Chapter 12

c-Fos in Differentiation and Development

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CONTENTS

INTRODUCTION

About ten years have passed since the first report (Müller et al., 1982) was published in which the expression of c-Fos was analyzed in different tissues in mice during development. Since that time several dozens of publications have addressed the expression of endogenous c-Fos as well as the analyses of gene transfer experiments in either tissue culture systems or mice.

IN VITRO: CELL CULTURE EXPERIENCES

Relatively few cell lines have been used to investigate the involvement of c-Fos in differentiation. Furthermore, some data have been derived from organ culture experiments. Most of the cell lines only differentiate *in vitro* upon induction. Since almost every external stimulus can induce expression of the *c-fos* gene (see Chapter 8, this volume), it was quite a complicated task to separate cause and consequence in the differentiating process. For example, the monomyelocytic cell line HL60 differentiates into macrophages upon treatment with the phorbol ester TPA (Rovera et al., 1979). This differentiation is accompanied by high expression of c-Fos (Müller et al., 1984; Mitchell et al., 1985). However, variants of the HL60 line can differentiate in the absence of c-Fos expression (Mitchell et al., 1986).

Another example are PC12 cells, which can be induced to differentiate by either nerve growth factor (NGF) or dexamethasone (Kruijer et al., 1985). However, only induction by NGF results in activation of the *c-fos* gene (Kruijer et al., 1985). Remarkably, c-Fos can activate expression of the NGF gene (Hengerer et al., 1990), which suggests the initiation of an autocrine mechanism. This, however, seemingly contradicts the result that constitutive overexpression of c-Fos blocks the differentiation of PC12 cells when induced by NGF (Ito et al., 1989). It is tempting to speculate that different expression levels of c-Fos elicit opposite effects in differentiation or that c-Fos has different functions during the course of differentiation.

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Another cell line system widely used to study differentiation are embryonal carcinoma (EC) cells. In undifferentiated EC cells, such as F9 or P19, c-Fos is expressed at very low levels, but is elevated in the course of differentiation to endoderm (Müller, 1983). The potential involvement of c-Fos in the differentiation of EC cells was tested by transfection of different *c-fos* gene constructs into F9 EC cells (Müller and Wagner, 1984; Rüther et al., 1985). Here, the spontaneous differentiation frequency of these cells was clearly increased as a consequence of c-Fos overexpression. These data are supported by *fos*–antisense experiments in F9 cells where blocking fos expression led to inhibition of the differentiation to endoderm (Edwards et al., 1988).

Further linkage of fos function to differentiation was investigated in B cells. One consequence of elevated expression of c-Fos in transgenic mice (Rüther et al., 1988) is that it appears to interfere with B cell function. This was analyzed in primary cultures of B cells isolated from different transgenic mouse lines. First, constitutive c-Fos expression blocked the differentiation of B cells to IgG_1-producing cells (Koizumi et al., 1993). Second, by using inducible *c-fos* constructs, differentiation of B cells to IgG_2b-producing cells was augmented when Fos was expressed only during the first 2 days after induction of differentiation. However, IgG_2b production was suppressed when c-Fos was further expressed at day 3 of differentiation (Takada et al., 1993). Thus, c-Fos might have different functions at various times during the differentiation of B cells.

Finally, organ culture systems have been used to investigate the pattern of c-Fos expression in the course of differentiation of osteogenic progenitors. In mouse mandibular condyles, cells of the progenitor zone differentiate and form new bone during *in vitro* cultivation. There is evidence that these cells express high levels of c-Fos prior to activation of genes characteristic of osteoblasts (Closs et al., 1990).

All these *in vitro* data suggest that c-Fos is a gene product that can either initiate or block certain differentiation processes. However, whether c-Fos is essential in differentiation by itself or just a component in one of several pathways could not be investigated in any of these systems.

IN VIVO: c-fos EXPRESSION IN MICE

PROFILE OF *c-fos* EXPRESSION

The first report about the expression of c-Fos in mice (Müller et al., 1982) described it as restricted to the extraembryonic tissues and placenta in mouse development and to bone and skin in adult mice. Later studies defined, by means of *in situ* analyses, the temporal and spatial pattern of c-Fos expression more precisely. Following ontogeny, c-Fos is first expressed in the trophectoderm of the preimplantation blastocyst (Whyte and Stewart, 1989). In the next stage analyzed, namely, late midgestation (day 13.5 to 14.5 of mouse development), c-Fos is expressed in the mesodermal web tissue of the digits, the growth regions of developing bones, and cartilage (Sandberg et al., 1988; Heckl and Wagner, 1989). In late gestation (day 17 of mouse development), high levels of c-Fos are found again in the mesodermal web tissue and the growth regions of long bones (Dony and Gruss, 1987; Togni et al., 1988; Heckl and Wagner, 1989). In addition, c-Fos is expressed in the intestine, developing cartilage, and the spinal cord as well as in certain structures in the peripheral nervous system (Caubet, 1989; Heckl and Wagner, 1989).

Just before birth, there is a marked expression of c-Fos in almost every organ (e.g., heart, liver, thymus, skin, lung, and gut) that declines one day after birth (Kasik et al., 1987). In healthy adult mice, c-Fos is only weakly expressed. Thus, c-Fos can be considered as a developmentally regulated gene with a precise spatial and temporal pattern. This suggests a specific function in certain developmental processes.

MICE EXPRESSING ADDITIONAL *c-fos*

If c-Fos is a key regulator in development, one would expect its activity to be dominant. Therefore, alteration of c-Fos expression, e.g., ectopically, should lead to consequences in development. Furthermore, ectopic c-Fos expression might help to unravel the function of c-Fos. Based on this idea, different transgenic and chimeric mouse lines have been generated that overexpress c-Fos in several organs at different levels. First, when c-Fos was expressed using the human metallothionein promoter in either transgenic or chimeric mice, chondro- and osteogenic hyperplasias and tumors developed (Rüther et al., 1987, 1989; Wang et al., 1991). The development of bone-associated tumors, however, was specific for transgenic constructs in which the proto-oncogene c-Fos had been converted into the transforming version. Second, when the nontransforming *c-fos* proto-oncogene was linked to the murine MHC class I promoter H-2 k^b, and, thereby, overexpressed in almost every organ, mice displayed a marked alteration of the thymus architecture and B cell function was impaired (Rüther et al., 1988; Takao et al., 1991).

Thus, only certain cell types are susceptible to a dominant action of c-Fos. They belong either to the chondro–osteo lineage or are part of the hematopoietic system. Since these cells are known to express endogenous c-Fos at certain stages in development, one can speculate that the level of c-Fos is crucial for their normal development.

MICE LACKING *c-fos*

The ultimate proof for the function of c-Fos in differentiation and development is the analysis of mice lacking c-Fos. This is performed by the inactivation of the *c-fos* gene via homologous recombination in embryonic stem (ES) cells. The ES cells carrying one mutant *c-fos* allele are then used to generate chimeric mice that can transmit the inactivated allele to offspring. These heterozygous F_1 mice will produce, by brother–sister mating, mice without functional c-Fos.

Using this approach, two groups recently published their findings about c-Fos-negative mice (Johnson et al., 1992; Wang et al., 1992). The predominant phenotype in mice without c-Fos was a disturbance of bone remodeling, called osteopetrosis. In almost every bone the bone marrow cavity was reduced because of massive production of new bone. The growth plates in bone were also affected, being reduced and highly irregular in the zone of proliferating chondrocytes. However, the zone of hypertropic chondrocytes was found to be increased.

The bone changes likely lead to other phenotypes. Teeth were present, but their eruption was apparently blocked by an abnormal amount of bone in the jaw. In the thymus, the total number of thymocytes was reduced about 10-fold, whereas the relative number of mature thymocytes was increased. In the spleen, B cells were found to be 75% reduced. However, myeloid cells showed a fourfold increase. All these findings can be interpreted to result from the drastic changes in bone, which might interfere with hematopoiesis. Bone marrow transfer studies now underway will explore the direct role of c-Fos in these phenotypes.

In addition, gametogenesis in both female and male mice was affected (Johnson et al., 1992). However, this finding was not consistent for all the homozygous animals analyzed. For spermatogenesis a disturbance might be expected, since c-Fos was found to be expressed throughout sperm development (Pelto-Huikko et al., 1991). Mating of heterozygous animals revealed a non-mendelian ratio of the different genotypes that likely represents a transmission distortion in the female germline (Wang et al., 1992).

Finally, both studies indicate that Fos-negative mice display abnormal behavior, such as no reaction to stress (Johnson et al., 1992; Wang et al., 1992). However, this behavior might be the consequence of systemic bone alterations. Furthermore, histological analysis did not show any gross changes in the brain.

CONCLUSIONS

The tissue culture experiments had previously indicated that c-Fos has a distinct function in differentiation. However, because of the restricted potential of the few *in vitro* differentiation systems, several of the results were inconsistent and could even be interpreted as resulting from artificial conditions. Nevertheless, these findings, as well as the c-Fos expression profile in development, have initiated several *in vivo* experiments.

The *in vivo* studies turned out to be more consistent. First, they documented that c-Fos is essential for normal bone development. Second, the correct amount of c-Fos is important for normal bone development, otherwise c-Fos exerts a dominant activity. Third, c-Fos seems not to have an essential role in proliferation and growth control, as was believed for several years. This also agrees with the growth and differentiation behavior of Fos-negative ES cells (Field et al., 1992). Fourth, although c-Fos is essential for normal development of certain structures, it is dispensable for embryonic development, since Fos-negative mice are viable and can even mate. Thus, c-Fos can be considered as a key regulator in specific tissues, such as bone and hematopoietic cells, were it can exert a dominant function.

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