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Invited Review

Fos family members: regulation, structure and role in oncogenic transformation

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Summary. The members of the Fos protein family might be subdivided in two groups, according to their ability to transform rodent fibroblasts, transforming (c-Fos and FosB) and non-transforming (Fra-l and Fra-2) proteins. Members of these groups are differently activated in response to external stimuli and posses different structural features. Importantly, whilst c-Fos and FosB contain multiple transactivation modules in their N- and C-terminal parts, transactivation domains are absent in the non-transforming Fos proteins. As a result, Fra-l and Fra-2 though efficiently form dimers with the Jun proteins, are weak transcriptional activators and inhibit the c-Fos-dependent activation in transient transfection assay. The numerous experiments performed with the different Fos mutant proteins with impaired transforming ability, as well as with chimeric proteins revealed the importance of the transactivation function for transformation. Fra-1 and Fra-2 proteins albeit ineffectively triggering oncogenic transformation, are abundant in *ras*- and src-transformed murine and chicken fibroblasts, in neoplastic thyroid cells and in highly malignant mouse adenocarcinoma cells, which underwent mesenchymal transition. The abundance of the non-transforming Fos proteins in these systems might be mediated by a positive AP-l-dependent feedback mechanism, as well as by *wnt* signals. Furthermore, the manipulation of the Fra-l expression level in thyroid and mammary tumor cells modulated the transcription of several tumor progression markers and affected cell morphology and invasiveness. These recent data demonstrate a novel function of non-transforming Fos proteins in the maintenance and progression of the transformed state. Interestingly, this function is independent of the documented invalidity of the Fra-l and Fra-2 proteins as transcriptional activators in rodent fibroblasts.

Key words: AP-1, Fos, Fra-l, Fra-2, neoplastic transformation

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Introduction

The Fos family consists of four cellular proteins, c-Fos, FosB, Fra-l and Fra-2. In addition, as a result of alternative splicing, a dominant negative mutant of FosB, FosB2, may naturally occur (Mumberg et al., 1991; Nakabeppu and Nathans, 1991). When activated by an external stimulus, Fos interacts with one of three cellular members of the Jun protein family (c-Jun, JunB, or JunD) to form an AP-1 (activating protein-l) complex (Angel and Karin, 1991). Fos-Jun dimers activate transcription by the binding to the TGAC/GTC/AA sequence elements, TREs (TPA-responsive elements), located in the promoters/enhancers of target genes (Lee et al., 1987). The transcriptional activation depends on direct contacts between AP-l and the basal transcriptional machinery (Metz et al., 1994a,b; Funk et al., 1997), and/or on the binding of transcriptional coactivators, CBP/p300 (Bannister and Kouzarides, 1995; Bannister et al., 1995) or JAB1 (Jun activation domain binding protein 1) (Claret et al., 1996).

The variety of stimuli inducing Fos synthesis and activity, as well as the detection of functional TREs in promoters of numerous genes with diverse functions suggest a complex and varied biological role of the Fos proteins. Indeed, members of this family have been implicated in most fundamental processes occurring in mammalian cells: cell cycle control (Kovary and Bravo, 1991, 1992, Balsalobre and Jolicoeur, 1995); apoptosis (Preston et al., 1996; Karin et al., 1997); cell differentiation (Lord et al., 1993; Baset-Séguin et al., 1994; Grigoriadis et al., 1994; Rutberg et al., 1996); oncogenic transformation (reviewed in Angel and Karin, 1991) and tumor progression (Reichmann et al., 1992; Saez et al., 1995). During the last few years of intensive studies, it has become clear that the Fos proteins, although being similar in terms of their affinity to TREs or dimerization with Jun, regulate different target genes and therefore have distinct biological functions. The specific functions of different Fos proteins in the transcriptional control of certain target genes may result from the peculiarities of the regulation of each particular member of the Fos family. Additionally, this specificity might reflect the differences in their biological features.

In this review I describe the regulation and function of the Fos family members focusing on their role in carcinogenesis. The functions of the Fos proteins in cell cycle control, differentiation and apoptosis will not be discussed.

Regulation of Fos proteins at transcriptional and post-translational levels

Though all Fos family members belong to a group of proteins, which are induced by serum in quiescent fibroblasts, the kinetics of the response is gene-specific. Whereas c-Fos and FosB, although very rapidly induced, become undetectable in 3 h, the expression of Fra-1 and Fra-2 increases significantly within the second hour and remains elevated for at least 12 h after stimulation (Kovary and Bravo, 1992; Schreiber et al., 1997). Fra-1 and Fra-2 but not c-Fos or FosB proteins are expressed in asynchronously growing cells, suggesting their importance for the maintenance of an active state of AP-1-regulated genes in cycling cells.

Numerous extracellular stimuli lead to the rapid and transient induction of c-Fos, which is a prototypical member of the Fos family. The activation of c-Fos occurs at several levels. The best-studied aspect of the c-Fos regulation is probably the modulation of its abundance. Different signals rapidly up-regulate transcription of the *c-fos* gene via several inducible enhancers, cAMP-responsive element (CRE) (Sheng et al., 1991), serum-response element (SRE) (Treisman, 1992) and *sis*-inducible enhancer (SIE) (Darnell et al., 1994). CRE mediates induction of *c-fos* via the binding of ATF or CREB transcription factors in response to cAMP- and Ca^{++}-dependent signals. SRE provides the transcriptional activation via the MAPK signaling pathway, which results in the phosphorylation of the Ets domain transcription factors belonging to the group of ternary complex factors (TCFs) (Treisman, 1994). A phosphorylated TCF binds as a dimer to the serum-response factor (SRF) leading to the formation of a ternary complex at SRE, thereby activating *c-fos* transcription. Another element, SIE is recognized by the STAT (signal transducer and activator of transcription) transcription factors, whose activity is modulated via the JAK (Janus kinase) pathway (Hoey and Schindler, 1998). Therefore, the presence of CRE, SRE and SIE causes the ability of the *c-fos* promoter to respond to different non-related ligands, such as, for example, interferons and growth factors.

In addition to the transcriptional control, the abundance of c-Fos is regulated at the level of protein stability by the ubiquitin-26S proteosome pathway (Tsurumi et al., 1995), which therefore contributes to the determination of the duration of the AP-1 response.

The induction of c-Fos also involves post-translational modifications of the protein. Upon stimulation by different ligands the protein becomes extensively phosphorylated in the carboxy-terminus. The phosphorylation pattern is rather complex and depends on the particular signal and cellular context. The cooperative phosphorylation of c-Fos by the MAP-kinase and 90 kDa-ribosomal S6 kinase contributes to the activation of the protein by increasing its stability and/or transactivation ability (Chen et al., 1996).

Control of the activity of the Fra-1 and -2 proteins has been studied in rat and chicken fibroblasts. Functional TREs were described in the promoter of *fra-2* (Sonobe et al., 1995), as well as within the intronic enhancer of *fra-1* (Bergers et al., 1995; Schreiber et al., 1997), suggesting that the transcription of both genes might be regulated via a positive autoregulatory loop. c-Fos and FosB have been implicated in the control of Fra-1 and Fra-2 transcription, hence explaining their delayed expression in response to the stimulation by serum of quiescent fibroblasts. The Fra-1 and Fra-2 proteins are significantly more stable than c-Fos and FosB (Gruda et al., 1994). This might further contribute to their predominance in asynchronously growing cells. In rodent fibroblasts, the activation of the Fra-1 and Fra-2 proteins involves their phosphorylation by the p44 MAP kinase. This modification affects the functional activity of both proteins by increasing their affinity to TREs (Gruda et al., 1994).

Fos family members: structure and functional differences

All members of the Fos protein family contain rather small domains, termed bZIP motifs, composed of leucine zipper and basic regions, which specify the hetero-dimerization with Jun and binding to TREs, respectively. These domains are highly conserved among Fos proteins (74-80% of homology) (Wisdom and Verma, 1993) (Fig. 1). In agreement with the high level of the sequence conservation in bZIP motifs, there are no significant differences between Fos family members in their ability to form dimers with different Jun proteins, although the Jun/FosB complexes were found to be more stable, than Jun/Fra-1, or Jun/c-Fos (Ryseck and Bravo, 1991). Similarly, there are no clear preferences in binding of specific AP-1 complexes to specific TREs, suggesting that the selective regulation of certain target genes by distinct Fos proteins cannot simply result from their selective binding to corresponding TREs. Two other regions, N- and C-terminal Fos domains are much less conserved showing only 15-30% of homology (Fig. 1). The functional difference between the C-terminal domains of Fos proteins was studied in rat 208F fibroblasts (Wisdom and Verma, 1993). C-termini of c-Fos and FosB proteins harbor transactivation function that was clearly shown to be absent in Fra-1 or Fra-2. Several motifs contributing to the transactivation ability of the C-terminus of c-Fos have been delineated, HOB1, HOB2, C-TM and TBM (Sutherland et al., 1992; Metz et al., 1994a; Brown et al., 1995; Funk et al., 1997). These motifs are directly involved in transcriptional activation via formation of multiple contacts with the components of basal machinery stabilizing the pre-initiation complex

and/or facilitating its assembly. HOB1 and HOB2 motifs are absent in FosB, where instead a proline-rich functional module, PRM has been identified (Metz et al., 1994b; Skinner et al., 1997) (Fig. 1). Another autonomous transactivation domain (N-TA) was mapped in N-terminal regions between amino acid residues 60 and 84 in c-Fos or 54 and 78 in FosB (Jooss et al., 1994). Though the overall homology in N-terminal regions of Fos proteins is very low (Fig. 1), N-TA domains exhibit high levels of similarity between c-Fos and FosB (80%). The corresponding peptides in Fra-1 and Fra-2 show correspondingly 40% and 68% homology with N-TA of c-Fos. The transactivation ability of Fra-1 and Fra-2-derived peptides homologous to N-TA has not been studied in direct experiments. However, N-TA synergistically cooperates with the C-terminal transactivation domains, which are not conserved in Fra-1 and Fra-2 (Funk et al., 1997). It is therefore not surprising that the entire Fra-1 protein fused to the DNA binding domain of Gal4 lacks transactivation function in NIH3T3 and Rat-1A cells (Metz et al., 1994b; Bergers et al., 1995). The multiple interactions between activation motifs and the general transcription factors together with the recruitment of co-activator complexes, underlie the mechanism of AP-1-dependent transcriptional activation.

Fra-1 and Fra-2 proteins recruit strong transcriptional activators, Jun family members, to TREs within the enhancers of target genes. However, due to the absence of transactivation function associated with the C- and likely with the N-terminal sequences of Fra-1 and Fra-2, these proteins are less potent transcriptional activators than c-Fos or FosB. Moreover, Fra-1 and Fra-2 inhibit the c-Fos-mediated activation of a synthetic AP-1-responsive promoter in transiently transfected

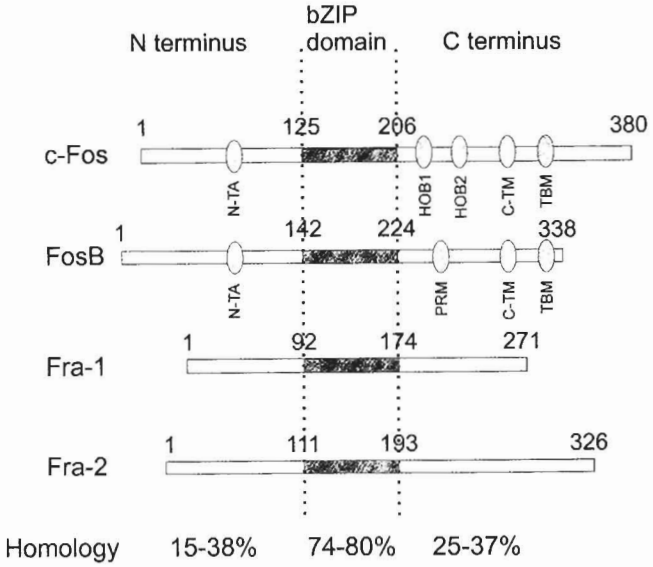

Fig. 1. Scheme of the Fos proteins with the characterized transactivation elements (Wisdom and Verma, 1993; Jooss et al, 1994; Metz et al., 1994a,b; Funk et al., 1997; Skinner et al., 1997).

fibroblasts (Suzuki et al., 1991). This data combined with the delayed synthesis of Fra-1 and Fra-2 in response to the stimulation by growth factors (Gruda et al., 1994) allowed to conceive them as inhibitory factors, which in certain circumstances may limit the duration of the AP-1 response. However, this model, though true in general, does not reflect the complexity of AP-1 function in the context of individual natural enhancers.

The targeted disruption of *c-fos* impaired the ability of growth factors to induce the expression of two matrix metalloproteases-encoding genes, *MMP-1* and *MMP-3*, though the abundance of AP-1 complexes formed with a TRE derived from the MMP-3 gene was not affected in *c-fos*-deficient fibroblasts (Hu et al., 1994). Three other AP-1 responsive genes, *MCP-1*, *c-jun* and metallothionein were equally expressed in *c-fos*-deficient and control cells (Hu et al., 1994). Therefore, whilst c-Fos is absolutely required for the activation of certain enhancers (such as the *MMP-1* and *MMP-3* enhancers), it might be functionally substituted by other Fos family members in the promoter/enhancer context of other AP-1 target genes. Another example of a gene differently regulated by the Fos proteins is cyclin D1. The study of the cyclin D1 promoter in a fibroblast culture generated from the *c-fos*$^{-/-}$ *fosB*$^{-/-}$ mice revealed the requirement of either c-Fos or FosB proteins for its activity. Moreover, these proteins may compensate each other in the context of this particular promoter, but they cannot be substituted by the weak activators, Fra-1 and Fra-2 (Brown et al., 1998). These data together with the decreased size of *fos*$^{-/-}$*fosB*$^{-/-}$ mice suggest the importance of c-Fos and FosB for cell cycle control, albeit that neither *c-fos* nor *fosB* knockouts produced any significant effects on viability. The expression of AP-1 target genes other than cyclin D1, (e.g. *c-jun*, *MCP-1* or metallothionein, whose transcription was not impaired in *c-fos*$^{-/-}$ mice) has not been studied in double knockout animals. However, the phenotypes of *c-fos*$^{-/-}$ and *c-fos*$^{-/-}$ fosB$^{-/-}$ mice were similar (osteopetrosis, lymphopenia and decreased size of animals) (Grigoradis et al., 1994; Brown et al., 1998). This suggests that in the context of certain AP-1-responsive genes in *c-fos*$^{-/-}$ mice, c-Fos is not compensated by FosB, but probably by Fra-1 and/or Fra-2. As the generation of Fra-1 and Fra-2 knockouts has not been reported to date, it is not clear whether these proteins may have their own target genes, distinct from those of c-Fos and FosB.

Fos family members in oncogenic transformation and tumor progression

The gene encoding the c-Fos protein was discovered as a normal cellular progenitor of oncogenes of two murine osteosarcoma viruses, FBR-MuSV and FBJ-MuSV. Overexpression of the *c-fos* protooncogene, as well as of its viral homologues, either the FBR or FBJ *v-fos* oncogenes is sufficient to transform rodent fibroblasts *in vitro*. Moreover, the *c-fos* activity is required for the transformation induced by sis, ras and

raf (Mercola et al., 1987; Jamal and Ziff, 1990; Ledwith et al., 1990; Litz-Jackson et al., 1992; Wick et al., 1992). Though the molecular mechanisms of the *c-fos*-mediated transformation of rodent fibroblasts are not clear in details, certain target genes relevant to this process were disclosed. These include genes coding for microfilament-associated proteins ezrin and tropomyosins-3 and -5B, whose deregulation may contribute to the morphological transformation and affect motility of rat fibroblasts (Jooss and Müller, 1995; Lamb et al., 1997). In addition, Hennigan et al. identified nine genes previously associated with invasion or metastasis to be up-regulated in *c-fos* or in *v-fos* transformed 208F fibroblasts (Hennigan et al., 1994).

Another feature of the Fos proteins that is relevant to the oncogenic transformation is the ability of the c-Fos-estrogen receptor chimera (FosER) to induce epithelial-fibroblastoid conversion in nontumorigenic mouse mammary epithelial cells (Reichmann et al., 1992). The observed breakdown of epithelial polarity in response to the activation of FosER was accompanied by dramatic changes in the gene expression program involving the down-regulation of epithelial markers E-cadherin, ZO-1 and cytokeratins and activation of the "mesenchymal" genes, vimentin and fibronectin. FosER chimera also induced the expression of several extracellular matrix degrading proteinases, whose activation has been associated with the invasive behavior of tumor cells. In general, the FosER mediated changes in non-tumorigenic mouse mammary epithelial cells resembled an epithelial-mesenchymal transition, which often occurs in the progression of epithelial tumors (Birchmeier and Behrens, 1994; Fish and Molitoris, 1994) implicating c-Fos in the control of this process (Reichmann et al., 1992). This concept was strongly supported by Saez et al., who applied the multistep skin carcinogenesis model to test the ability of *c-fos*$^{-/-}$ mice to develop cancer (Saez et al., 1995). Though some yet unidentified genes could functionally complement the absence of *c-fos* at the early stages of cancer development, initiation and promotion, the *c-fos*-deficient papillomas failed to undergo malignant conversion. Taken into account that the wild-type papillomas eventually progressed into malignant tumors, these results demonstrated an absolute requirement of *c-fos* for the malignant progression (Saez et al., 1995).

Cellular transformation by Fos proteins is envisaged to depend on dimerization with the Jun component, the DNA-binding activity of the basic region and the transactivation function. This hypothesis is supported by the observation that *fos* and *jun* cooperate in the induction of transformation (Neuberg et al., 1991). In addition, the studies of numerous *c-fos* mutants demonstrated that their ability to activate transcription correlates with their transforming potential in rodent fibroblasts. Consistent with this view, FosB that harbors transactivation domains (Fig. 1) efficiently transforms rodent fibroblasts, whereas no transforming potential could be assigned to the proteins lacking transactivation function, Fra-1, Fra-2 and FosB2, upon their overexpression in 208F rat fibroblasts (Wisdom and Verma, 1993). Similarly, neither Fra-1, nor FosB2 protein was able to induce morphological alterations in Rat-1A cells. However, Rat-1A fibroblasts or chicken embryo fibroblasts (CEF), which correspondingly overexpressed exogenous Fra-1 or Fra-2, acquired to a certain extent the capability of anchorage-independent growth in soft agar (Nishina et al., 1990; Bergers et al., 1995).

The data quoted above may serve as a basis of a model explaining the role of different Fos proteins in oncogenic transformation and tumor progression. Transforming proteins, c-Fos, FosB, and v-Fos that harbor transactivation domains at the N- and C- termini may contribute to different aspects of carcinogenesis. This involves the deregulation of the cell cycle control (activation of cyclin D1); morphological transformation (deregulation of the expression of the cytoskeleton-associated proteins); tumor cell invasiveness (up-regulation of extracellular matrix degrading proteinases, their activators and receptors). The inability of Fra-1 and Fra-2 to transform 208F fibroblasts might be explained by the absence of transactivation domains in their N- and C-termini. The certain sensitivity of Rat-1 and CEF fibroblasts to Fra-1 and Fra-2 may result from the enhanced expression of the Jun component in these cells. Jun can be targeted to the responsive promoters by non-transforming Fos proteins affecting the ability of these cells to grow in soft agar. One may suggest that in other cell systems, where the Jun component is deficient, Fra-1 and Fra-2 play even a tumor-suppressive role by competing with transforming Fos proteins for the interaction with Jun. Recently obtained data, however, show that the real contribution of different Fos proteins to the oncogenic transformation is different from that suggested by this model.

AP-1 is essential for *v-src*-mediated transformation of chicken embryo fibroblasts (CEF) (Suzuki et al., 1994). Major changes in the AP-1 composition induced by *v-src* involve the elevation of Fra-2 level with its subsequent phosphorylation by ERK2 (Murakami et al., 1997). Moreover, the transfection of CEF with a constitutively active mutant of MEK1 (MEK-DD) was sufficient for induction of AP-1-dependent cellular transformation, as well as the activation of Fra-2 synthesis via a positive autoregulatory loop (Murakami et al., 1999) (Fig. 2a). As ERK-2-mediated phosphorylation of Fra-2 significantly enhances the transactivation potential of the protein, probably by the increasing of its affinity to TREs, these data implicate Fra-2 in cellular transformation by *v-src* and MEK-DD.

Mechta et al. have identified Fra-1 as a predominant Fos component in *ras*-transformed murine fibroblasts, whereas no traces of transforming Fos proteins, c-Fos or FosB, were detected in these cells (Mechta et al., 1997). Though the downstream target of the *ras*-signaling is c-Fos, Fra-1 was suggested to be a protein that mediates the ras-transformation in NIH3T3 cells. The study of the

transition of small cell lung cancer cells to more malignant non-small cell lung carcinoma also revealed the accumulation of Fra-1 in AP-1 complexes during malignant progression of this type of tumor (Risse-Hackl et al., 1998). Similarly, elevated Fra-1 level was detected in oncogene-transformed rat and human thyroid cells (Vallone et al., 1997; Battista et al., 1998) and in mouse malignant E-cadherin-negative adenocarcinoma cell lines (Kustikova et al., 1998). Therefore, *fra-1* has been implicated in the maintenance and progression of the transformed state in several non-related cell systems, ras-transformed murine fibroblasts and different epithelial tumor-derived cell lines. In contrast, c-Fos transcription was shown to be inhibited as a result of malignant transformation of human bronchial epithelial cells (Lee et al., 1998), as well as in human colon cancer (Zhang et al., 1997). Interestingly, *c-fos* is known to down-regulate its own promoter in transient transfection assay (Lucibello et al., 1989), and Kessler et al. reported the down-regulation of *c-fos* transcription by Fra-1 in *ras*-transformed NIH3T3 fibroblasts (Kessler et al., 1999). This observation may provide an explanation of how the ras stimuli determine the composition of AP-1

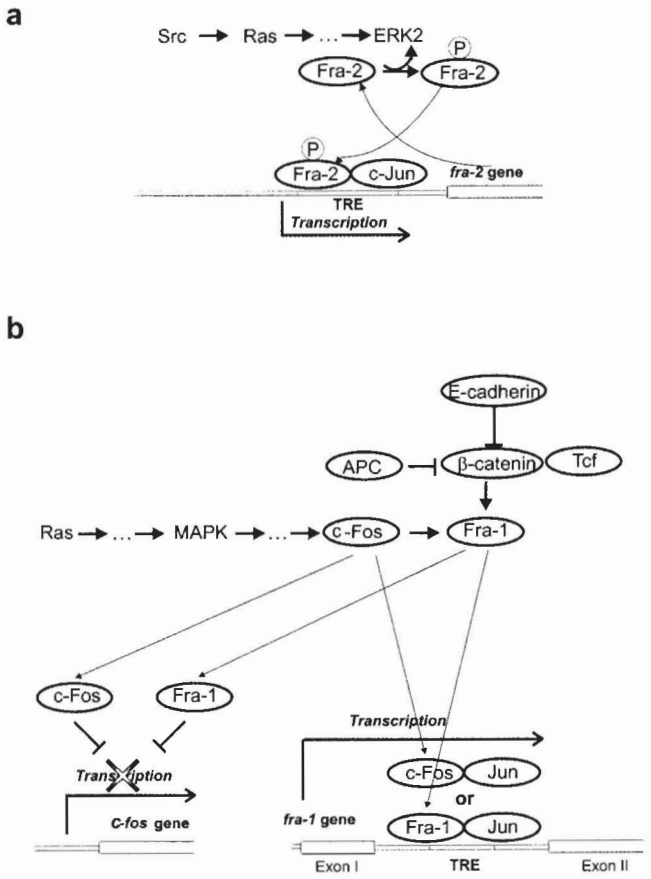

Fig. 2. Hypothetical models illustrating the accumulation of Fra-2 **(a)** and Fra-1 **(b)** as predominant AP-1 components in neoplastic cells. The schemes are based on the data presented in the following publications. a) Murakami et al., 1997, 1999. b) Mechta et al., 1997; Battista et al., 1998; Kustikova et al., 1998; Kessler et al., 1999; Mann et al., 1999.

complexes in transformed cells. Activation of ras induces *c-fos* transcription via the MAPK pathway. c-Fos activates Fra-1 synthesis through an AP-1-responsive intronic enhancer. c-Fos and then Fra-1 down-regulate the *c-fos* transcription. Simultaneously, both proteins may hetero-dimerize with the product of another AP-1-responsive gene, *c-jun* to maintain the elevated level of Fra-1 synthesis (Fig. 2b). This feedback regulation of the expression of different AP-1 factors in response to continual ras-signals leads to the accumulation of Fra-1 as a predominant Fos component in transformed cells. The down-regulation of *c-fos* transcription may contribute to the escape from the c-Fos-dependent apoptosis.

Activation of *ras* is not the only signaling event, which may lead to the accumulation of Fra-1 protein. Recently, Mann et al. described *fra-1* as a gene which can be induced in human colorectal carcinomas by ß-catenin, a key protein in *wnt* signaling (Mann et al., 1999). In response to *wnt* signals ß-catenin releases from the complex with the adenomatous polyposis coli (APC) tumor suppressor protein, translocates to the nucleus and binds LEF/TCF transcription factors to activate transcription of target genes (Gumbiner, 1997). The activation of the *wnt* signal transduction pathway occurs in several types of cancer as a result of mutations in molecules playing a central role in this signaling: loss of function mutations in APC (Kinzler and Vogelstein, 1996) and gain of function mutations in ß-catenin (Morin et al., 1997; Rubinfeld et al., 1997). In addition, E-cadherin, a cell adhesion molecule that is often inhibited in epithelial malignancies (Birchmeier and Behrens, 1994), can antagonize *wnt* signals by sequestration of ß-catenin to the membrane (Orsulic et al., 1999). Therefore, the activation of *fra-1* by *wnt* establishes a causative link between the loss of tumor suppressor proteins (APC and E-cadherin) and activation of AP-1 and explains the induction of Fra-1 synthesis during progression of thyroid and mammary tumors, which is accompanied by the loss of E-cadherin (Fig. 2b).

The direct physiological consequences of the *fra-1* activation has been studied in transformed rat thyroid and mouse mammary cell lines. The inhibition of *fra-1* synthesis in neoplastic thyroid cell lines by using an antisense strategy caused a partial reversion of the transformed phenotype as evaluated by two criteria: morphological appearance and the ability to form colonies in soft agar (Vallone et al., 1997). These data suggested that Fra-1 is necessary for the development of the fully transformed phenotype of thyroid cells. The overexpression of Fra-1 in epithelioid mammary mouse adenocarcinoma cells was sufficient for the appearance of certain, albeit restricted mesenchymal characteristics, including the ability to invade through an artificial basement membrane, morphological alterations and changed type of motility (Kustikova et al., 1998). In addition, ectopic Fra-1 activated the expression of five genes associated with malignancy, *S100A4*, *HMGI(Y)*

and three components of the urokinase system, *uPA*, *uPAR* and *PAI-1* (Kustikova et al., 1998). However, it is not clear whether the activation of these genes occurred directly, or via the induction of other Fra-1 immediate targets. Interestingly, Fra-2 was much less effective in inducing these alterations in the same cell line (our unpublished data). This physiological difference between two proteins, which are very similar in terms of transcriptional activation via AP-1 recognition sites, suggests that they may posses some yet unknown specific functional features. This speculation is supported by the observation that Fra-1 but not other Fos proteins are capable of physical interaction with the bHLHZip USF transcription factor (Pognonec et al., 1997). The further study of the functions of non-transforming Fos proteins relevant to oncogenic transformation and tumor progression seems to be a matter of great importance.

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