Bone matrix to growth factors: location, location, location

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The demonstration that fibrillin-1 mutations perturb transforming growth factor (TGF)–β bioavailability/signaling in Marfan syndrome (MFS) changed the view of the extracellular matrix as a passive structural support to a dynamic modulator of cell behavior. In this issue, Nistala et al. (2010. *J. Cell Biol.* doi: 10.1083/jcb.201003089) advance this concept by demonstrating how fibrillin-1 and -2 regulate TGF-β and bone morphogenetic protein (BMP) action during osteoblast maturation.

The ECM consists primarily of collagenous and microfibrillar elastic polymers, their associated adaptor molecules, and hydrophilic proteoglycans that together assemble into complex multiprotein structures (Hynes, 2009; Ramirez and Rifkin, 2009). These extracellular macroaggregates of similar composition but distinct tissue-specific morphologies provide cell support, organize individual tissues, impart structural integrity, and orchestrate cell behavior through interactions with a variety of cell surface receptors. Recently, it has become apparent that the ECM is also a dynamic modulator of growth factor bioavailability and signaling (Ramirez and Rifkin, 2003, 2009; Hynes, 2009).

Fibrillins are major components of the ECM and form either microfibrils or, together with tropoelastin, elastic assemblies (Ramirez and Sakai, 2010). Additionally, fibulins and latent TGF-β–binding proteins (LTBPs), molecules structurally related to fibrillins, are crucial elements of nascent microfibril assemblies. The instructive function of fibrillin assemblies is mediated primarily by bound TGF-β and BMP (Ramirez and Rifkin, 2009). TGF-β is usually released from cells as a large latent complex (LLC) consisting of TGF-β, the TGF-β propeptide (latency-associated protein), and an LTBP (Annes et al., 2003; Rifkin, 2005). The LLC is targeted to microfibrils by noncovalent interaction between specific domains of fibrillin-1 and -2 and LTBP. The release and/or activation of TGF-β from ECM-sequestered LLC enables ligand–receptor binding (Annes et al., 2003; Kang et al., 2009). BMPs, which are members of the TGF-β family and are intimately involved in connective tissue modeling and remodeling, also assemble noncovalently with fibrillins (Sengle et al., 2008a), but BMP–propeptide association does not prevent the ligand from interacting with its receptors (Sengle et al., 2008b). Interaction with fibrillins targets BMPs to the ECM, from which they are subsequently released, and probably protects BMPs from neutralization by soluble inhibitors.

Insight into the function of fibrillin-1 and -2 has derived from the study of two genetic diseases, MFS and congenital contractural arachnodactyly (CCA). MFS is caused by mutations in fibrillin-1 and is associated with both structural and regulatory deficits of microfibrillar assemblies in multiple tissues (Judge and Dietz, 2008). Fibrillin-1 mutations impair tissue integrity by perturbing formation of microfibrillar assemblies, thereby promoting improper LLC sequestration and, consequently, indiscriminant activation of latent TGF-β (Neptune et al., 2003; Habashi et al., 2006). This concept that fibrillin-1 modulates the specificity of growth factor action also extends to fibrillin-2. Mutations in fibrillin-2 cause CCA, a condition akin to but clinically distinct from MFS (Putnam et al., 1995). In addition to recapitulating the human phenotype, *Fbn2*$^{-/-}$ mice display a bone patterning defect caused by reduced BMP signaling in the interdigital mesenchyme (Arteaga-Solis et al., 2001). The unique phenotypes of MFS and CCA and their distinct TGF-β and BMP signaling abnormalities support the notion that the fibrillins have discrete regulatory properties in spite of both being part of the same ECM macroaggregate and both binding TGF-β and BMP.

In this issue, Nistala et al. identify fibrillin microfibrils as extrinsic factors that impart regulatory specificity to TGF-β and BMP signals. The authors addressed the question of how fibrillin-1 or -2 regulates TGF-β and BMP signaling during osteogenic differentiation, as both MFS and CCA display decreased bone mineral density. Both cytokines are abundant in the bone matrix but are known to exert opposing actions on osteoblast maturation (Fig.1 A). Using *Fbn2*-null mice and osteoblasts, the authors show that bone formation and osteoblast maturation are impaired. Furthermore, cultured osteoblasts from these mice form fewer mineralized nodules because of decreased levels of both the transcription factor osterix and osterix-driven stimulation of collagen I deposition. BMP restores osterix and collagen expression and, consequently, ECM mineralization in the mutant cultures. Latent TGF-β is inappropriately activated by *Fbn2*$^{-/-}$ osteoblasts, and the inhibition of TGF-β signaling enhances maturation of the

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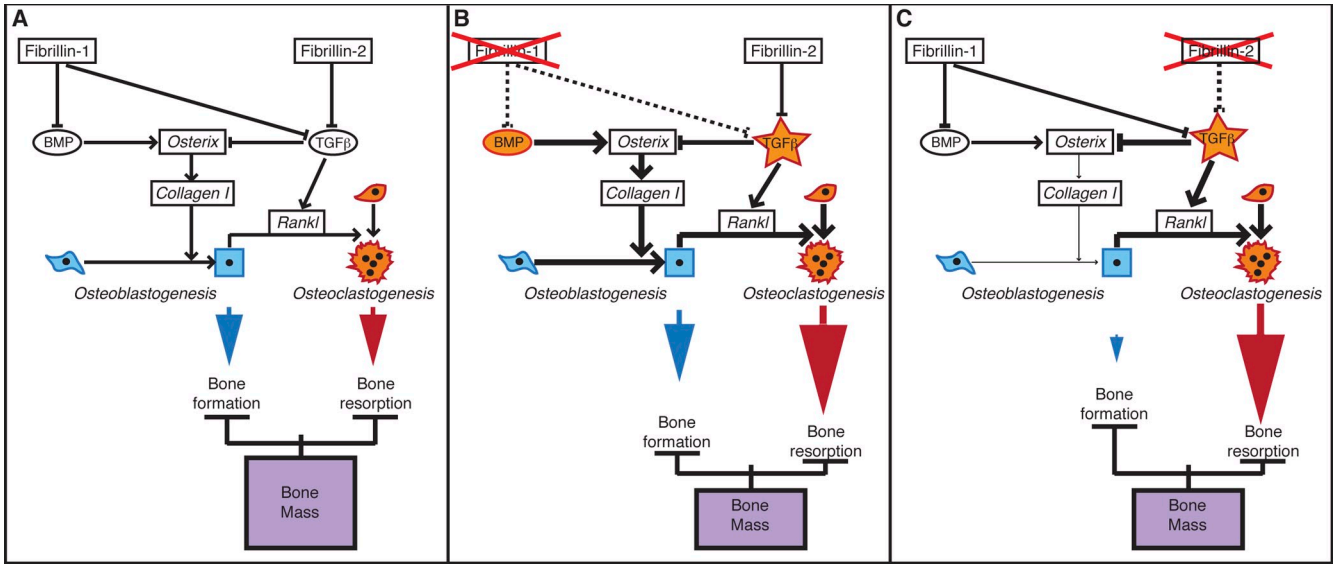

Figure 1. **Fibrillin control of bone metabolism.** (A) Fibrillin-1 and -2 deposition in bone matrix controls active TGF-β and BMP levels, yielding appropriate osteoblastogenesis and osteoblast-supported osteoclastogenesis. (B) Fibrillin-1 loss heightens active TGF-β and free BMP concentrations, mildly accelerating osteoblast maturation. Increased TGF-β signaling also stimulates osteoblast production of RANKL, thereby increasing matrix degradation by osteoclasts. (C) Fibrillin-2 loss maintains normal BMP levels and raises active TGF-β levels, yielding decreased osteoblast maturation and increased osteoblast-supported osteoclastogenesis. Thus, bone anabolism and catabolism are both integral parts of fibrillin-modulated TGF-β and BMP signaling.

mutant osteoblasts (Fig. 1 C). These findings demonstrate that fibrillin-2 microfibrils normally restrict TGF-β signaling during osteoblast maturation.

In contrast, *Fbn1*$^{-/-}$ osteoblasts express more osterix and collagen and mature more rapidly than control cells (Fig. 1 B). As with *Fbn2*$^{-/-}$ cells, latent TGF-β is inappropriately activated in *Fbn1*$^{-/-}$ cultures, which additionally display augmented BMP signaling associated with reduced ligand sequestration in the ECM. These findings suggest that the elevation of osteoinductive BMP signals in *Fbn1*$^{-/-}$ osteoblast cultures overrides the inhibitory action of improper TGF-β activity. As such, the results identify fibrillin-1 microfibrils as negative regulators of TGF-β and BMP bioavailability in the forming bone.

These observations support several important conclusions. First, the sum rather than the relative amounts of TGF-β and BMP signaling determines the rate of bone formation. Both *Fbn1*- and *Fbn2*-null cells display enhanced signaling by TGF-β, an inhibitor of osteoblast maturation, but the level of BMP, a potent osteoinductive factor, modifies the final outcome. Second, microfibril association with TGF-β and BMP complexes operates both positively by concentrating the signaling molecules (Arteaga-Solis et al., 2001) or negatively by restricting the activity of the protein (Nistala et al., 2010b). Third, cytokine-directed changes in osteoblasts affect their coupling with osteoclast-degradative activities. These same investigators have found that increased latent TGF-β activation is responsible for enhanced osteoblast-supported osteoclastogenesis by *Fbn1*$^{-/-}$ and *Fbn2*$^{-/-}$ cells through the elevation of receptor activator of NF-κB ligand (RANKL) production (Fig. 1; Nistala et al., 2010a). Thus, fibrillin-1 and -2 microfibrils control both anabolic and catabolic phases of bone homeostasis and perhaps lineage determination of marrow stem cells. Finally, organ-specific manifestations of MFS and CCA suggest that microfibrils function in a tissue-specific rather than in a universally predictable manner. The mechanistic basis for this remains unknown.

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