

Cellular Mechanotransduction in Bone: Moving Towards a Molecular Mechanism

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INTRODUCTION: Bone cells occupy fluid filled voids (lacunae) in the mineralized matrix, interconnected by small tubes (canaliculi). As the bone matrix is cyclically loaded, fluid flows in the lacunar-canalicular network from regions of high matrix strain to low matrix strain and back in an oscillatory fashion. We have shown that this dynamic fluid flow is a potent signal for bone cell¹ regulations and that there appears to be important differences between the response to oscillatory flow and flows that do not incorporate a reversal of flow direction. We have characterized the biochemical signaling pathway activated by oscillatory fluid flow as involving IP3 mediated calcium signaling and MAP kinase signaling leading to osteogenic gene regulation.² Finally, we found that PGE2 release (critical to bone adaptation to mechanical loading) occurs in response to oscillatory fluid flow independent of intracellular calcium signaling, but does involve membrane associated extracellular proteoglycans³ suggesting that at least two cellular signaling pathways are activated by oscillatory fluid flow. Currently our work is focused on uncovering the molecular mechanism by which these signaling pathways are activated.

DYNAMIC VS STEADY FLOW AND THE CYTOSKELETON: Interestingly, we have found that in contrast to unidirectional flow, the signaling pathway activated by oscillatory fluid flow does not involve the stretch activated membrane calcium channel. We speculate that one reason for this might be the viscoelastic mechanical nature of cells. Chronic unidirectional flow is likely to result in much larger cellular deformations than short-term reversing flow, thereby activating different cellular signaling pathways, perhaps associated with fracture healing. We have also recently found that while an hour of unidirectional flow leads to the formation of actin stress fibers in the cytoskeletons of bone cells, this does not occur with reversing oscillatory flow supporting the view that cellular viscoelasticity may be an important consideration for mechanotransduction.

STEM CELLS: Mechanical disuse is known to result in decreased numbers of bone forming cells. Thus, loading induced fluid flow may be an important regulator of osteoprogenitors as well as mature bone cells. In our recent work, we have found that oscillatory fluid flow does indeed increase the proliferation rate of bone marrow stromal cultures as well as the expression of markers of osteogenic differentiation.⁴

PRIMARY CILIA AS MECHANOSENSORS: Primary cilia are non-motile flagella-like structures found in most mammalian cell types. Although their existence has been known for over a century, they have had no clear function suggesting that they may be vestigial. However, recently they have been found to be involved in polycystic kidney disease and may act as sensors of fluid flow. This has prompted us to examine the potential role of the primary cilium as a mechanotransducer in bone. In our preliminary studies we have verified the existence of primary cilia in bone cells *in vivo*. Furthermore, we have found primary cilia in MC3T3-E1 osteoblastic and MLO-Y4 osteocytic cell lines and found that removal of the cilia results in reduced flow sensitivity in the cells. We have recently created a bone-specific conditional knock-out of the cilia protein Kif3A and are examining the osteogenic response of these mice to loading.

REFERENCES: ¹C Jacobs et al (1998) *J Biomech* **31**:969-976. ²J You et al (2001) *J Biol Chem* **276**:13365-13371. ³G Reilly et al (2003) *Biorheology*, **40**:591-603. ⁴Y Li et al (2004) *J Orthop Res* **22**:1283-1289.

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