophobic amino acids are critical for transactivation (8, 13). In agreement with these observations, we found that the triple mutation in the Fos C-TM has the most deleterious effect on transactivation.

Conclusions. The observations reported in this study taken together with previously published observations are corroborated in the model shown in Fig. 8A. Apart from the bZip domain, four different regions have been shown to contribute to transactivation. Three of these domains, the N-TM, C-TM, and TBD, represent context-independent activation modules (Fig. 8). The N-TM and C-TM are the most crucial elements involved in both transactivation and transformation (Fig. 8B). The proteins interacting with the N-TM and C-TM are unknown but may belong to a class of basal factors, TBP-associated factors or coactivators (43, 44). The TBD interacts with TFII D and directly contacts TBP, while the C-TM may contact TFII B as in VP16. A fourth domain, HOB2, does not function on its own but cooperates with the C-TM. The precise function of a fifth domain, HOB1, is unclear, but there is some evidence to suggest a negative modulatory role. These findings raise the intriguing question of how the different domains in Fos contribute to activated transcription. It is conceivable that these different interactions do not occur simultaneously but contribute to different steps in the formation of the preinitiation complex; they may even affect other stages of transcription such as promoter clearance or elongation (43, 44). In this context, the C-TM identified in this study may trigger distinct essential early steps in the stepwise formation of an actively transcribing RNA polymerase II. With our increasing knowledge of the domain structure of Fos protein, the identification of interacting proteins, and the characterization of basal transcription factors, it should be possible to address such questions in the near future, e.g., by using reconstituted in vitro transcription systems.

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REFERENCES


28. Metz, R., T. Khouzadis, and R. Bravo. 1994. A C-terminal domain in FosB, absent in FosB/SF and Fra-1, which is able to interact with the TATA

FIG. 8. Domain structure and cooperations in Fos. (A) Scheme of c-Fos with the characterized transactivation and modulatory elements. Arrows mark either positive or negative cooperations between different domains. (B) Properties of different Fos domains with respect to transactivation and transformation.