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TRANSCRIPTIONAL REGULATION OF OSTEOBLAST DIFFERENTIATION DURING DEVELOPMENT

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The osteoblast is the bone-forming cell. The molecular basis of osteoblast-specific gene expression and

characterization of OSE2, an osteoblast-specific cis-acting element present in the promoter Osf2/Cbfa1, the protein that binds to OSE2, was identified. Osf2/Cbfa1

expression is initiated in the mesenchymal condensations of the developing skeleton and is strictly restricted to cells of

regulates the expression of multiple genes expressed in osteoblasts, and forced expression of Osf2/Cbfa1 in

osteoblast-specific genes. Osf2/Cbfa1 gene inactivation in mice leads to failure of mesenchymal progenitor cells to

mutations in the Osf2/Cbfa1 gene cause Cleidocranial dysplasia in human and mice, a condition marked by result demonstrate that Osf2/Cbfa1 is an osteoblast-specific transcriptional

redundant with the function of other gene products during development.

The skeleton comprises two tissues: cartilage and bone, and three cell types: the chondrocyte in cartilage, the

studies of organogenesis in vertebrates, skeleton developmental biology comprises two fields of research

on the genetic control of skeleton patterning i.e. the presence of all the skeletal elements in a given animal and

numbers of cartilages and bones in all skeletal elements. Many genes encoding either growth factors or transcription

patterning of various skeletal elements. Importantly, these genes have not been shown to mediate, , the differentiation of the three specific cell types of the

understanding the genetic and molecular basis of cell differentiation in the chondrocytic, osteoblastic, and lineages. Multiple studies have shown that differentiation in all cell
pituitary cells (2), erythrocytes (3), hepatocytes (4),
(5) and adipocytes (6), is controlled by cell-specific

various lineages. On the assumption that this would also be the long-term search for OSFs) that could also act as differentiation factors during development.

OSTEOBLAST-SPECIFIC TRANSCRIPT OF CBFA1

To search for
the only osteoblast-specific gene, was used as a tool to identify osteoblast-specific cis-acting OSEs) (7). Two
was extensively characterized (7). Two groups (8,9) showed that the nuclear activity binding to OSE2, called Cbfa proteins

Cbfa proteins are the mammalian homologues of the Drosophila runt protein, a transcription factor implicated in

proteins, along with their *C. elegans*
counterparts, form a new family of transcription factors whose hallmark is a highly conserved 128 amino acid

date, three distinct Cbfa proteins encoded by different

of them, termed Cbfa1, has been initially proposed to be expressed in T lymphocytes only, although its expression in

encoding the conserved runt domain of the various Cbfa

library the only cDNA that hybridized encoded a modified Cbfa1 transcript, termed Osf2/Cbfa1. This Osf2/Cbfa1

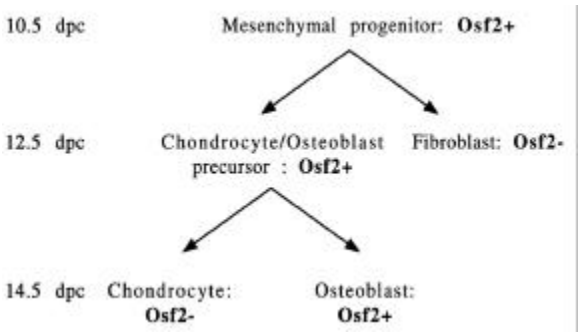

Figure 1. Schematic representation of Osf2/Cbfa1 expression during mouse skeletogenesis.

transcript is larger than the originally described Cbfa1 transcript (14). As presented below several molecular and developmental biology arguments indicate that Osf2/Cbfa1 is expressed at high level only in osteoblastic cells and that it is a transcriptional activator of osteoblast differentiation.

The first argument comes from the pattern of expression of Osf2/Cbfa1. During development Osf2/Cbfa1 is expressed at high levels in the cells of every mesenchymal condensations that will give rise to a skeletal element. Its expression is detectable as early as 11.5 day post coitum (dpc) by *in situ* hybridization and at about 10.5 dpc when analyzed by expression of a β-galactosidase reporter gene driven by the endogenous Cbfa1 promoter (15). At these stages, there is not yet differentiated skeletal cells or definite cartilage and bone tissues, indicating that Cbfa1 expression identifies a cell type that is a common progenitor for the chondrocytic lineage and the osteoblastic lineage (14). By contrast, at 14.5 dpc, throughout the rest of embryonic development and in postnatal life Osf2/Cbfa1 expression is restricted to cells of the osteoblastic lineage and absent in cells of the chondrocytic lineage suggesting the existence of a mechanism to turn off its expression in differentiated chondrocytes (see figure 1). The second argument came from molecular biology experiments showing that Osf2/Cbfa1 binds to the promoter of all the genes expressed predominantly in osteoblasts, such as alpha1(I) collagen, bone sialo protein, osteopontin and osteocalcin, and regulates positively their expression in tissue culture and in vivo (14). The third, and most compelling argument that Osf2/Cbfa1 acts as a differentiation factor in the osteoblastic lineage is that forced expression of Osf2/Cbfa1 into fibroblastic cell lines or into primary skin fibroblasts leads to the acquisition of an osteoblastic phenotype by these cells (14). All of these arguments provide compelling evidence that Osf2/Cbfa1 is a transcriptional activator of osteoblast differentiation.

To define whether Cbfa1 acts alone or whether other genes could replace it during skeleton development in vertebrates, genetic experiments are required. In skeleton biology there are only two systems in which to perform genetic analysis: mouse and man. Fortunately, experiments in both systems have led to the same conclusions that Osf2/Cbfa1 function is dominant and is not redundant with that of any other genes during development.

4. Osf2/Cbfa1 REGULATES OSTEOBLAST DEVELOPMENT FROM MESENCHYMAL PRECURSORS.

As discussed above, the Osf2/Cbfa1 gene product was identified biochemically as a transactivator of the Osteocalcin gene, suggesting that it plays a role in osteoblast-specific gene expression (14). This notion was underscored by *in situ* hybridization studies that revealed its expression pattern restricted to cells of the osteoblastic lineage (14). A formal genetic demonstration of the importance of Osf2/Cbfa1 in osteoblast differentiation and bone formation was provided by gene targeting experiments in which Cbfa1-deficient mice were generated (15,16). Remarkably, the skeletons of these mice showed a complete absence of osteogenesis and consequent lack of both endochondral and intramembranous ossification, with no alteration of skeletal patterning. This lack of bone resulted in early postpartum death from respiratory distress, presumably due to inadequate support for respiratory effort from a soft, cartilaginous rib cage, thus restricting analysis of these mice to embryonic development.

Detailed histological analysis of the skeleton of Cbfa1-/- mice revealed a lack of detectable osteoblast differentiation (15,16). This conclusion was consistent with the results of histochemical and in situ hybridization studies assessing the expression of a series of osteoblast marker genes. Cbfa1-/- mice showed weak or absent staining for alkaline phosphatase, an early marker of osteoblast differentiation, in perichondrial mesenchyme and the predicted epiphyseal areas, and a lack of expression of osteopontin and osteocalcin. Taken together, these data demonstrate that the Cbfa1 gene is essential for the differentiation of osteoblasts and thus for bone formation during the development of the skeleton.

5. PHENOTYPE OF Cbfa1+/- MICE

Although mice heterozygous for the Cbfa1 mutation were viable and outwardly healthy, more detailed analysis revealed specific defects in bone formation (15). The abnormalities in bone development were confined to those bones formed by intramembranous ossification directly from mesenchymal precursors. Endochondral ossification was unaffected in Cbfa1+/- mice. The most prominent defects observed in intramembranous ossification were hypoplasia of the clavicle and delayed ossification of the cranial bones resulting in opening of the fontanelles.

The specific defects in intramembranous ossification apparent in Cbfa1+/- mice are reminiscent of a human heritable disease of the skeleton called Cleidocranial dysplasia (CCD). This disease is an autosomal dominant disorder, and the main symptom of heterozygous patients is defective intramembranous ossification. Some years ago, a mouse model for CCD was generated by gamma irradiation, a form of mutagenesis that generates large chromosomal deletions (17). By analysis of mice heterozygous for the Ccd mouse on a C57BL/10 background and for the wildt ype gene on a variety of different strain backgrounds using a Cbfa1 cDNA probe, Otto *et al.* (15) demonstrated that the Cbfa1 gene was at

least partially deleted in Ccd mice. More detailed genomic analysis of these strains using microsatellite markers established that the Ccd and Cbfa1 loci mapped to a region on mouse chromosome 17 that was syntenic with human chromosome 6p21, the location of the human locus for CCD (15). These studies also defined the limits of the deletion at the Ccd locus and positioned the Cbfa1 locus within the middle of this deletion. This study provides compelling evidence that inactivation of the Cbfa1 gene generates the phenotype of the radiation-induced Ccd mouse mutant and also identifies CBFA1 as a candidate gene for human CCD.

6. THE GENETIC BASIS FOR THE HUMAN CCD SYNDROME

Two recent studies have provided direct evidence for mutation of the CBFA1 gene in CCD (18,19). A variety of mutations were detected in the CBFA1 gene from unrelated CCD patients, including large deletions, smaller deletions and insertions that introduced stop codons into the DNA-binding runt domain, missense mutations and an in-frame expression of a poly-alanine tract immediately N-terminal to the runt domain (19). Importantly, two missense mutations within the runt domain resulted in substitutions of highly conserved residues and abolished DNA-binding of CBFA1 to its target sequence (18). Taken together, these studies strongly suggest that mutations in the CBFA1 gene cause CCD and that heterozygous loss of function (i.e. haploinsufficiency) of CBFA1 is sufficient to produce the disease.

7. PERSPECTIVES

The discovery of Osf2/Cbfa1 as the major if not the only transcriptional activator of osteoblast differentiation indicates that the genetic mechanisms controlling osteoblast differentiation are much simpler than those governing differentiation of the myoblast, another mesenchymal cell type. This is in agreement with the fact that the osteoblast is a much more recent cell to have appeared during evolution and that, unlike what is the case in myoblasts, only one gene, Osteocalcin, has so far been shown to be osteoblast-specific. This discovery also demonstrates that skeletogenesis is controlled by the same gene, in the same way, in mouse and human. This provides the necessary tool to begin unraveling the link between chondrogenesis and osteogenesis and to understand the transcriptional mechanisms controlling bone growth, remodeling and repair.

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