The complexities of skeletal biology

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For a long time, the skeleton was seen as an amorphous tissue of little biological interest. But such a view ignored the large number of genetic and degenerative diseases affecting this organ. Over the past 15 years, molecular and genetic studies have modified our understanding of skeletal biology. By so doing this progress has affected our understanding of diseases and suggested in many instances new therapeutic opportunities.

The skeleton is an organ of unappreciated complexities. These complexities affect patterning and cell differentiation during development, and physiology and pathology postnatally. Its peculiar distribution in more than 220 locations in the human body and the extreme conservation of the function of genes affecting skeletal development and physiology between mouse and human explains why our ability to generate genetically modified mice has so profoundly transformed skeletal biology. Probably no other organ in vertebrate biology has benefited as much from our ability to generate, at will, mutant mouse strains.

The skeleton is made of two tissues (cartilage and bone), three cell types (chondrocytes, osteoblasts and osteoclasts) and more than 200 different skeletal elements spread out throughout the body. These properties raise questions about the location and shape of skeletal elements as well as allocation of cell lineage. Beyond development, the skeleton has to fulfil a series of functions about which we have little understanding in molecular terms, but which are of critical importance as they are often affected in common degenerative diseases. Among these functions one can cite the molecular mechanisms determining the extent of longitudinal growth, the spatial restriction of extracellular matrix mineralization to bone, and the maintenance of a constant bone mass.

Although still incomplete, our molecular understanding of skeletal physiology has advanced considerably by analysing skeletal cells themselves as well as how hormones and the nervous system affects their proliferation and function. As described in this timely issue, significant progress has been made in addressing the developmental biology, physiology and pathology of the skeleton. This progress in turn has raised a series of questions relative to each of the different aspects of skeletal biology.

Development of the skeleton

The aspect of skeletal biology that first received attention from molecular geneticists was developmental biology. One reason for this, and a key feature of the skeleton, is that the shape of skeletal elements varies greatly from one location to another in the body. Thus, the study of patterning of skeletal elements or of a group of skeletal elements such as limbs or the skull has been an object of study of embryologists even before the era of molecular biology. The earlier studies were performed mostly in chicken, whereas the more recent molecular studies were done using either chicken or mouse models[1–3]. Another factor favouring research on developmental biology of the skeleton in the past 15 years is the emerging notion that regulatory genes are often conserved throughout evolution. And even if the function of a given gene is not conserved from lower organisms to mice, the genetic pathways in which it resides very often is. In many instances this has accelerated the elucidation of the molecular bases for a given skeletal phenotype observed in mouse embryos.

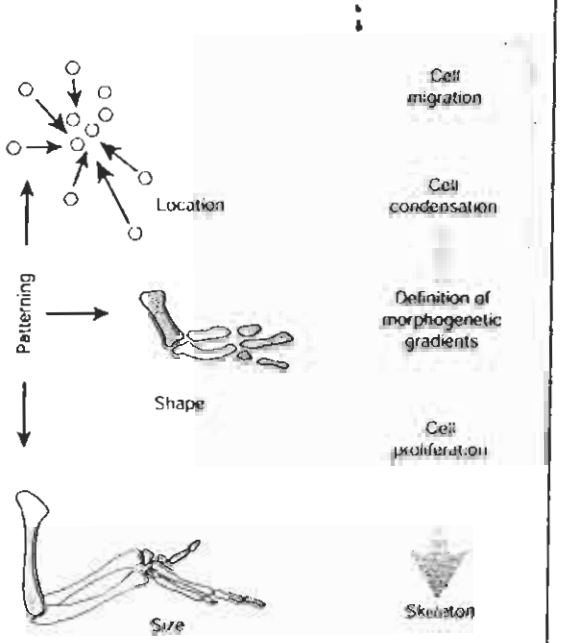

Figure 1 Key issues in skeletal development. The three main issues are: what triggers mesenchymal cells to migrate to a given location and to aggregate in the shape of a future skeletal element; how are morphogenetic gradients defined and regulated to determine location and shape of skeletal elements; and how do skeletal cells differentiate and proliferate?

The first event studied during development of the skeleton was patterning — the size, shape, number and arrangement of bones of the limbs and of the skull (see reviews in this issue by Mariani and Martin, page 319, and Helms and Schneider, page 326). The embryonic limb emerges from the lateral-plate mesoderm as a bud of mesenchymal cells covered by a layer of ectoderm. These anatomically distinct zones of the developing limb bud — the apical ectodermal ridge, the zone of polarizing activity and the progress zone — concur to establish over time the proximal–distal, anterior to posterior and dorsal-to-ventral axes of the limb. Many of the genes that are expressed in each area and that control limb patterning have been identified and their function characterized[4].

The principal debate in this field now centres on how cells in the progress zone acquire positional information. One model proposes that acquisition occurs progressively

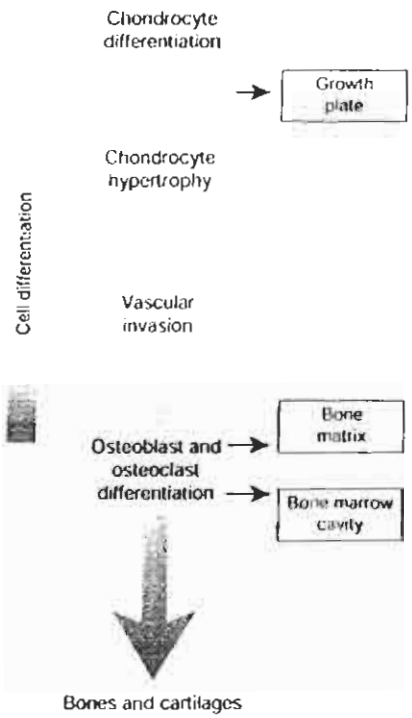

Figure 2 Each of the three specific cell types of the skeleton have particular spatial distributions. Chondrocytes are found in the growth plate and joints; subsequently they hypertrophy and die through apoptosis. Vascular invasion follows, bringing osteoblast progenitors from the bone collar into the centre of the future bone. A bone marrow then forms and osteoblasts favour osteoclast differentiation.

in a proximal-to-distal sequence[5]. A second, more recent model suggests that specification to form a given skeletal element in the developing limb occurs very early, at the time or even before the time that limb bud outgrowth is initiated[6]. This question is not fully resolved, and the outcome will undoubtedly require addressing in molecular terms another crucial yet almost unexplored question of skeletal development: what are the mechanisms leading mesenchymal cells to aggregate to form these condensations prefiguring each skeletal element at the onset of cell differentiation in the skeleton? Understanding this process will affect our understanding both of skeletal patterning and of chondrocyte differentiation.

The difficulties encountered in assembling coherent genetic pathways accounting for limb or skull patterning have been enormous and have not all been overcome. This explains why we still know so little about the genetic pathway regulating patterning of other skeletal elements such as ribs, hands and feet.

Skeletal cell differentiation

Once patterning of a given skeletal element is achieved, mesenchymal cells in this element differentiate in most cases into chondrocytes, the cell type specific of cartilage. The cartilaginous template is eventually replaced by bone containing osteoblasts and osteoclasts. This process of bone formation is called endochondral ossification. In some skeletal elements, mesenchymal cells differentiate directly into osteoblasts in a process called intramembranous ossification.

In the past ten years a combination of mouse and human genetics has provided a sophisticated understanding of how cells differentiate into chondrocyte, osteoblast (or bone-forming cells) and osteoclast (or bone-resorbing cells). Two of these lineages, the chondrocyte and the osteoblast, are of mesodermal origin and originate from a common progenitor cell called an osteochondroprogenitor[7]. Beyond this stage, differentiation along the chondrocyte lineage will eventually give rise to resting, proliferating and hypertrophic chondrocytes that will be organized in columns in the growth plate (ref. 7, and see review in this issue by Kronenberg, page 332). Many genes encoding growth factors or transcription factors contribute to the regulation of chondrocyte differentiation. Figures 1 and 2 summarize the key issues in skeletal development. Unresolved questions concerning chondrocyte differentiation relate mainly to the molecular determinants of the columnar organization of the growth plate and how it controls longitudinal growth of the skeleton.

In contrast to what is known of chondrogenesis, only a handful of genes have so far been shown to control osteoblast differentiation. What seems to emerge from this ongoing work is that different genes control osteoblast differentiation and proliferation. For instance, *Runx2/Cbfa1* and *Osterix* control osteoblast differentiation, whereas the LRP5 (low-density lipoprotein receptor-related protein 5) signalling pathway controls osteoblast proliferation. *Runx2* is necessary and sufficient for osteoblast differentiation, although there is an unexplained delay between its expression and the appearance of bona fide osteoblasts. Currently, the only gene known that might explain this long delay is *Osterix*[8], which seems to act downstream of *Runx2* (see reviews in this issue by Harada and Rodan, page 349, and Zelzer and Olsen, page 343). The relative paucity of transcription factors known to affect osteoblast differentiation contrasts with what is known regarding the role of these factors in other cell differentiation processes. Thus, it is likely that other transcription factors affecting osteoblast differentiation and proliferation, positively or negatively, remain to be identified. This may have important therapeutic consequences, as modulating the activity of these factors could lead to an increase in bone formation, the function that is affected in osteoporosis.

Knowledge of how osteoclast differentiation occurs is more detailed than that of osteoblast differentiation and has come from two independent lines of research that has converged over the past ten years on identical transcription factors. Gene-deletion experiments have identified a series of transcription factors — PU1, c-Fos, nuclear factor (NF) κB, NF-αTc and Mitf[9–13] — that control osteoclast differentiation. Another line of research identified a soluble tumour-necrosis factor receptor called osteoprotegerin as an inhibitor of osteoclast differentiation. This led rapidly to the identification of RANKL — the receptor activator of NF-κB (RANK) ligand — as an osteoclast differentiation factor binding to the RANK receptor in osteoclasts. The RANKL signalling pathway eventually leads to NF-κB and c-Fos activation, thus merging two aspects of osteoclast biology (see review in this issue by Boyle *et al.*, page 337). The fact that RANKL is produced by osteoblasts lends support to the concept that osteoblasts control osteoclast differentiation, not function. This, however, may be a simplistic view as RANKL is produced by many cells and is probably a circulating molecule. Nevertheless, identification of these genes has completely changed our understanding of bone resorption and may in the future affect the treatment of osteoporosis.

Skeletal physiology and pathology

Compared with our knowledge of other organs, our molecular understanding of many skeletal functions is limited. As a result, we still know little about the pathophysiology of degenerative diseases of the skeleton. One obvious difficulty in studying molecular physiology of the skeleton is that one cannot rely on conservation of regulatory pathways between *Caenorhabditis elegans*, *Drosophila* and humans, as skeleton was acquired late during evolution. Thus, answers to physiological questions have to be found using vertebrate models, mostly in mice.

The importance of studying skeletal physiology is illustrated by the incidence of degenerative diseases affecting functions such as the control and arrest of skeletal longitudinal growth, bone mineralization and the control of bone mass (Fig. 3). Osteoporosis, a disease of

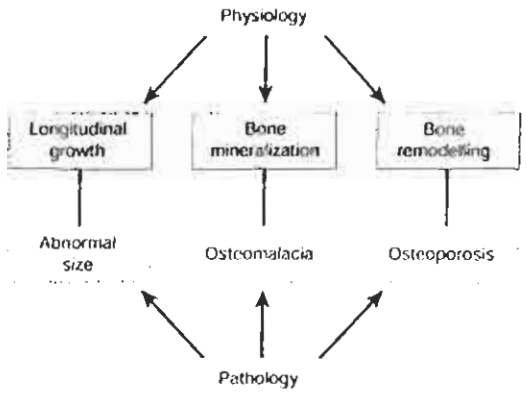

Figure 3 Unresolved issues in skeletal physiology. Three outstanding issues are: how does bone growth stop; why is mineralization restricted to bone; and how is bone mass maintained constant through bone remodelling? These impact on three types of disease in the skeleton: abnormal size, rickets and osteomalacia, and osteoporosis. In addition, understanding the molecular bases of intracellular matrix mineralization may affect our understanding of other disease such as atherosclerosis and osteoarthritis.

low bone mass, is the most frequent of all degenerative diseases and exerts a profound influence on research in skeletal biology. Furthermore, its incidence will undoubtedly increase with the ageing of the general population. At present, only symptomatic treatment is available in the form of inhibitors of bone resorption. Some of the genes affecting the control of bone mass also affect cell differentiation and proliferation during development, while other regulatory loops seem more specific to the adult skeleton. For instance, *Runx2* controls bone formation after birth by affecting osteoblast differentiation. Likewise, mutations in *LRP5*, which encodes a surface receptor, affect high bone mass in humans[14,15]. Given the ubiquitous expression of *LRP5*, the challenge will be to identify the ligand or signal-transducing molecules that account for the skeletal action of the LRP5 signalling pathway. Another molecule that functions during development and after birth is RANKL, which helps produce osteoclasts throughout life.

In contrast to pathways affecting skeletal differentiation, other pathways seem to affect only skeletal physiology. One of these critical regulatory loops is the negative regulation of bone resorption exerted by oestrogen, which explains the increase in bone resorption and the bone loss observed following gonadal failure at menopause. At present we do not have a clear understanding of how oestrogen regulates bone resorption and this is probably one of the most important challenges remaining in the field of skeletal physiology. It is possible that the hormone may control osteoclast function locally through a nonspecific mode of action[16,17].

Recent research has identified a novel means of regulation of bone mass that includes a hypothalamic relay. As discussed by Harada and Rodan on page 349, leptin is a powerful inhibitor of bone formation. Leptin acts by binding to its signal transducing receptors present on hypothalamic neurons, with the sympathetic nervous system as its peripheral mediator. Leptin regulation of bone formation is dominant over the influence of gonads on bone resorption[18,19]. Indeed, animals deprived of leptin signalling have non functional gonads, and bone resorption increases as expected owing to a lack of oestrogen. But if leptin signalling is removed in this gonad-free animal, what is observed in terms of bone pathophysiology is the consequence of the absence of leptin signaling (that is, high bone mass), not the consequence of the gonadal failure. This regulation is also important for therapeutic reasons, as β adrenergic antagonists can increase bone mass and prevent osteoporosis in ovariectomized mice. This aspect of the regulation of bone mass has now entered the field of clinical investigation.

Lastly, how mechanical stress affects bone mass is another important aspect of skeletal physiology[20], and its study is taking full advantage of the many mutant mouse strains available. We have no real clues of how bones sense and transduce mechanical forces and how this may affect gene expression.

Cartilage, especially articular cartilage biology, is often ignored in reviews about skeleton biology. This is partly because we still know little about joint formation (see review in this issue by Mariani and Martin, page 319) and even less about the physiopathology of osteoarthritis, the most frequent degenerative disease of the joints. Besides osteoarthritis, autoimmune disease represents another category of joint disease, with rheumatoid arthritis being the most common example of this disease type. We have no certainty about the nature of the molecular mechanisms leading to the development of a rheumatoid arthritis (see review in this issue by Firestein, page 356). As a result, treatments are often empirical. Better understanding of the role played by cytokines involved in inflammatory processes should lead eventually to a clearer understanding of these diseases (as illustrated, for example, by studies of the role of RANKL in the development of rheumatoid arthritis). This area of skeletal biology, which lies at the frontier between bone biology and immunology, will expand rapidly as our knowledge of the molecular bases of inflammation improves.

Owing to space constraints, this Insight does not cover all aspects of skeletal biology and pathology. However, by formalizing the various questions still confronting developmental biologists, molecular physiologists and clinicians it serves to define the challenge ahead in each field. A trend that is apparent in the accompanying reviews and that should become more evident in the future is that progress in skeletal biology is having an increasing effect directly on the management of cartilage and bone diseases. □

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