1. Introduction

1. 1. Bone: a complex dynamic organ.

Bone is often considered as a static structure which simply serves as the basic material to build up the skeleton shaping our body. This assumption must be corrected; bone is an active and dynamic tissue that is constantly renewed all through the life by a process called remodeling. Bone remodeling is the result of the activities of two specialized cells: resorption of old bone mineralized matrix by the osteoclasts and deposition of new bone matrix by the osteoblasts. Remodeling is not only essential for body growth until puberty but, even after completion of longitudinal growth, in order to maintain stability, to respond to changes in load and to permit fracture healing. Building the skeleton is only one aspect of bone function. Additionally bone plays an important role in calcium- and phosphorus-homeostasis since it is the major source of storage for these components which can be released by bone remodeling. Furthermore bone is also an essential haematopoietic organ that provides the environment for haematopoiesis including the immune system which takes place in the bone marrow.
Thus, bone is a complex organ that requires tightly regulated mechanisms to ensure proper function. Imbalanced remodeling activity leads to well known pathologies that are causing increased or decreased bone mass. Decreased bone mass can cause osteoporosis due to increased bone resorption or osteopenia due to decreased bone formation. Increased bone mass can cause osteopetrosis due to decreased bone resorbtion or osteosclerosis due to increased bone formation. In addition, bone can also be affected by tumors such as osteosarcoma and osteoclastomas.

1. 2. Bone development: 2 systems and 4 cell types.

The human skeleton consists of more than 200 bones which differ in shape and composition. This pinpoints to the developmental aspect of bone tissue. There are 4 major cell types which are in charge for synthesizing and resorbing bone matrix and thereby maintaining the bone, namely the osteoblasts, the osteocytes, the

chondrocytes and the osteoclasts. How can these cells contribute to such complex structures and how are they regulated? Part of our current knowledge stems from studying naturally occurring mouse mutants which developed specific bone phenotypes. In addition, the development of techniques to manipulate the mouse genome provided a powerful tool for further elucidating the molecular determinants of bone development and homeostasis. Identifying the responsible gene loci in mice, and thereby facilitating finding the human counterparts demonstrated a strong conservation of the involved genes. In the following, a brief summary of the work in the bone field that led to the current concept of bone biology will be given with emphasis on the role of the transcription factor AP-1.

1. 2. 1. The 2 ways of bone development

The skeleton is established by two different ways (figure 1.01). Most bones of the skull and flat bones are generated by a process called intra-membranous ossification. Here, condensations of mesenchymal cells assemble at the site of the future bone. These mesenchymal cells directly differentiate into osteoblasts which in turn depose the mineralized bone matrix.

Long bones and most of the other skeletal elements are formed in a different manner. In the mechanism known as endochondral ossification, the bone is generated through an intermediary scaffold made of cartilage which is synthesized by chondrocytes. This developmental process which requires the orchestrated interaction of all bone cells will be described in detail.

The site of the future bone is, analogous to the beginning of intra-membranous ossification defined by mesenchymal cell condensations. In the center of these condensations, cells differentiate into chondrocytes which express specific markers like collagen II (the main constituent of cartilage) and aggrecan. These cells differentiate further into pre-hypertrophic and hypertrophic chondrocytes which can be distinguished by morphology and altered expression level of collagen II. While pre-hypertrophic chondrocytes express a very low level of collagen II, hypertrophic chondrocytes do not express it anymore but instead express collagen X which is specific to this cell population (Linsenmeyer et al, 1991). At the periphery of the condensation (now termed as perichondrium) the mesenchymal cells differentiate into osteoblasts which surround the newly formed cartilaginous scaffold and establish

a structure called bone collar (St-Jaques et al., 1999). The extracellular matrix of the cartilage attracts endothelial cells from the mesenchyme which invade via the bone collar and establish vascularization of the cartilage. Vascularization permits the invasion of the cartilage scaffold by osteoclasts that resorb most of the mineralized cartilage matrix. Additionally osteoblast progenitors which are brought in from the bone collar replace the cartilage matrix by deposing collagen I, the main component of the bone matrix.

At the distal ends of the bone, the remaining chondrocyte population is organized in a columnar setting termed growth plate. Resting chondrocytes are situated at the most distal sites and serve as a pool for the proliferating chondrocytes which are spatially followed by pre-hypertrophic and hypertrophic chondrocytes. The activity of the growth plate (i.e. sequential deposition of preliminary cartilage matrix followed by resorbtion by osteoclasts and bone matrix deposition by osteoblasts) is essential for the longitudinal growth of the bone. Thus, cartilage is playing two functions, shaping the bone and promoting its growth. In the following sections the molecular mechanisms controlling bone development will be discussed.

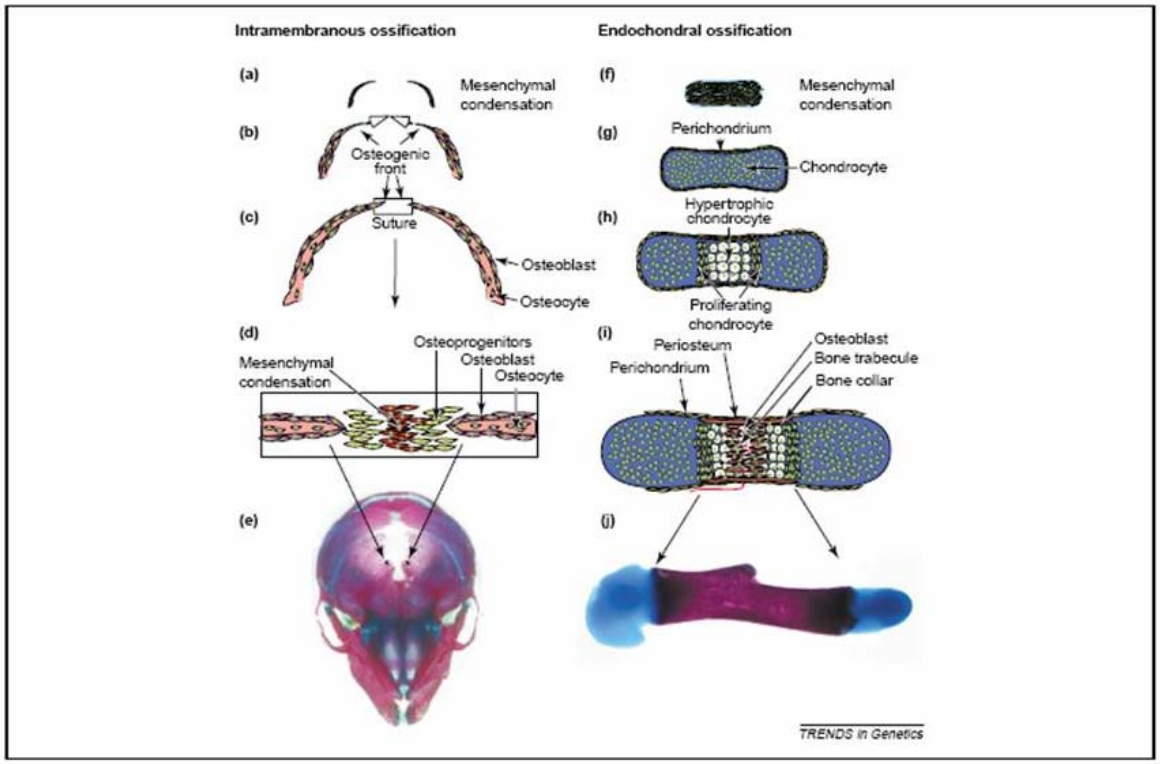

Figure 1.01. Intra-membranous and endochondral bone formation. Both processes are initiated by mesenchymal condensations. Intra-membranous bone is synthesized directly by osteoblasts while endochondral bone is established via an intermediate cartilage template. The skull and the long bone were stained with alcian blue (cartilage) and alizarine red (bone). From Nakashima and De Crombrugghe, 2003.

1. 2. 2. The cells

Chondrocytes and chondrogenesis

Non-hypertrophic chondrocytes

Non-hypertrophic chondrocytes include the resting and proliferating chondrocytes. The first factor found to play a crucial role in non-hypertrophic chondrocyte proliferation was fibroblast growth factor receptor 3 (FGFR3). It was shown that an activating mutation of this receptor was the cause for a human disorder called achondroplasia (ACH) (Naski et al., 1996). This condition is characterized by a nearly complete absence of non-hypertrophic chondrocytes leading to a disturbed growth plate regulation and finally to dwarfism. FGFR3 that belongs to the family of tyrosine-

kinase transmembrane proteins is expressed in proliferating chondrocytes. The mutation in FGFR3 in ACH leads to constitutive activation which in turn inhibits proliferation of chondrocytes. Thus FGFR3 is a negative regulator of chondrocyte proliferation. This was further demonstrated by inactivating FGFR3 signaling in mice leading to increased chondrocyte proliferation and subsequently to an extended zone of proliferating chondrocytes within the growth plate (Deng et al., 1996). There are more than 20 FGF proteins, that could serve as ligands (Ornitz and Itoj, 2001) and none of them has been clearly identified as FGFR3 ligand in cartilage. Nevertheless it could be shown that FGF18-deficient mice display a very similar phenotype with an expanded zone of proliferating chondrocytes which indicate that FGF18 might be the preferred ligand for FGFR3 in chondrocytes (Liu et al., 2002). Activating mutations in FGFR3 induce phosphorylation and nuclear localization of the transcription factor STAT1 and initiation of transcription of cell cycle inhibitor p21.

Another key signaling molecule involved in chondrocyte proliferation is Indian hedgehog (Ihh). Ihh deficient mice show a marked decrease in non-hypertrophic chondrocyte proliferation (St-Jaques et al., 1999). It is a secreted factor which activates the G protein-coupled transmembrane protein smoothened (Smo) by binding to its repressor patched-1 (Ptc1). CyclinD1 is one of the genes known to be positively regulated by Ihh signaling. CyclinD1 is a positive regulator and its down-regulation observed in Ihh deficient mice may explain the observed phenotype. Interestingly, activation of FGFR3 has been shown to down-regulate of Ihh expression, therefore establishing a loop between the two signaling pathways (Liu et al., 2002).

An important role in chondrocyte development has been demonstrated for the Sox family of transcription factors by generating mouse chimera. Embryonic stem cells harboring inactivated Sox9 were injected into wild-type female mice. A block in the chondrocyte lineage commitment was observed in the generated chimeras (Bi et al., 1999). So far Sox9 is the earliest known differentiation marker of chondrocytes. While upstream factors are not clearly identified, *in vitro* experiments suggest that Sox9 is increased upon FGF-mediated signaling via activation of the mitogen-activated protein kinase pathway (MEK/ERK) (Murakami et al., 2000). Sox5 and Sox6 were also identified as crucial transcription factors for chondrocyte development downstream of Sox9. Sox5/Sox6 double knock out mice exhibit chondrodysplasia as a result of a distorted growth plate.

In addition to the Sox family, there are several other transcription factors which play an important role for chondrocytes such as Hif1α, which is important for chondrocyte survival (Schipani et al., 2001) or CREB (Long et al., 2001) and ATF-2 (Reimold et al., 1996) important for their differentiation.

The differentiation step from non-hypertrophic to hypertrophic chondrocytes is regulated by the Wnt signaling pathway. The Wnt family of secreted factors comprises 19 members. Out of these, Wnt5a seems to control the transition to hypertrophic chondrocytes (Yang et al., 2003). It is expressed in the early limb perichondrium and its forced expression at sites determined to become hypertrophic leads to failure in the transition. Notch signaling is also implicated in the transition control as shown by its expression in non-hypertrophic chondrocytes that inhibits subsequent differentiation.

Hypertrophic chondrocyte differentiation

Hypertrophic chondrocytes include the two subpopulations of pre-hypertrophic chondrocytes and hypertrophic chondrocytes. Pre-hypertrophic chondrocytes are larger in size than proliferating chondrocytes and express less collagen II, while expressing FGFR1, Ihh and Runx2/Cbfa1. The terminally differentiated hypertrophic chondrocytes are the largest cells among the chondrocyte subpopulations and they are unique in expressing collagen X. Hypertrophic chondrocytes also express Runx2/Cbfa1 albeit at low level and VEGF which is important to initiate the vascularization of the bone.

Hypertrophic chondrocyte differentiation is regulated by parathyroid hormone-related peptide (PTHrP). PTHrP is a secreted factor expressed in the perichondrium. It binds the PTH/PTHrP receptor (PPR), a G protein coupled receptor expressed in pre-hypertrophic chondrocytes. The function of PTHrP in cartilage was shown by the analysis of PTHrP deficient mice that display a dwarfism owned to a premature transition from pre-hypertrophic chondrocytes to hypertrophic chondrocytes (Karpalis et al., 1994). Thus PTHrP is a negative regulator of hypertrophic chondrocytes. This is further documented in *in vitro* cultures of metatarsal bones. Addition of PTHrP to the media resulted in a delayed hypertrophic chondrocyte differentiation (Lanske et al., 1996). Activating mutations of the PPR in mice and man also result in a delayed differentiation of hypertrophic chondrocytes (Schipani et al., 1995; Schipani et al.,

1997). PTHrP expression in turn is up-regulated by Ihh which is expressed in pre-hypertrophic chondrocytes and signals to the perichondrium. Owned to the lack of vascularization of the growth plate, a gradient of PTHrP concentration is established by diffusion. This gradient determines the onset of hypertrophy of chondrocytes and as a consequence the location of the bone collar (Chung et al., 2001).

Expression of Ihh expression is in part controlled by Runx2/Cbfa1 and mice deficient for this transcription factor display some skeletal elements devoid of hypertrophic chondrocytes (Inada et al., 1999). Ihh in turn has been shown to be necessary for Runx2 expression in the bone collar where it is required to induce osteoblast differentiation (St-Jaques et al., 1999). These results emphasize the need for a tight interaction between chondrocytes and osteoblasts for endochondral bone formation.

The elongation of the growth plate defines the longitudinal growth of the bone elements. This is achieved by the regulation of chondrocyte proliferation which is mainly controlled by the two hormones insulin like growth factor 1 (IGF1) and growth hormone (GH). GH is produced in the pituitary gland and acts as a classical hormone while IGF1 exhibits a broader expression pattern including expression in proliferating and hypertrophic chondrocytes. Mice deficient for either one of the factors are severely growth retarded (Lupu et al., 2001). Deficiency in these hormones is observed in human with similar consequence and some forms of human growth disorders are treated by IGF 1 and GH application.

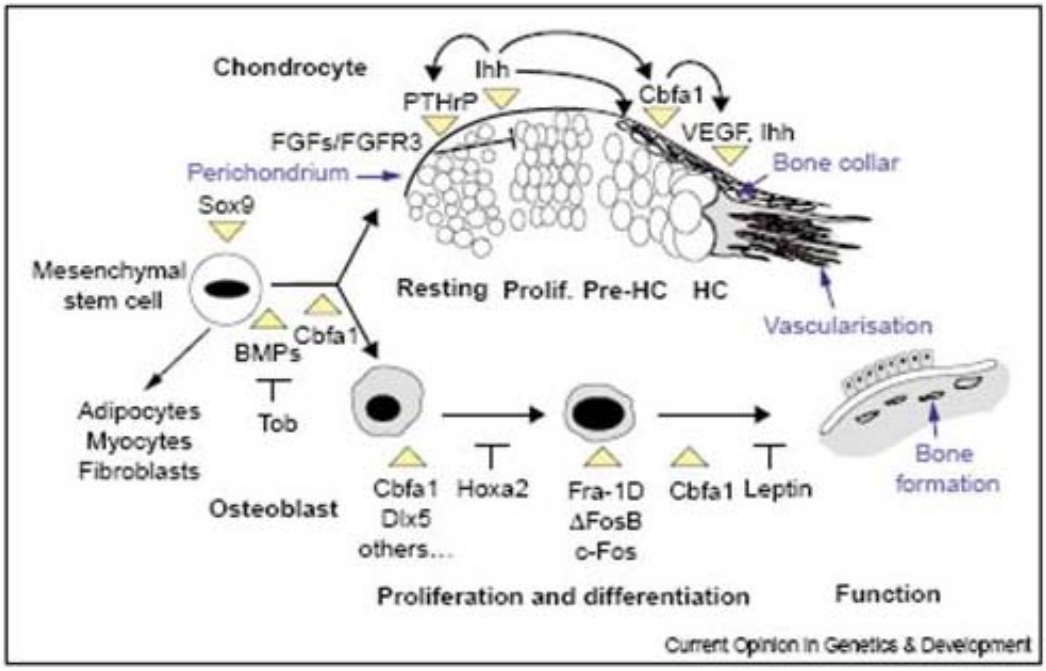

Figure 1.02. Chondrocyte and osteoblast differentiation. Major factors involved in chondrocyte and osteoblast differentiation and their interaction are shown. From Wagner and Karsenty, 2001.

Vascular invasion of hypertrophic cartilage

During endochondral ossification the preliminary cartilage scaffold has to be replaced by bone. Thus, it is necessary to first resorb the cartilage in order to subsequently form the bone matrix. These processes require two cell types namely the osteoclast and the osteoblast. Both cell types are brought in from the bone collar by the blood stream. Therefore vascularization is essential for bone development. The vascularization process is largely dependant on the protease MMP9 and on the master regulator for angiogenesis VEGFs. MMP9 deficient mice have an extended zone of hypertrophic chondrocytes that could be rescued by bone marrow transplantation of wild type cells. These experiments indicated that the phenotype was due to a defect in cells of haematopoietic origin. Among them, the bone resorbing cells osteoclasts were shown to highly express MMP9 (Vu et al., 1998). Furthermore through degradation of the cartilage, VEGF which is expressed by hypertrophic chondrocytes and deposed in the cartilaginous matrix is now released and can bind to its receptors on endothelial cells, thereby inducing vascularization.
The important function of VEGF on bone development was demonstrated by blocking VEGF with a soluble receptor (Gerber et al., 1999) or by specifically inactivating

VEGF in chondrocytes (Haigh et al., 2000), both leading to a phenotype similar to the one observed in MMP9 deficient mice.
Expression of VEGF is dependant on Runx2 as shown by lack of vascular invasion due to absence of VEGF that is observed in Runx2 deficient mice. *In vitro* experiments proved that Runx2 is able to induce VEGF (Zelzer et al., 2001).

1. 2. 3. Osteoblast, osteocytes and Osteogenesis

Osteogenesis is the process by which bone matrix is synthesized and mineralized by the cells of the osteoblastic/osteocytic lineages. Osteoblasts are the cells that depose and mineralize the matrix. Osteocytes are the most abundant cells of the bone; they are the late stage of differentiation of the osteoblasts embedded into the mineralized bone. Their function is so far still unknown.

Osteoblast differentiation

Osteogenic growth factors

Growth factors which have been demonstrated to take part in osteogenesis include the group of bone morphogenetic proteins (BMPs) belonging to the TGF beta superfamily. The osteogenic properties of the BMPs were demonstrated by showing that ectopic endochondral bone formation can be induced by administration of BMPs into organs which normally do not form bone (Reddi 1997). The exact mode of action of the BMPs is not entirely elucidated but the current data suggest that they contribute to osteogenesis as skeletal mesoderm inducers and therefore are important for skeletal patterning (Capdevila and Izpisua Belmonte, 2001).
The growth factors IGF1 and 2 which are abundant in the bone extra-cellular matrix (ECM) can induce osteoblasts proliferation *in vitro* (Canalis, 1993). *In vivo*, over-expression of IGF1 in osteoblasts leads to increased bone mass (Zhao et al., 2000). From the Wnt family, Wnt10a was shown to be a positive regulator of bone mass.
The Wnt signaling is also controlling osteogenesis, as shown by the over-expression of Wnt10a in mice that leads to increased trabecular bone mass while the knock out mice display reduced bone mass (Bennet et al., 2005). Wnt proteins bind to frizzled and Lrp and, via activation of a signaling cascade including kinases, Wnt signaling is

integrated by beta catenin to induce the binding of the TCF/Lef transcription factors to Wnt target genes. The essential role of this cascade for the commitment of mesenchymal osteo-chondroblast progenitors to differentiate to osteoblasts was demonstrated by tissue specific deletion of beta catenin resulting in ectopic chondrocyte differentiation at the expense of osteoblast differentiation (Hill et al., 2005; Day et al., 2005). In contrast ectopic expression of beta catenin suppresses chondrocyte differentiation (Hill et al., 2005; Day et al., 2005).

Transcriptional control of osteogenesis

Runx2/Cbfa1 was genetically identified in mouse and human as an essential transcription factor necessary for osteoblasts differentiation. Runx2 innactivation in mice results in a complete absence of osteoblasts in all skeletal elements independent of the developmental pathway determining skeletogenesis (Komori et al., 1997; Otto et al., 1997). In addition, mice harboring only one intact Runx2 allele display defects in bones originating from intra-membranous ossification (i.e. delay fontanel closure and absence of clavicle). This phenocopied a human disease called cleidocranial dysplasia (CCD) in which inactivation of one Runx2 allele occurs (Mundlos et al., 1997). Runx2 over-expression in mice can lead to endochondral ossification of skeletal elements which would not ossify under normal conditions (Takeda et al., 2001; Ueta et al., 2001). Runx2 can also induce osteoblast differentiation *in vitro* and its forced expression in fibroblastic cells resulted in an osteoblasts-specific gene signature (Ducy et al., 1997). These facts clearly identifying Runx2 as an essential player in osteoblastogenesis and raises the question of its own regulation.

Runx2 activity, a temporal and spatial regulation

Expression analysis in mice has shown that Runx2 is already detectable at embryonic day 10.5 (E10.5) in the mesodermal condensation that prefigure skeletal elements. Since no osteoblastogenesis takes place at this stage, there must be a mechanism preventing Runx2 to promote osteoblast differentiation. Indeed Runx2 activity was shown to be blocked by the Twist proteins at these stages of

development providing one explanation for the delay in Runx2-induced osteoblast phenotype (Bialek et al., 2004). The transcription factor Stat1 was also shown to block Runx2 activity by sequestering it in the cytoplasm (Kim et al. 2003). Smad3 was also reported to bind Runx2 and thereby decreasing its transcriptional activity (Alliston et al., 2001). These data indicated, that while being expressed, Runx2 activity is spatially and temporally controlled by interaction with other proteins.
Another explanation for the discrepancy between the timing of expression and the timing of action of Runx2 might be that its specific target genes may be only accessible at later stages of development. One such gene was identified as the transcription factor osterix (Osx). Mice deficient for Osx resemble to *runx2* knock out mice. Runx2 expression is not affected by Osx deficiency. In contrary, Osx absent in mice lacking Runx2, indicating that Osx must lay downstream of Runx2 (Nakashima et al., 2002).

Transcriptional regulation of Runx2

Several transcription factors were shown to regulate Runx2 expression. Among them are the homeobox-containing transcription factors Msx2 and Bapx1. Msx2 deficient mice display delayed ossification in the skull and an overall decreased bone volume. In these mice, Runx2 was found to be down-regulated (Satokata et al., 2000). In addition, activating mutations of Msx2 in human lead to increased bone formation in the skull (Ma et al., 1996). Bapx1 which is required for axial skeleton formation also positively regulates Runx2 as shown by the reduced level of Runx2 expression found in Bapx1 knock out mice (Triboli and Lufkin, 1999). Inactivation of another homeobox transcription factor, Hox-2, results in elevated Runx2 expression levels (Kanzler et al., 1998). Hence Hox-2 is a negative regulator of Runx2.
Despite the key role played by Runx2 during osteoblast development, pathways independent of Runx2 which are important for osteoblastogenesis have been described. The Wnt signalling pathway which activates target genes by the Lef/Tcf family of transcription factors was identified as an essential regulatory mechanism in osteoblast biology in both mice and man. Wnt proteins bind to their receptor frizzled and to the co-receptor LDL-receptor-related protein 5 (LRP5). Inactivation of LRP5 in mice is associated with low bone mass phenotype due to decreased osteoblast proliferation (Kato et al., 2001). In human, while deletion or inactivation of the LRP5

leads to low bone mass, activating mutations were identified in high bone mass syndromes (Gong et al., 2001; Little et al., 2002).
Dlx5 is a homeobox containing transcription factor expressed in mesenchymal condensations undergoing intra-membraneous ossification. In mice, Dlx5 deficiency causes a delayed skull formation that was shown to be independent of Runx2 (Acampora et al., 1999).
A role for several members of the AP-1 transcription factor in osteoblastogenesis, at least in postnatal development, has been demonstrated. Analysis of transgenic mice revealed that overexpressing the fos related transcription factor 1 (Fra-1) induced a drastic acceleration of osteoblast differentiation. As a consequence, these mice developed a strong osteosclerosis (Jochum et al., 2000). In contrast, osteopenia is developing in *fra-1* deficient mice in part due to reduced osteoblast differentiation (Eferl et al., 2004). Osteosclerosis was also observed in mice overexpressing another AP-1 member namely ΔfosB (Sabatakos et al., 2000). A decrease in bone mass is also evident in mice lacking *junB* (Kenner et al., 2004). Analysis of the function of this transcription factor revealed its essential roles in promoting osteoblast differentiation and osteoclastogenesis.

1. 3. 4. Osteoclast differentiation

Osteoclasts derive from the monocyte/macrophage lineage and possess the unique capacity of resorbing bone matrix. The role of this cell type in bone homeostasis is illustrated by diseases caused by deregulation of osteoclasts. Increased osteoclast activity observed in aging women following menopause is the major cause of osteoporosis, causing bone fragility and inducing high risk for fractures. In contrast, decreased or impaired osteoclastogenic function results in osteopetrosis, a rare disease in human characterized by absence of the bone marrow cavity. In the following the main genes and pathways involved in controlling osteoclastogenesis will be introduced.

The earliest factor identified in osteoclastogenesis is the transcription factor PU.1. The commitment of hematopoietic stem cells to the macrophage lineage is dependent on PU.1, as illustrated by the lack of macrophage and osteoclast observed in PU.1 deficient mice (Tondravi et al., 1997). Two cytokines were identified

as being essential for osteoclast differentiation *in vivo* namely macrophage colony stimulating factor (M-CSF) and receptor activator of nuclear factor kappaB ligand (RANKL). Mice with inactivating mutation of M-CSF (op/op mice) lack macrophages and osteoclasts and develop osteopetrosis (Yoshida et al., 2000). This phenotype can be rescued by administration of M-CSF but not by bone marrow transplantation (Felix et al., 1990), revealing a paracrine mode of action for M-CSF signaling. M-CSF that binds to the receptor encoded by *c-fms* which signals via the PI3K and ERK MAPK pathway, is important for survival and proliferation of the monocytes.

RANKL is expressed by osteoblasts and several immune cells including activated T-cells. RANKL binds to RANK and activates the transcription factors NF-kappaB and c-Fos, both essential for osteoclastogenesis. RANKL administration in mice results in increased bone resorption (Burgess et al., 1999), while RANKL deficient mice fail to develop osteoclasts (Kong et al., 1999). RANKL availability is regulated by the level of its decoy receptor osteoprotegerin (OPG) (Simonet et al., 1997; Yasuda et al., 1998), which when over-expressed titers out RANKL and subsequently induces osteopetrosis. In turn, OPG knock out mice are osteoporotic due to increased osteoclast differentiation (Bucay et al., 1998; Mizuno et al., 1998). Signaling of the RANK/RANKL axis requires the adaptor protein TNF receptor associated protein 6 (Traf6) which binds to the cytoplasmic domain of RANK. Overexpression of dominant negative Traf6 or deletion of Traf6 in mice caused a block in osteoclastogenesis by inhibition of NF-kB activation (Lomaga et al., 1999). RANK also signals via the JNK pathway and disruption of JNK1 but not JNK2 decreased RANKL-induced osteoclast differentiation *in vitro* (David et al., 2002).

As mentioned above the final executioners of RANK signalling are NF-kB and c-Fos. p50/p52 double knock out mice are osteopetrotic due to the lack of osteoclasts demonstrating that these two NF-kB members are essential for osteoclastogenesis (Franzoso et al., 1997). Similarly, inactivation of the AP-1 member c-Fos is also leading to osteopetrosis due to a block in osteoclast differentiation (Grigoriadis et al., 1994). Moreover other AP-1 members have been shown to be implicated in osteoclastogenesis. The knock in of *fra1* in *c-fo*s locus demonstrated that Fra-1 can substitute c-Fos in osteoclasts (Fleischmann et al., 2000), although Fra-1 is not essential for osteoclastogenesis *in vivo* (Eferl et al., 2004).

Finally there are a number of genes which are not necessary for osteoclast differentiation but for their resorptive function such as TRAP, Cathepsin-K, chloride

channel-7 and carbonic anhydrase 2. These genes, essential for the activity of the cells, are often found to be mutated in osteopetrotic patients. Theses genes among many others enable the osteoclast to execute its resorptive activity which involves motility, synthesis of collagenases, acidification of bone matrix and finally resorption of the bone matrix.

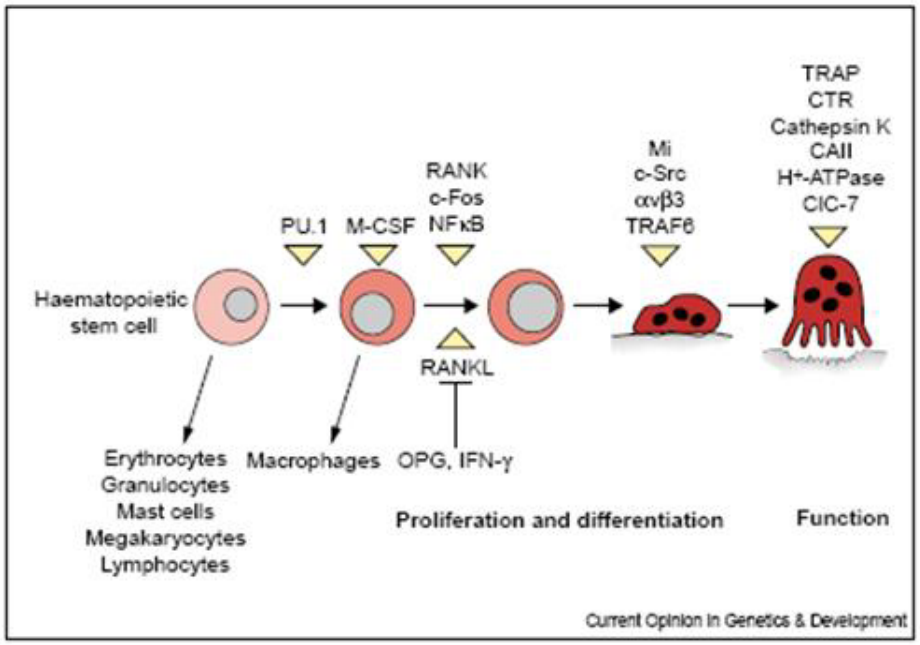

Figure 1.03. Osteoclast differentiation. From Karsenty and Wagner, 2001.

1. 3. The AP-1 transcription factor

1. 3. 1. Structure and regulation of AP-1

The prototype of AP-1 members was first identified as the oncogene encoded by the Finkel-Biskins-Jenkins virus that caused osteosarcomas in mice.

The cellular counterpart of AP-1 was first identified as an essential factor to bind at the human metallothionein IIa (hMTIIa) promoter and to initiate its transcription (Lee et al., 1987). Further investigation demonstrated that AP-1 activity is induced upon stimulation with 12-O-tetradecanoylphorbol 13-acetate (TPA) with subsequent transcription of the genes collagenase, SV40, stromelysin, polyoma and interleukin 2

(Angel et al., 1987). Analysis of the promoter regions of the aforementioned genes revealed a conserved binding element with the palindromic heptameric sequence 5`TGA G/C TCA-3 termed TPA responsive element (TRE) (Lee et al., 1987). Insertion of the TRE upstream of the thymidine kinase gene (TK) renders it TPA-inducible while mutations of the TRE shut off TPA induced transcription. TPA as well as other growth factors and cytokines activate Protein Kinase C (PKC) which in turn induces AP-1 activity, thus inhibition of PKC also abolish AP-1 dependent transcription of target genes (Imbra and Karin, 1987).

Biochemical approaches led to a more accurate picture of AP-1 composition. The use of affinity columns coupled to synthetic TRE oligonucleotides revealed that proteins from nuclear Hela cell extracts bound to the column, were not homogenous. Instead multiple proteins bound to the column that could later be identified as proteins encoded by members of the Fos and Jun family (Bohmann et al., 1987; Franza et al., 1988). Sequential immunoprecipitation studies further proved that the major AP-1 complex consists of heterodimers made of Fos and Jun.

By now 4 members of the Fos family of transcription factor are known, namely c-Fos, FosB, Fra-1 and Fra-2. The Jun family comprises the 3 proteins c-Jun, JunB and JunD.

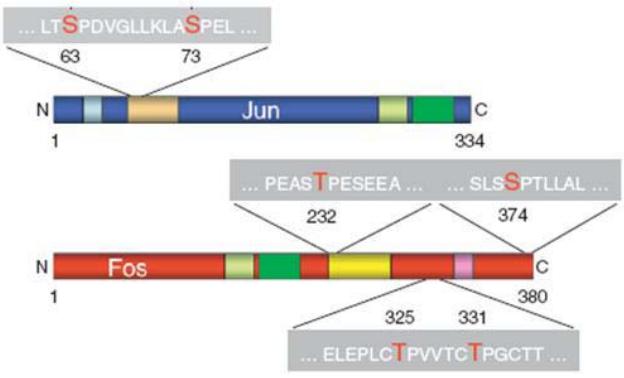

Figure 1.04. Structure of AP-1. Green:Leucine zipper, light green:basic region, light blue in Jun: delta domain, pink in Fos: Erk-binding domain. Some of the phosphorylation sites present in Fos and Jun are shown. From Hess et al., 2004

Both protein family members share characteristic domains including the leucine zipper, a basic domain and a nuclear localization sequence (NLS). The leucine zipper is a structure that arises from a specific amino acid sequence forming an alpha helix

where every 7th amino acid is a leucine. As a result of this arrangement the leucine side chains protrude from one side of the alpha helix and form a hydrophobic surface. These hydrophobic surfaces mediate dimerization (Landschutz et al., 1989). The basic domain is a prerequisite for DNA binding. Since AP-1 is only active as a dimer it is conceivable that members of the Fos family can homodimerize with other Fos proteins and heterodimerize with Jun members. However, it has been shown that Fos can only form heterodimers. Homodimerization is not possible due to the high abundance of negatively charged side chains within the leucine zipper region. These residues can form additional salt bridges with residues from the leucine zipper of a Jun member which further stabilize dimer formation. This is also an explanation for the higher thermostability of heterodimers (Smeal et al., 1989). In contrast, Jun members can form homo- and heterodimers.

In addition to structural domains necessary for dimerization and DNA binding, c-Fos and FosB as well as c-Jun and JunD harbor strong transactivation domains. These transactivation domains are located at the C-terminus domain of c-Fos and FosB and at the N-terminus domain of c-Jun and JunD.

c-jun harbors two conserved serine residues at position 63 and 73 and two threonine residues at position 91 and 93 which serve as phosphoacceptors. These residues are phosphorylated by the MAP kinase members Jun N-terminal kinase (JNK). The JNK pathway integrates signals from growth factors and stress (cytokines, UV, ROS, osmotic shock). JNKs bind to a specific docking site located between residues 30 and 60 (delta domain) of c-Jun (Kallunki et al., 1994). The close vicinity of the docking site to the phosphoacceptor seems to facilitate the physical interaction of the catalytic pocket of the kinase with the serines. The lack of delta domain may be one reason for the weak transactivational capacity of JunD. The conformational change induced by c-Jun phosphorylation allows recruitment of CREB binding protein (CBP). CBP can potentiate c-Jun transcriptional activation by connecting it to the basal transcription machinery (Kwok et al., 1994). Moreover CBP is able to acetylate histone thereby opening the chromatin and enhancing c-Jun binding to DNA (Ogryzko et al., 1996). Phosphorylation of N-terminal phosphorylation sites in c-Jun also affects protein stability. It was shown that the phosphorylation is priming the protein for proteasomal degradation by the ubiquitin pathway in neuronal cells (Nateri et al., 2004).

Phospho-acceptor sites that regulate its activity are also present in c-Fos. These include the threonines 232, 325, 331 and serines 362 and 374. Threonines 325, 331 and serine 374 are phosphorylated by extracellular regulated kinases (ERKs) while the kinase responsible for threonine 232 is still under debate. Additionaly serine 362 is phosphorylated by ribosomal kinase S6 (Rsk2) (David et al., 2005). Phosphorylation of specific residues can increase the transcriptional activity of c-Fos by stabilizing the protein *in vitro* and *in vivo* thereby increasing c-Fos transforming activity (David et al., 2005; Okazaki and Sagata, 1995).
Apart from posttranslational modifications, the expression of the various AP-1 members is also transcriptionally regulated. In addition, mechanisms affecting the stability of their mRNA have been described. Finally AP-1 activity can be regulated by dimer composition and interaction with other transcription factors and cofactors.

1. 3. 2. Biological functions of AP-1

AP-1 in mouse development

The relevance of AP-1 for regulating important biological functions during mouse development and its involvement in tumorigenesis were extensively studied by means of transgenic and knock out mouse models and different cell lines derived from these animals. Loss of function studies revealed that Fra-1, Fra-2, c-Jun and JunB are indispensable for development since their absence leads to embryonic lethality between E8.5 and E12.5 or death at birth (*fra-2* KO) (Schreiber et al., 2000; Hilberg et al., 1993; Johnson et al., 1993; Schorpp-Kistner et al. 1999). Interestingly, when overcoming the early lethality, many AP-1 mutants exhibit a specific bone phenotype which indicates that osteoblasts and osteoclasts are AP-1 target cells. The current knowledge of functional aspect of each AP-1 member will be summarized in the following paragraphs.

c-Fos

c-Fos deficient mice are viable and fertile but develop osteopetrosis and are lacking teeth due to a block in osteoclast differentiation (Johnson et al., 1992; Wang et al.,

1992). In addition, extramedullary haematopoiesis is developing as a consequence of the lack of bone marrow cavity (Okada et al., 1994).
c-Fos over-expressing stem cells injected into wild type blastocyst contributed to chimeric mice with no overt phenotype indicating c-Fos did not affect differentiation potential of embryonic stem cells *in vivo* (Wang et al., 1991). c-Fos over-expression from a ubiquitous promoter induced transformation of the osteoblast lineage with subsequent osteosarcoma formation (Grigoriadis et al., 1993).

FosB

Mice lacking *fosB* are viable and fertile and display no gross abnormality except from nurturing defects (Brown et al., 1996; Gruda et al., 1996). Ubiquitous expression of FosB does not interfere with development (Grigoriadis et al., 1993) but interestingly over-expression of the splice variant ΔFosB result in osteosclerosis due to increased osteoblast activity.

Fra-1

Fra-1 deficient mice die at E9.5 due to defects in the placenta and yolk sac. Mutated placentas display morphologic alterations, have reduced size and lack of vascularization. The embryonic lethality can be overcome by crossing conditional *fra-1* knock out mice with the MORE-*cre* mouse. MORE-*cre* mice express Cre ubiquitously except in extra-embryonal tissues (Tallquist and Soriano, 1999). These mice are viable and fertile and develop osteopenia due to decreased osteoblast differentiation (Eferl et al., 2004).
Ectopic Fra-1 expression can also rescue embryonic lethality of *fra-1* knock out mice (Schreiber et al., 2000). Similar to ΔFosB, over-expression of Fra-1 from an ubiquitous promoter result in osteosclerosis due to accelerated osteoblast differentiation with splenomegaly as secondary effect (Jochum et al., 2000).

Fra-2

Mice with inactivated Fra-2 die at birth (Eferl and Wagner, 2003). Over-expression of Fra-2 induces tumour formation in pancreas, thymus and lung (Eferl and Wagner,

2003). Over-expression from the CMV promoter results in ocular malformations (McHenry et al., 1998).

c-Jun

c-jun deficient mice die between E12.5 and E13.5 due to malformations of the cardiac outflow tract. In addition mutant mice display a pathologic liver structure with increased apoptosis in hepatoblasts (Eferl et al., 1999). The function of c-Jun in liver is also emphasized by the fact that *c-jun* knock out stem cells contribute to all tissues in chimera mice except for the liver (Hilberg et al., 1993). More precise insights of c-Jun role in development were obtained by tissue specific inactivation using the Cre-loxP system. Postnatal *c-jun* inactivation in hepatocytes demonstrated no biochemical and histological abnormalities, nevertheless liver regeneration capacity was severely affected upon partial hepatectomy (Behrens et al., 2002). *c-jun* inactivation in the chondrocytic lineage resulted in malformation of the vertebrae (scoliosis) due to defective invertebral disc formation (Behrens et al., 2003)
The influence of transactivation potential of c-Jun during development was analyzed by generating knock in mice carrying *c-jun* alleles with mutated phosphoracceptors (Serine 63 and 73 were mutated to alanine). Despite reduced transcriptional activity, the mice developed normally but some other c-Jun specific functions like kainate induced neuronal apoptosis were impaired (Behrens et al., 1999).
Ectopic expression of c-Jun did not show any phenotype in mice (Grigoriadis et al., 1993). Over-expression of a dominant negative c-Jun under the control of the osteoclasts specific TRAP-promoter leads to osteopetrosis due to a strong decrease in osteoclast number (Ikeda et al., 2004).

JunB

Mice deficient for JunB die between E8.5 and E10 due to defects in extra embryonic tissues which resemble the phenotype of *fra-1* knock out mice (Schorpp-Kistner et al., 1999). Conditional inactivation of *junB* in the myeloid lineage gives rise to myeloid leukaemia suggesting an essential role for JunB in negatively regulating myeloid proliferation (Passegue et al., 2001). Deletion of *junB* in osteoblast results in osteopenia, a disorder charaterized by low bone mass.

Ectopic JunB expression had no overt consequence on development whereas JunB over-expression in T lymphocytes altered T helper cell differentiation (Li et al., 1999).

JunD

There is no strong overt phenotype in mice lacking JunD. The mice are born in mendelian ratio and appear healthy. Knock out mice are a bit smaller and male homozygous knock out mice become sterile due to defects in spermatogenesis (Thepot et al., 2000). Ubiquitious expression of JunD leads to a decreased number of peripheral T and B cells (Meixner et al., 2004).

Additionally to the knock out and over-expression studies of individual AP-1 members, gene replacement studies were performed. *fra-1* was knocked in the *c-fos* locus and *junB* or *junD* were recombined into the *c-jun* locus (Fleischmann et al., 2000; Passegue et al., 2002; Eferl and Wagner, 2003). *c-fos* replacement by *fra-1* could completely rescue the osteopetrotic phenotype, nevertheless not all the known c-Fos target genes could be switched on by Fra-1 in embryonic fibroblast. *junB* and *junD* knock ins could rescue the embryonic lethality caused by the absence of c-Jun.

Conclusions can be drawn from these studies. First, only the *fra-1* and *junB* phenotypes have shown some similarities. All other knock out lines exhibit distinct phenotypes indicating that the AP-1 members have no redundant functions *in vivo*. In addition, based on phenotype similarities, this approach did not allow to define clear partnership between Fos and Jun members. The second observation is the fact over-expression of JunB and c-Jun that are both essential for development did not result in a specific phenotype. In contrast the dispensable gene JunD exhibit phenotypes.
The gene replacement experiments suggest, that AP1 members could share some but not all functions *in vivo* and therefore some specificity may be due to the spatio-temporal pattern of expression of each member.
Finally nearly all the Fos members and JunB exhibit a specific function in bone development. For c-Jun, a bone cell related function was reported only reported *in vitro* (David et al., 2002). A bone specific function for JunD was not reported to date.

1. 3. 3. AP-1 in bone cell biology

c-Fos

The function of c-Fos for bone development was demonstrated by generation of *c-fos* knock out mice. These mice are born at Mendelian ratio, viable and fertile. They can easily be distinguished from their wild type littermates due to their decreased body size and absence of teeth (Johnson et al., 1992; Wang et al., 1992). Histological analysis revealed that osteoclasts were absent in *c-fos* knock out mice. A series of elegant experiments were performed to elucidate the cause of lack in osteoclasts (Grigoriadis et al., 1994). Osteoclasts require factors from the stromal environment and osteoblasts to differentiate. Therefore, the block of osteoclastogenesis caused by the lack of c-fos may not have been cell autonomous but rather due to a defect in the supportive cells. To this end embryonic limbs were transplanted under the kidney capsule of adult hosts. In this setup, the mesenchymal cells including osteoblasts derive from the donor limb, while the haematopoietic cells (including osteoclast progenitors) are provided by the microenvironment of the host kidney capsule. Transplantation of mutant and wild type limbs to wild type host gave rise to normally developed bone with bone marrow cavity and TRAP-positive multinucleated osteoclasts. Unlikely mutant and wild type limbs in a mutant host environment did not develop a bone marrow cavity and osteoclasts were not visible. Moreover bone marrow cells from wild type mice were injected into irradiated newborn mutant mice. Bone and teeth development were rescued whereas injection of mutant splenocytes in irradiated wild type mice did not rescue the osteopetrotic phenotype. All of the results prove that the defect in osteoclastogenesis lies in the haematopoietic compartment and not in the stromal environment. This conclusion is further supported by *in vitro* experiments. Wild type osteoblast feeder cells are able to induce osteoclastogenesis of wild type but not of mutant bone marrow cells. In contrast mutant osteoblast feeders support wild type bone marrow cells to differentiate into osteoclasts.

Fra-1

The *fos* family member *fra-1* is also playing a role in bone development. Transgenic *fra-1* mice are osteosclerotic and smaller than their wild type littermates (Jochum et al., 2000). Bone histomorphometric analysis revealed a more than 2- fold increased

trabecular bone volume. *In vivo*, osteoclast numbers are comparable to wild type mice, however *in vitro,* osteoclast differentiation is increased when bone marrow cells isolated from *fra-1* transgenic mice are co-cultured with osteoblasts (Jochum et al., 2000). Thus the dramatic increase in bone mass is likely to be caused by the increased osteoblast activity. Osteosclerosis was also observed when transgenic femoral anlagen were transplantated under the kidney capsule of wild type or transgenic recipients. In contrary, transplantation of wild type anlagen under the kidney capsule of *fra-1* transgenic mice did not result in trabecular thickening. *In vitro* cultivated transgenic osteoblast progenitors differentiated much faster than wild type progenitors (Jochum et al., 2000). Finally, mutant osteoblasts were shown to deposit higher amounts of bone matrix including Osteoclacin, Collagen I and mGlap (Eferl et al., 2004). Since *fra-1* is an essential gene for placenta development, mice were generated where *fra-1* is inactivated in every tissue except in the placenta thereby rescuing embryonic lethality. The mice displayed a low bone mass phenotype (Eferl et al., 2004). Bone histomorphometric analysis revealed that the number of osteoclasts and osteoblasts were not altered *in vivo*. Low bone mass was resulting of reduced matrix deposition by osteoblast, classifying the observed phenotype as osteopenia. *In vitro* differentiation experiments revealed a severe defect of mutant osteoblasts to form bone matrix. Analysis of various bone matrix marker genes identified Osteocalcin, Collagen I and matrix Gla protein (mglap) as Fra-1 target genes down-regulated in knock out mice. Mutanted bone marrow cells grown on culture dishes showed a reduced osteoclast differentiation capability compared to wild type bone marrow cells, confirming previous studies, which have identified Fra-1 as an important c-Fos target gene during osteoclastogenesis (Matsuo et al., 2000). The osteoclastogenic defect could be rescued by cultivating the Fra1 deficient bone marrow cells directly on bone slices (Eferl et al., 2004). In conclusion fra-1 is a non-essential positive regulator of bone formation and resorbtion.

Fos B

Overexpression and knock out studies on FosB did not show any bone specific phenotype. However, ectopic expression of ΔfosB, a naturally occurring splice variant lacking the transactivation domain of FosB leads to osteosclerosis (Sabatakos et al., 2000). The phenotype is quite similar to the one observed in *fra-1* transgenic mice

with accelerated osteoblast differentiation. In addition to increased osteoblast differentiation ΔfosB also inhibits adipogenesis, a phenotype so far not described in *fra-1* transgenics. Since adipocytes and osteoblasts are deriving from common mesenchymal progenitors it was hypothesized that ΔFosB is involved in the control of the switch for osteoblast commitment. Further studies revealed that ΔFosB influences osteoblastogenesis and adipogenesis independently in a cell autonomous manner (Kveiborg et al., 2003).

Fra-2

Fra-2 is expressed in epithelial and cartilaginous tissues (Carrasco and Bravo, 1995). Fra-2 knock out embryos exhibit reduced zones of hypertrophic chondrocytes and impaired matrix deposition in the growth plate due to a differentiation defect of hypertrophic chondrocytes (Karreth et al., 2004). Analysis of primary chondrocytes from knock out embryos revealed a prolonged proliferation phase and delayed differentiation into hypertrophic chondrocytes compared to wild type chondrocytes. Since mice lacking Fra-2 die at birth, postnatal development of Fra-2 deficient cartilage was investigated by crossing mice harboring floxed *fra-2* alleles with chondrocyte specific Cre expressing ColIα1-*cre* mice.
ColIα1-*cre*/*fra-2*$^{fl/fl}$ mice were found to be growth retarded and the analysis of the growth plate has shown a reduced zone of hypertrophic chondrocytes (Karreth et al., 2004). Thus Fra-2 is a positive regulator for the initiation of hypertrophic chondrocyte differentiation.

Jun

From the Jun family members only JunB have been analyzed in detail concerning bone related functions. Due to the fact that *junB* is indispensable for embryonic development two different strategies were attempted to overcome lethality and to study JunB role in embryonal and postnatal development. One group generated viable *junB* deficient mice expressing low level of JunB by generating *junB* knock out mice that ectopically expressed JunB under the control of a ubiquitous promoter albeit at very low levels (Hess et al., 2003). A more elegant approach was performed by crossing conditional *junB* mice with the already mentioned MORE-*cre* mice, where

Cre is expressed in every tissue except in the placenta (Kenner et al., 2004). Thus JunB expression in the tissue essential for embryogenesis is not affected and mice are viable. Both mouse models exhibit bone specific abnormalities, namely osteopenia implying a role for JunB in bone development. Reduced bone mass in *junB* deficient mice is already detectable one week after birth and progressively worsened within age. Cortical bone thickness in mutants at 6 months of age was approximately half of cortical bone thickness in control littermates. Consequently bone stability was also reduced in mutants as demonstrated by mechanical tests. Bone matrix specific markers Collagen I, Osteocalcin and Bone Sialoprotein (bsp) were reduced in mutated bone. Analysis of osteoblasts *in vitro* demonstrated a proliferation defect in mutated cells due to a block in the transition from G1 to S-phase along with increased expression of the negative cell cycle regulator p16 and reduced expression of cyclinD1. Apart from proliferation capacity, differentiation potential of osteoblasts was also affected by the lack of JunB. There were less mature osteoblasts and expression of matrix markers was markedly reduced. In addition to bone formation defects, it could also be demonstrated that the bone resorbing properties were altered in *junB* knock out mice. The numbers of osteoclasts in bone were reduced in mutants and measurement of molecules arising upon bone resorption revealed a low bone turnover phenotype. Bone marrow cells from mutant mice were severely comprised in differentiating into osteoclasts as shown by reduced numbers of TRAP positive mature osteoclasts and reduced levels of matrix metalloproteinase-9. Resorptive activity of individual cells was not affected indicating that osteoclast function is normal. Results from co-culture experiments favor a cell autonomous defect of osteoclastogenesis in *junB* deficient mice (David et al., 2002). To support this notion, conditional *junB* mice were crossed with Cre expressing mice under the control of the monocyte specific lysozyme-M promoter. These mice were osteopetrotic due to reduced osteoclast number. *In vitro* differentiation from mutant bone marrow cells was also less efficient. All the data characterized JunB as an essential gene for both, bone forming and bone resorbing activity by regulating osteoblast and osteoclast function.

c-Jun

To study the impact of c-Jun on bone development, conditional *c-jun* mice were crossed with the CollagenIIα1-*cre* line, resulting in *c-jun* deletion in the chondrocyte lineage. The mutant mice developed scoliosis due to fusions of vertebral bodies (Behrens et al., 2003). These fusions arose from an impaired development process of the notochord. The notochord is a structure which prefigures the vertebral bodies. During development the notochordal cells vanish from the sites of future vertebral body formation and localize to the sites between the vertebral bodies to become vertebral discs. In mutants, the number of notochordal cells is reduced due to a higher apoptosis rate resulting in incomplete separation of the vertebral bodies and subsequently to fusion of some vertebral elements.

JunD

So far no bone specific phenotype has been reported in *junD* deficient mice (Thepot et al., 2000).

1. 3. 4. AP-1 in tumorigenesis

Fos and Jun were initially identified by their viral counterparts of the oncogenes v-Fos and v-Jun. Since v-Fos and v-Jun induced tumors, therefore, it was speculated that the cellular genes may act as oncogenes. However by comparing the protein structure of viral and cellular Fos and Jun, it became clear that there were deviations in some domains of the proteins. The N-terminal phosphorylation domain of c-Jun is not present in the viral protein due to an in-frame deletion and thus the viral Jun can not be phosphorylated by JNK anymore. At first glance this modification appears to be paradoxical since the viral protein can not be transactivated and as a consequence, potential oncogenic target genes will not be amplified efficiently. Actually this assumption does not reflect reality because in contrast to the cellular Jun, the viral Jun harbors transforming properties. It is assumed that the lack of the transactivation domain results in a protein that is independent of signaling events and therefore renders v-Jun constitutively active. Recent work has also shown that protein turnover is abolished in v-Jun since phosphorylation site serine-243 present in c-Jun is mutated to phenylalanine in v-Jun. Thereby v-Jun can not be primed by GSK3 for ubiquitination and subsequent degradation (Wei et al., 2005).

The viral Fos protein is also lacking specific phospho-acceptors in the transactivation domain due to a frame shift mutation. Analogous to v-Jun it is thought that through mutation of the transactivation domain, v-Fos is escaping regulatory mechanisms which prevent sustained activation of Fos.

Overexpression of c-Fos induces osteosarcoma in mice due to transformation of the cells of the chondro-osteoblastic lineage (Grigoriadis et al., 1993). Cyclin A and E, two positive regulators of cell cycle progression (Sunters et al., 2004) were identified as target genes of c-Fos induced tumorigenesis. Other target genes identified so far share functions involved in tumor invasiveness such as MMP1, MMP3, Cathepsin L and Ezrin (Hu et al., 1994; Hennigan et al., 1994; Jooss and Muller, 1995). Vascularization is a prerequisite for tumorigenesis since the growing tumor is highly dependent on nutrition and oxygen supply. VegfD a member of the angiogenesis promoting factors is also positively regulated by c-Fos (Marconcini et al., 1999). Prior metastasizing, transformed epithelial cells require a transition step to mesenchymal morphology characterized by loss of polarity. This epithelial-mesenchymal transition (EMT) can also be induced by c-Fos (Reichmann et al., 1992).
Over-expression of c-Jun in mice does not induce tumorigenesis but c-Fos-induced osteosarcomas develop faster and are larger in a *c-jun* transgenic background (Grigoriadis et al., 1993; Wang et al., 1995). Although c-Jun alone is not sufficient to initiate transformation, it was demonstrated that RAS-induced transformation is suppressed in fibroblasts lacking c-Jun (Johnson et al., 1996). Transactivation of c-Jun plays an important role, since in a mouse model for skin tumor induction, tumorigenesis was impaired in mice carrying c-Jun alleles with mutated phosho-acceptors. c-Fos induced osteosarcomas in *c-fos* transgenic mice were also smaller in size when crossed with mice expressing a c-Jun mutant that cannot be phosphorylated (Behrens et al., 2000).
c-Jun can induce target genes which were shown to support different hallmarks of cancer. Most of c-Jun regulated genes are involved in proliferation control such as epidermal growth factor receptor (EGFR), its ligand HB-EGF, keratinocyte growth factor (KGF) and cyclinD1 (Zenz et al., 2003; Park et al., 1999; Bakiri et al., 2000; Shaulian et al., 2000). Anti-apoptotic genes controlled by c-Jun include BCL3 (Rebollo et al., 2000). Furthermore proliferin which promotes angiogenesis is also positively regulated by c-Jun (Toft et al., 2001). The tumour suppressor protein p53

often found to be mutated in tumors is also regulated by c-Jun (Schreiber et al., 1999; Stepniak et al., 2006).

In contrast to the well established tumor promoting functions of c-Jun, the other Jun members seem to play the opposite role. Several genes which are induced by c-Jun are down-regulated by JunB. This may be achieved by JunB binding to c-Jun and thereby generating a transcriptionally less or even inactive dimer than the heterodimer c-Jun-c-Fos (Bakiri et al., 2000; Chiu et al 1989; Deng and Karin, 1993). Despite tittering out of c-Jun, JunB also induces target genes which are implicated in suppressing tumors like p16 (Passegue and Wagner, 2000). *In vivo* evidence for the anti-oncogenic function of JunB stems from mice lacking JunB in the myeloid lineage resulting in chronic myeloid leukaemia (Passegue et al., 2001).

The role of JunD in tumorigenesis is controversial since JunD deficient immortalized fibroblasts display increased proliferation while JunD deficient primary fibroblasts proliferate less (Weitzmann et al., 2000). This result highlights the dual role of AP-1 in tumorigenesis in general. Contrary to the textbook descriptions, AP-1 is not always acting as a tumor promoter. Tumor inducing and suppressing functions of AP-1 are determined by the cellular context. This is nicely illustrated by a mouse model where the tumour repressor gene p53 is inactivated and hence the mice are prone to tumor initiation. Crossing of *p53* knock out mice with *c-fos* deficient mice resulted in p53-deficient mice that develop a new form of tumor: rhabdomyosarcoma (Fleischmann et al., 2003).

Cell context specific functions were also reported for c-Jun. In neuronal cells, c-Jun induces apoptosis while it is necessary for survival in hepatocytes (Behrens et al., 2002; Raivich et al., 2004).