Functions of AP1 (Fos/Jun) in bone development

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Genetically modified mice and cells have provided important insights into the biological functions of the dimeric transcription factor complex AP1, in particular into its role in skeletal development. Data obtained from knockout mice revealed that some components, such as c-Fos are key regulators of bone cell differentiation, whereas others, like c-Jun, JunB and Fra-1 are essential in embryonic and/or postnatal development. Apart from identifying specific roles of AP1 proteins in developmental processes, researchers are beginning to obtain a better molecular understanding of their cell-context dependent functions, their downstream target genes and how they regulate bone cell proliferation, differentiation, and apoptosis.

A great variety of dimers composed of members of the Fos, Jun, and ATF families of proteins constitute the transcription factor complex AP1 (for review see references1). While the Fos proteins (c-Fos, FosB, Fra-1, Fra-2) can only heterodimerise with members of the Jun family, the Jun proteins (c-Jun, JunB, JunD) can both homodimerise and heterodimerise with Fos members to form transcriptionally active complexes. Some members of the ATF and CREB families of proteins are also part of AP1 complexes. AP1 converts extracellular signals into changes in the expression of specific target genes, which harbour AP1 binding site(s) in their promoter or enhancer regions. The activity of AP1 is modulated by interactions with other transcriptional regulators and is further controlled by upstream kinases that link AP1 to various signal transduction pathways. AP1 has been implicated in a large variety of biological processes including cell differentiation, proliferation, apoptosis, and oncogenic transformation. Here I will summarise the current understanding of AP1 functions in bone development, which was largely obtained from the analysis of genetically modified mice and cells in which specific AP1 genes have been ectopically expressed, inactivated, mutated, or replaced by each other.

Most important insights regarding the specific functions of AP1 proteins in mouse development were obtained from loss of function experiments using ES cell technology. A summary of the phenotypes of mice harbouring genetic modifications of the different fos and jun genes is given in table 1. These analyses showed that AP1 proteins have specific functions during embryogenesis and organogenesis. With regard to embryonic development, the Fos and Jun proteins can be grouped into two categories: some such as c-Fos, FosB, and JunD are dispensable, whereas others like c-Jun, JunB and Fra-1 are essential for embryonic development. In the following I will discuss the specific phenotypes of knock out mice in particular of c-Fos and Fra-1 as well as studies in transgenic mice expressing individual AP1 components with an emphasis on the effects observed on bone development.

AP1 IN BONE DEVELOPMENT

The growth and the maintenance of the skeleton depends on the coordinated function of osteoblasts and osteoclasts, the two principal cell types of bone tissue.1,2 Osteoblasts, which derive from mesenchymal progenitors, produce the extracellular matrix of the bone that later undergoes mineralisation. In contrast, osteoclasts belong to the monocyte/macrophage lineage and reduce bone mass by resorbing the mineralised extracellular matrix. Recent studies have shown that AP1 components, mainly members of the Fos family such as c-Fos and Fra-1, have important functions in both of these cell types.

<table>
<thead>
<tr>
<th>Knockout</th>
<th>Phenotypes</th>
<th>Affected organs and cell types</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-Fos</td>
<td>osteopetrosis</td>
<td>bone, osteoclasts</td>
</tr>
<tr>
<td>FosB</td>
<td>nurturing defect</td>
<td>brain, hypothalamus</td>
</tr>
<tr>
<td>Fra-1</td>
<td>embryonic lethality (E9.5)</td>
<td>extra-embryonic tissue, placenta</td>
</tr>
<tr>
<td>Fra-2</td>
<td>lethal at birth (unpublished)</td>
<td></td>
</tr>
<tr>
<td>c-Jun</td>
<td>embryonic lethality (E12.5)</td>
<td>liver, hepatoblasts and heart outflow tract</td>
</tr>
<tr>
<td>JunB</td>
<td>embryonic lethality (E8-10)</td>
<td>extra-embryonic tissue, placenta</td>
</tr>
<tr>
<td>JunD</td>
<td>male sterility</td>
<td>testis, spermatogenesis</td>
</tr>
</tbody>
</table>

Table 1 Analysis of genetically modified fos and jun mice

<table>
<thead>
<tr>
<th>Transgene</th>
<th>Promoters</th>
<th>Phenotypes</th>
<th>Affected organs and cell types</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-Fos</td>
<td>H2kA</td>
<td>osteosarcoma</td>
<td>bone, osteoblasts</td>
</tr>
<tr>
<td>FosB</td>
<td>H2kA</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>ΔFosB</td>
<td>TCRβ</td>
<td>impaired T cell differentiation</td>
<td>thymus, immature thymocytes</td>
</tr>
<tr>
<td>Fra-1</td>
<td>NSE</td>
<td>osteosclerosis</td>
<td>bone, osteoblasts</td>
</tr>
<tr>
<td>Fra-2</td>
<td>CMV</td>
<td>ocular malformations</td>
<td>eye, anterior eye structures</td>
</tr>
<tr>
<td>c-Jun</td>
<td>H2kA</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>JunB</td>
<td>ubiquitin C</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>JunD</td>
<td>not reported</td>
<td>enhanced Th2 maturation</td>
<td>thymus / CD4 thymocytes</td>
</tr>
</tbody>
</table>

For details and further references please see Jochum et al.1
Osteoblasts and the role of c-Fos and Fra-1

In osteoblasts, AP1 activity can be induced by transforming growth factor β, parathyroid hormone and 1,25-dihydroxy vitamin D, which are potent regulators of osteoblast differentiation and proliferation. The various members of the AP1 complex are differentially expressed during osteoblast maturation in vitro with all Fos and Jun proteins initially being highly expressed. Subsequently, during the period of extracellular matrix production and mineralisation, their levels decline, and Fra-2 and JunD become the major components of the AP1 complex in fully differentiated osteoblasts. The pattern of c-Fos expression during development suggests a critical role in endochondral ossification, although the analysis of c-Fos deficient mice indicates that c-Fos is dispensable for the differentiation of osteoblasts.

When c-Fos was ectopically expressed in various cell types and in transgenic mice, specific effects on the skeleton were observed. Chimeric mice obtained from c-Fos overexpressing embryonic stem cells develop chondrogenic tumours implying a function of c-Fos in chondrogenesis in vivo. Surprisingly, c-Fos overexpression in an in vitro model of chondrogenesis inhibits the differentiation of chondrocytes. Ectopic c-Fos expression from a ubiquitous promoter in transgenic mice has no noticeable effects on cell differentiation in most organs, but results in the specific transformation of osteoblasts leading to osteogenic sarcomas reminiscent of human osteosarcomas.

Importantly, transgenic mice overexpressing Fra-1 in osteoblasts as well as in other cell types exhibit again a specific bone phenotype. These mice show increased bone formation and develop osteosclerosis of the entire skeleton. This phenotype is attributable to a cell autonomous increase in the number of mature osteoblasts indicating that Fra-1 increases osteoblast differentiation. A similar osteosclerotic phenotype is observed in transgenic mice expressing ΔFosB in osteoblasts. These mice also show reduced adipogenesis, a phenotype that is not observed in Fra-1 transgenic mice.

Given the osteoblastic phenotype of the Fra-1 transgenic mice it was of interest to next ask whether the absence of Fra-1 affects osteoblast or osteoclast differentiation. Inactivation of Fra-1, however, results in embryonic lethality around day 10 of development because of defects in the placenta thereby precluding the analysis of bone cell development. The labyrinth layer of mutant placenta is reduced in size and largely avascular suggesting that the invasion of allantoic vessels into the chorionic plate is impaired in the absence of Fra-1. The development of mutant fetuses can be rescued up to birth by providing wild type extra-embryonic tissues using tetraploid blastocyst injection. This suggests that Fra-1 is dispensable for the differentiation along most, if not all, cell lineages in the fetus. Moreover, the lethality of Fra-1 deficient mice was fully rescued by the ectopic expression of Fra-1 from transgenic mice demonstrating that AP1/Fra-1 activity during development does not have to be tightly regulated. These rescued mice still develop osteosclerosis, which was indistinguishable from the disease observed in the Fra-1 transgenic mice. However, it cannot be excluded that Fra-1 has a critical role in osteoblast homeostasis during postnatal life. We recently demonstrated using a conditional allele of fra-1 that mice without Fra-1 can indeed be born and develop osteopenia, which has to be further characterised (Robert Eferl, unpublished data).

Osteoclasts and the role of c-Fos and Fra-1

Mice lacking c-Fos are viable and fertile but lack osteoclasts and are therefore osteopetrotic. This phenotype is strain dependent, as on a 129/sv background much fewer pups are born and reach weaning age, when compared with C57Bl/6 mutant mice (unpublished observations). Mutant mice also show abnormalities of the haematopoietic system including extramedullary haemato poiesis and lymphopenia, which are both secondary to the bone phenotype. Although c-Fos is rapidly induced in T cells upon activation and it regulates the transcription of various cytokines including interleukin 2, the analysis of c-Fos deficient mice has shown that c-Fos is not required for the differentiation and activity of peripheral T cells.

The lack of osteoclasts in c-Fos deficient mice is accompanied by increased numbers of bone marrow macrophages, which is specific for this osteopetrotic mutant. The osteopetrosis is characterised by increased bone mass attributable to reduced bone resorption. Recent data using retroviral gene transfer into c-fos mutant osteoclast precursors in vitro have shown that all Fos proteins, most efficiently Fra-1, can complement for the absence of c-Fos. Further structure–function analysis also demonstrated that the major C-terminal transactivation domains of c-Fos and FosB are dispensable for the rescue of osteoclast formation in vitro. Interestingly, Fra-1, which lacks a transactivation domain, has the highest rescue activity. Moreover, the osteoclast differentiation factor RANKL also known as ODF, OPGL, TRANCE (for review see Karsenty and Wagner) induces besides c-Fos also Fra-1 transcription in a c-Fos dependent manner, thereby establishing a link between RANK signalling and the expression of AP1 proteins during osteoclast differentiation (see below).

The osteopetrotic phenotype can be partly cured in c-Fos mutant mice by expressing a Fra-1 transgene, one target gene of c-Fos in the osteoclast lineage. Importantly, total restoration of osteoclast differentiation was achieved in knockout mice generated by the insertion of the fra-1 gene into the c-fos locus. Moreover, Fra-1 was able to rescue c-fos dependent functions, in particular bone development and light induced photoreceptor apoptosis. Interestingly, the rescue of bone cell differentiation was gene-dose dependent and Fra-1 was unable to substitute for the expression of AP1 target genes in fibroblasts. These results show that c-Fos and Fra-1 have maintained functional equivalence during vertebrate evolution and further suggest that functional divergence of Fos genes is a result of divergence of regulatory rather than protein coding sequences.

In addition to the effects on osteoblasts, ectopic Fra-1 expression also increases the differentiation of osteoclasts, both in progenitor cell lines and primary osteoclast progenitors. This strong osteoclastogenic effect is not apparent in Fra-1 transgenic mice in vivo. Osteoclast differentiation on the other hand does not seem to require the presence of Fra-1, as the skeleton of rescued Fra-1 deficient fetuses contains functional osteoclasts at birth. Moreover, recently established Fra-1 knockout mice using a conditional gene targeting approach demonstrate that a normal skeleton can be formed (Robert Eferl, unpublished data and see above) suggesting that Fra-1 is dispensable for the formation and differentiation of the major bone cell types, the osteoblasts and the osteoclasts. The role of c-Fos in signalling during osteoclast differentiation

To maintain bone homeostasis the signalling by the osteoclastogenic ligand RANKL, a member of the TNF family and an essential factor for osteoclast differentiation, has to be tightly controlled, as deregulated osteoclast activity leads to severe bone diseases such as osteoporosis. In a genomic search for c-Fos target genes induced by RANKL in osteoclast precursor cells using wild type and c-fos-/- cells, a group of mRNAs whose expression is dependent on interferon α/β signalling was found to be specifically induced by c-Fos. The induction was decreased in cells lacking c-Fos and the down regulated genes included interferon responsive genes such as Ifi-3, Ifi-I, Mx-1, Scy-10 and Irf-7 (unpublished and Takayama et al.). Although the integral role of the interferon α/β system in RANKL signalling has been documented, it was unknown whether this system is linked to RANKL signalling and bone metabolism. We found that
RANKL directly induced the interferon β gene in a c-Fos dependent manner in osteoclast precursor cells. However, interferon β inhibits the differentiation of osteoclasts by interfering with the RANKL induced expression of c-Fos, which is essential for osteoclast formation. The mechanism of interferon β gene induction by c-Fos is distinct from that induced by viruses and implies that an autoregulatory mechanism is operating in fine tuning the activity of osteoclasts. RANKL induces in the same progenitor cell c-Fos, which in turn induces the expression of positive regulators such as Fra-1 as well as its own inhibitor interferon β (fig 1). The inhibition of c-Fos by interferon α/β signalling occurs at the post-transcriptional level and the molecular mechanism has yet to be defined. The importance of this regulatory mechanism for bone homeostasis is supported by the observation that mice deficient in interferon β signalling exhibit severe bone loss accompanied by increased osteoclastogenesis. Moreover, we were able to show in a model of LPS induced inflammatory bone destruction that the application of interferon β had a therapeutic effect in this disease model. Therefore, this study may offer new approaches to the treatment of bone diseases caused by excessive osteoclastogenesis, such as autoimmune arthritis and osteoporosis.

CONCLUSIONS

Through the extensive analysis of the individual AP1 components using genetically modified mice and cells we are beginning to unravel the specific functions of these proteins in development and/or in the adult organism. The initial studies have largely focused on c-Fos and c-Jun, but have been extended to other family members. As the absence of c-Jun, JunB and Fra-1 results in embryonic lethality, the analysis of these proteins during later stages of development and in postnatal life were not possible without the development of new genetic tools. With the conditional inactivation of AP1 genes in a cell type specific and/or inducible manner using the established Cre/loxP and tet regulated systems, novel functions are being assigned to the individual members of AP1 (for example, Behrens et al). However, we still do not understand the enormous specificity of proteins such as c-Fos and Fra-1 for osteoblast and/or osteoclast function. Nor do we know the individual partners of these molecules responsible for their transcriptional activity and specificity. In addition, we only have a very vague picture about the important downstream genes controlled by these factors. The genomic analysis explained by the discovery of the interferon β system as a c-Fos regulated gene cascade illustrates the power of these approaches. Finally, we will have to come back to classic biochemical and/or proteomic approaches to precisely define the molecular mechanisms how a transcription factor complex such as AP1 influences the decision of a cell to divide, differentiate, or die.

ACKNOWLEDGEMENTS

The IMP is supported by Boehringer-Ingelheim and I thank PD for comments on the manuscript.

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REFERENCES
