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A Model for Mechanotransduction in Bone Cells: The Load-Bearing Mechanosomes

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Abstract The skeleton's response to mechanical force, or load, has significance to space travel, the treatment of osteoporosis, and orthodontic appliances. How bone senses and processes load remains largely unknown. The cellular basis of mechanotransduction, however, likely involves the integration of diffusion-controlled signaling pathways with a solid-state scaffold linking the cell membrane to the genes. Here, we integrate various concepts from models of connective membrane skeleton proteins, cellular tensegrity, and nuclear matrix architectural transcription factors, to describe how a load-induced deformation of bone activates a change in the skeletal genetic program. We propose that mechanical information is relayed from the bone to the gene in part by a succession of deformations, changes in conformations, and translocations. The load-induced deformation of bone is converted into the deformation of the sensor cell membrane. This, in turn, drives conformational changes in membrane proteins of which some are linked to a solid-state signaling scaffold that releases protein complexes capable of carrying mechanical information, ''*mechanosomes*'', into the nucleus. These mechanosomes translate this information into changes in the geometry of the 5′ regulatory region of target gene DNA altering gene activity; bending bone ultimately bends genes. We identify specific candidate proteins fitting the profile of load-signaling mechanosomes. J. Cell. Biochem. 88: 104–112, 2003.

Key words: adhesion complex; architectural transcription factors; connective membrane skeleton proteins; nuclear matrix; tissue matrix; stretch-activated channels

SKELETON'S RESPONSE TO GRAVITY: THE MOLECULAR MECHANICS OF INFORMATION TRANSFER AND THE PHYSICAL LINK BETWEEN THE BONE AND ITS GENES

A Principal Question Concerning the Skeleton's Response to Load Is the Means of Information Transfer and Processing

The skeleton is exquisitely tuned for sensing and responding to mechanical forces, which bears considerable significance to clinical medicine [Turner, 2000 and references therein]. The cellular basis for bone's sensitivity to load is not understood and several key issues need to be addressed before a comprehensive theory of bone adaptation can be proposed. Foremost is how mechanical information is transferred from the bone to the target gene and rendered into changes in gene activity. We address this question by proposing a mechanism that identifies specific candidate proteins that carry mechanical information from the bone cell membrane to the load-responsive genes. The key and novel aspect of this model involves the load-induced formation of *mechanosomes*, multiprotein complexes comprised of focal adhesion-associated or adherens junction-associated proteins. The mechanosome contains a nucleocytoplasmic shuttling DNA-binding protein that typically moves between the adhesion complexes and the nucleus and one or more adaptor proteins

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acquired from the focal adhesions or adherens junctions. Mechanosomes translocate to the nucleus and there effect changes in gene transcription through the concerted action of the DNA-binding protein, the associated adaptor proteins, and other local trans-acting factors associated with the target gene. We refer to them as *load-bearing* mechanosomes to indicate their capacity for carrying mechanical information from the adhesion complex to the target gene and not in the sense of providing structural support.

Physical Links Between the Bone Extracellular Matrix, the Bone Cell, and the Bone Gene Provides Clues to the Molecular Mechanics of Load

The concept of the mechanosome derives from the question concerning how observed load-induced changes in bone cell shape and adhesion integrate with diffusion-controlled cellular signaling pathways capable of effecting changes in gene transcription. To provide the context for our mechanotransduction model, we briefly review recent information on cell structure, the mobility of selected cell adhesion proteins, and the architecture of transcription.

Bending bone deforms bone cells. The compression and tension forces acting on bone through the application of mechanical load not only distort the cells but also exposes them to shear stress via the movement of interstitial fluid through the canalicular spaces [Turner and Pavalko, 1998]. Flow-induced electric potentials are also generated through streaming effects [Chakkalakal, 1989]. Ultimately, the bone cell membrane is further deformed in response to these mechanical and electrical forces. The osteocyte is considered a likely candidate for the primary mechanical sensor cell in bone and the osteoblast the effector cell [Turner and Pavalko, 1998], although both types of cells are sensitive to mechanical stimuli. In addition, osteoblasts respond to the released paracrine signals of the osteocyte putatively including prostaglandins and nitric oxide [Chow, 2000]. In this discussion, we will not distinguish between a sensor and effector cell in the detection, processing, and response to mechanical signals since these molecular pathways are likely operative in both osteocytes and osteoblasts.

Changes in cell shape and adhesion are functionally and physically coupled to the genes by the tissue matrix [Bidwell et al., 1998, and references therein]. The cell tissue matrix is defined as the inter-linking proteins of the extracelluar matrix (ECM), the proteins of the focal and cell adhesion complexes, the cytoskeleton, and the nucleoskeleton or nuclear matrix [Bidwell et al., 1998 and references therein; Fig. 1]. The integrins of the focal adhesion complex are heterodimeric transmembrane proteins that link the extracellular matrix with the cytoskeleton through several actin-associated proteins including α-actinin, vinculin, talin, and tensin [Turner and Pavalko, 1998]. The N-cadherins, expressed in bone cell adherens junctions, are single chain transmembrane glycoproteins that interlink the cytoskeletons of neighboring cells via the calcium-dependent homophilic interactions between the cadherin extracellular domains [Marie, 2002]. The cytoplasmic domains of the cadherins bind to the submembranal plaque proteins β- or γ-catenin which are in turn linked to the cytoskeleton via α-catenin [Conacci-Sorrell et al., 2002]. Changes in cell shape and adhesion, like those observed with the application of load or shear stress, derive from a change in the organization of the tissue matrix. This involves alterations in the links between the cytoskeleton and adhesion proteins, the links between the integrins and the ECM, and/or the links between cadherin proteins of neighboring cells. These couplings regulate the transcription of genes, some of which are load-responsive, in endothelial cells, osteoblasts, and fibroblasts although the underlying mechanisms are not known [Dhawan et al., 1991; McDougall et al., 1996; Takahashi et al., 1997; Damsky, 1999; Aplin and Juliano, 2001; Marie, 2002]. We interpret this as indicative that load-induced changes in bone cell shape and adhesion are functionally significant.

Connective membrane skeleton (CMS) proteins are signal transducers that associate with the cell adhesion complexes and act to organize the cytoskeleton and initiate biochemical cascades in response to extracellular cues [Lelièvre and Bissell, 1998]. As signal transducers these molecules transfer information from the membrane to the cytoskeleton, and, in some cases, to the nucleus [Lelièvre and Bissell, 1998]. The number of identified CMS proteins is extensive and expanding. Some of the CMS proteins that interact with the focal adhesion complex include $p130^{cas}$, zyxin, moesin, paxillin, fimbrin, VASP, and the actin-associated proteins [Lelièvre and Bissell, 1998]. The adherens junctions associate with zyxin, vinculin, the catenin molecules,

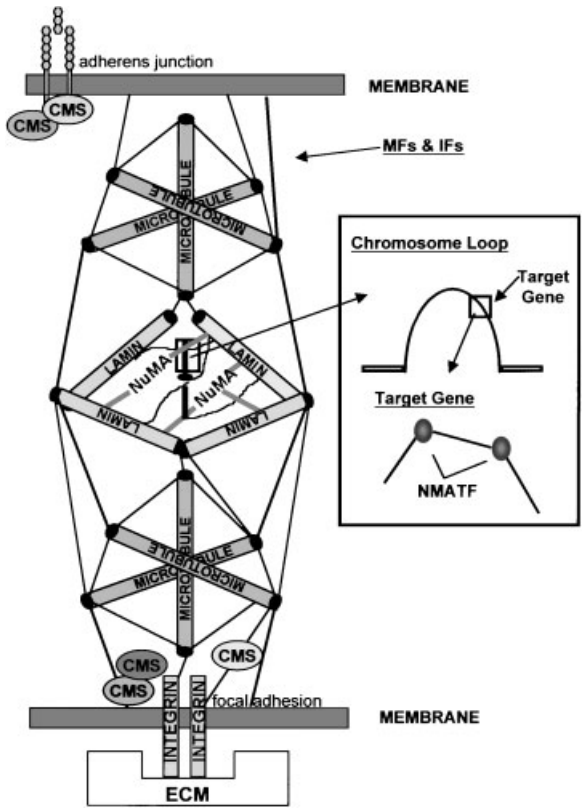

Fig. 1. Schematic representation of the bone cell tissue matrix. The tissue matrix consists of the interlinking proteins of the extracellular matrix (ECM), the focal adhesion proteins (represented as integrins), the adherens junctions, the cytoskeleton (microtubules, actin microfilaments (MF), intermediate filaments (IF)), and the nuclear matrix (represented with the lamin proteins and NuMA). The organization of the tissue matrix is depicted in a tensegrity-like arrangement of the cytoskeletal and nucleoskeletal scaffolds [Ingber, 1997]. The connective membrane skeleton (CMS) proteins associate with the adhesion complexes. This cellular substructure culminates with nuclear matrix architectural transcription factors (NMATF) that couple target gene promoter geometry to cell shape and adhesion.

symplekin, and radixin, to name a few [Lelièvre and Bissell, 1998]. Additionally, there are numerous classical signal transduction molecules that can localize at or in the vicinity of these adhesion complexes [Vuori, 1998]. These include the Crk family adaptor proteins, Src tyrosine kinases, focal adhesion kinase (FAK), MAP kinase, protein kinase C (PKC) and the small G proteins Ras, Rho and Rac, which take part in diverse cellular processes including cytoskeleton and microtubule reorganization [Vuori, 1998; Matozaki et al., 2000].

The nuclear matrix is the nuclear component of the tissue matrix [Bidwell et al., 1998 and references therein]. This unique biochemical fraction is operationally defined as the proteinaceous substructure that resists nuclease digestion and high salt extraction [Bidwell et al., 1998 and references therein]. One interpretation of the low solubility of these proteins is that some of them, e.g., NuMA and the lamins, form a nuclear scaffold in the living interphase cell upon which active genes, transcription factors, and splicing molecules assemble [Stein et al., 2000]. Other nuclear matrix proteins themselves act as trans-acting proteins, splicing factors, and agents of DNA replication [Stein et al., 2000]. Nuclear matrix transcription factors recently identified as significant to osteoblast gene expression are Nmp4/CIZ (nuclear matrix protein 4/cas interacting zinc finger protein), Cbfa1/Runx2, and YY1 [Bidwell et al., 2001 and references therein]. Furthermore, Nmp4/CIZ may act as an architectural transcription factor, a protein that regulates gene expression by bending DNA and thus altering the interactions between other classical trans-acting proteins [Bidwell et al., 2001 and references therein]. The association of Nmp4/CIZ with the nuclear matrix may provide a molecular pathway for translating the physical changes in tissue matrix organization and adhesion into changes in promoter geometry and ultimately gene expression [Bidwell et al., 1998; Bidwell et al., 2001].

A MODEL FOR MECHANOTRANSDUCTION IN BONE: THE LOAD-BEARING PROTEINS OF THE OSTEOCYTE/OSTEOBLAST

The Dectection of the Mechanical Signal: Deformation of the Bone Cell Membrane Induces Conformational Changes in Mechano-Sensitive Channels

The load-induced deformation of the bone cell membrane opens the stretch-activated cation channel (SA-CAT) (Fig. 2). The activation of the SA-CAT results in an immediate increase in cytosolic calcium ($[Ca^{2+}]_i$) via a release of internal stores and entry through the SA-CAT and/or an L-type, voltage-sensitive calcium channel (VSCC). This mobilization of $[Ca^{2+}]_i$ initiates numerous kinase cascades converting the mechanical forces that induce conformational changes in the channel into a biochemical signal. In the present model, the $[Ca^{2+}]_i$-induced mobilization of kinases drives the activation of selected early response genes and, via kinases such as c-Src, ultimately play a role in the elaboration of signaling complexes that

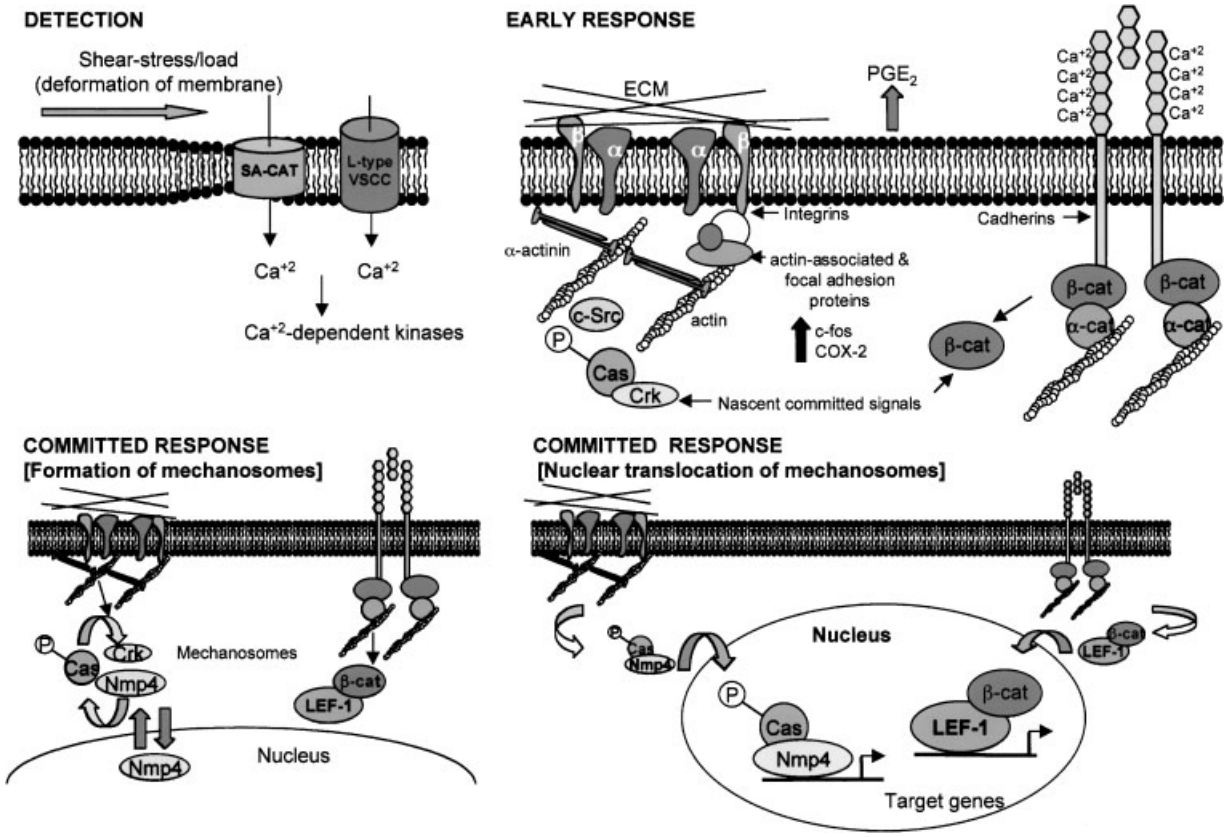

Fig. 2. Mechanotransduction in bone cells. Detection of the mechanical signal is primarily via the response of the stretch-activated cation channel (SA-CAT) and the L-type voltage senstive calcium channel (VSCC) to the deformation of the cell membrane. Early response events include the transfer of mechanical energy from the tissue matrix to adhesion proteins including the integrins and cadherins. This energy is converted to chemical energy via the c-Src-mediated phosphorylation of $p130^{cas}$ and its complexation with the adaptor protein Crk and the translocation of β-catenin to the cytoplasm, as selected examples. The upregulation of c-fos and COX-2 expression and release of PGE_2 also occur at this time. The bone cell is committed to the mechanical response with the formation of mechanosomes, shown here with the complexation of a nucleocytoplasmic shuttling isoform of Nmp4/CIZ (Nmp4) with $p130^{cas}$ and the association of β-catenin with LEF-1. The mechanosomes translocate to the nucleus to effect changes in target gene expression. Both Nmp4/CIZ and LEF-1 mediate changes in promoter geometry by bending or looping target gene DNA. The proteins $p130^{cas}$ and β-catenin modulate the action of Nmp4/CIZ and LEF-1, respectively, through allosteric control.

carry mechanical information to the committed response target genes.

Although numerous studies indicate the presence of a mechanically sensitive ion channel in both osteocytes and osteoblast-like cell lines [Kizer et al., 1997; Miyauchi et al., 2000; Ryder and Duncan, 2001], its molecular identity remains elusive. The α_{1C} pore-forming subunit of the L-type VSCC appears to have a significant role in mediating Ca^{2+} influx in mechanically activated cells [Miyauchi et al., 2000; Ryder and Duncan, 2001]. We have recently shown that inhibition of the VSCC channel significantly attenuates load-induced bone formation in rats [Li et al., in press]. Whether this acts as the SA-CAT or that the SA-CAT is an independent entity which is regulated by the L-type VSCC is not clear [Miyauchi et al., 2000; Ryder and Duncan, 2001].

The epithelial sodium channel (ENaC) shares significant homology with the "touch sensitive" genes identified in *Caenorhabditis elegans* [Travernarakis and Driscoll, 1997]. In mammals, these ENaC channels mediate sodium reabsorption in the distal part of the renal tubule, the ducts of several exocrine glands, the airway epithelium of the lung, and in the distal colon [Rossier, 1998]. The α-ENaC subunit is expressed in UMR 106-01 rat osteosarcoma cells and primary osteoblasts in culture [Kizer et al., 1997]. However, the expression of the α-ENaC subunit in either planar lipid bilayers, a null fibroblast for SA-CAT activity, or in the Xenopus oocyte expression system

has yielded contradictory results concerning this channel's mechanosensitivity in any cell type [Rossier, 1998]. Data from a recent study suggest that ATP, acting through a phospholipase C-dependent purinergic pathway, masks stretch-induced ENaC activity [Ma et al., 2002]. Because mechanical stretch can induce cellular ATP release [Sauer et al., 2000] the failure to take into account the purinergic regulation of the ENaC channel may have contributed to the apparent differences in results of the earlier studies [Ma et al., 2002].

The Immediate Early Response to the Mechanical Signal: The Load-Induced Strain on the Tissue Matrix Is Focused to the Adhesion Complexes, Selected CMS Molecules, to the Nuclear Matrix, and to the Early Response Target Genes

The load-induced strain on the bone cell tissue matrix focuses changes in lipid membrane tension to the adhesion complexes and induces a recruitment and re-organization of both the integrin and cadherin proteins (Fig. 2). This mechanically-induced remodeling of the focal adhesions and adherens junctions involves the activation of numerous tissue matrix-associated CMS proteins and their organization into signaling complexes. The phosphorylation of $p130^{cas}$, through a c-Src associated pathway, and its resultant complexation with the adaptor molecules such as Crk is an early step in the translation of focal adhesion remodeling into a signaling complex. Additional CMS activation steps in this model include changes in α-actinin association with the cytoskeleton and the translocation of β-catenin from the adherens junction into the osteoblast cytoplasm. The concomitant mobilization of FAK, MAP kinase and the small G protein signaling molecules Ras, Rac, and Rho mediate the early bone cell cytoskeletal response to load and contribute to the elaboration of the nascent signaling complexes as well as integrating the mechanical signal with the G protein-based response limbs.

In the present model, this early, but non-committed response of the bone cell to load also includes an upregulation of the transcription factor *c-fos*, a regulator of numerous genes that support the osteoblast phenotype. There is a concomitant increase in the expression cyclooxygenase-2 (COX-2), an enzyme that is required for prostaglandin production. Another early response to fluid shear stress is the release of prostaglandin E_2 (PGE_2) and prostacyclin (PGI_2) within minutes of a cell receiving the incoming mechanical signal. The release of prostaglandins into the extracellular environment appears to occur prior to the upregulation of COX-2 protein expression and can function as an autocrine and paracrine signal activating the effector cells and sensitizing the sensor cells. The load-induced strain on the tissue matrix positions nuclear matrix architectural transcription factors, e.g., selected Nmp4/CIZ isoforms, along the 5′ regulatory regions of target genes to be altered once the committed response is initiated.

Recent data provide evidence for some of the proposed load-induced early events occurring at the osteoblast cell adhesion complexes. Our own studies have demonstrated that fluid flow on bone cells induced a recruitment of integrins to focal adhesions and a reorganization of the osteoblast actin filaments into bundles called stress fibers [Pavalko et al., 1998]. Shear stress also mediated the upregulation of COX-2, c-fos, and the release of PGE_2 [Pavalko et al., 1998; Cheng et al., 2001]. Additionally, the injection of a truncated α-actinin fragment into these cells inhibited the rapid upregulation of COX-2 and c-fos normally seen after just 1 hr of fluid shear stress [Pavalko et al., 1998]. We have also shown that the response of COX-2 and c-fos to shear stress is dependent on the mobilization of $[Ca^{2+}]_i$ [Chen et al., 2000]. Shear stress also induced the translocation of β-catenin to the osteoblast cytoplasm and ultimately the nucleus [Norvell and Pavalko, 2002]. Under similar experimental conditions, the exposure of endothelial cells to shear stress has been shown to induce the activation of c-Src, the phosphorylation of $p130^{cas}$ and its complexation with Crk, all within 5 min of exposure [Okuda et al., 2001]. Although it is presently unknown whether $p130^{cas}$ is responsive to shear stress in osteoblasts, this protein is phosphorylated in MC3T3-E1 osteoblast-like cells upon exposure to certain G-protein activators, such as fluoroaluminate and lysophosphatic acid, via a c-Src-dependent pathway [Freitas et al., 2002]. The culture of these cells on a type I collagen substrate, as compared to plastic, also upregulates the basal level of tyrosine phosphorylated $p130^{cas}$ [Freitas et al., 2002].

Our proposed mechanism for the bone cell response to load incorporates a concept employed in tensegrity, a model for the general architectural basis of cellular mechanotransduction [Ingber, 1997]. This is the notion that the

strain-bearing tissue matrix transfers focused mechanical energy to molecules associated with this macromolecular scaffold and converts this to chemical energy through stress-induced changes in molecular conformation [Ingber, 1997]. In turn these changes in molecular shape drive alterations in the phosphorylation of numerous proteins and the bending of DNA through nuclear matrix architectural transcription factors [Bidwell et al., 1998; Ingber, 1997]. There is growing experimental evidence for this solid-state or hard-wired cellular signaling pathway. Applying a mechanical tug to the integrin receptors on the surface of endothelial cells resulted in the reorientation of cytoskeletal filaments, the distortion of the nucleus, and redistribution of the nucleoli along the axis of the applied tension field [Maniotis et al., 1997]. Similarly, the twisting and occupation of these integrin receptors activated the cAMP-PKA signaling pathway driving gene transcription through the phosphorylation of the transcription factor CREB [Meyer et al., 2000]. Using an in vitro model of human mammary epithelial cell acinar morphogenesis, a dynamic interaction between the extracellular matrix, nuclear matrix organization, and tissue phenotype was demonstrated [Lelièvre et al., 1998]. In these experiments, the subnuclear localization of specific nuclear matrix proteins including NuMA, the splicing factor SRm160, and the cell cycle regulator Rb was governed by the composition of the basement membrane supporting tissue development and organization [Lelièvre et al., 1998]. These proteins exhibited distinct subnuclear distribution patterns specific for proliferation, growth arrest, and acini formation [Lelièvre et al., 1998]. Disruption of the nuclear localization of these proteins resulted in profound phenotypic changes in these cells indicative that nuclear organization itself can modulate the cellular and tissue phenotype [Lelièvre et al., 1998].

The Committed Response to the Mechanical Signal: Connective Membrane Skeleton Proteins Form Mechanosomes That Translocate to the Nucleus and Govern the Load Response Gene Program

A critical step in the present bone cell mechanotransduction scheme is the formation of mechanosomes, the association of specific load-induced CMS complexes with proteins capable of translocating to the nucleus and effecting changes in gene expression (Fig. 2). In this model, the $p130^{cas}$ complex binds to a nucleocytoplasmic shuttling isoform of Nmp4/CIZ, translocates to the nucleus, and there contributes to the formation of enhanceosomes, higher-order trans-acting complexes that mediate changes in transcription. We propose that Nmp4/CIZ displaces Crk in the nascent mechanosome before nuclear translocation (Fig. 2). In like manner, cytoplasmic β-catenin associates with the LEF-1/TCF transcription factor and enters the nucleus. As architectural transcription factors both Nmp4/CIZ and LEF-1/TCF modulate the geometry of the 5′ regulatory regions of target genes, thereby converting the initial deformation of the cell membrane into a bend in DNA. In this model, the role of β-catenin and $p130^{cas}$ in the nucleus is to govern the specific function of LEF-1/TCF and Nmp4/CIZ, respectively, through allosteric control. The target genes in the load response program will be primarily those that encode ECM proteins and proteins that are involved in matrix remodeling, such as the matrix metalloproteinases.

Cell adhesion molecule control of nucleocytoplasmic shuttling has been recently established with a burgeoning list of known proteins that interact with the cytoplasmic domains of cell adhesion molecules and shuttle to the nucleus to act as transcription factors [Aplin and Juliano, 2001]. Proteins that fit this profile include zyxin, JAB1, Hic-5, TRIP6, and CASK [Aplin and Juliano, 2001]. The nuclear translocation of the beta-catenin/LEF-1/TCF complex and its role in the regulation of gene expression is well established in other cell types [Eastman and Grosschedl, 1999]. These proteins are key components of the Wnt signaling pathway that controls embryonic patterning and cell fate decisions during development and has recently been implicated in the regulation of postnatal bone formation [Eastman and Grosschedl, 1999; Kato et al., 2002].

The LEF-1/TCF proteins are architectural transcription factors that contain an HMG (high mobility group)-box, a DNA-binding domain that induces a sharp bend in the DNA helix [Eastman and Grosschedl, 1999]. As true architectural factors, these proteins cannot independently activate transcription, but do so only within the context of a DNA regulatory region that supports interactions with other classical transcription factors [Eastman and Grosschedl, 1999]. The LEF-1/TCF proteins mediate the

organization of higher-order trans-activating complexes by overcoming the energetic barriers preventing the bending and looping of the DNA. Interestingly, association with β-catenin allows the LEF-1/TCF proteins to act like classical transcription factors [Eastman and Grosschedl, 1999] and we speculate that this may be through an allosteric-induced change in the LEF-1/TCF conformation. Finally, like some of the Nmp4/CIZ isoforms, LEF-1 localizes to the nuclear matrix under certain physiological conditions [Sachdev et al., 2001], but whether this occurs in bone cells in response to load has not yet been determined.

Nmp4/CIZ are a family of Cys_2His_2 zinc finger transcription factors, all derived from a single gene, of which some appear to be nucleocytoplasmic shuttling proteins that interact with $p130^{cas}$ [Bidwell et al., 2001; Nakamoto et al., 2000]. These proteins have from 5–8 zinc fingers [Bidwell et al., 2001]. The number and arrangement of these fingers target some of these proteins to specific osteoblast nuclear matrix domains [Bidwell et al., 2001 and references therein]. Although these proteins contain an AT-hook, a domain common to many architectural transcription factors, and bend DNA, they also contain a transactivation domain that appears to be under allosteric control via binding to DNA [Torrungruang et al., 2002]. These proteins themselves regulate the expression of type I collagen and numerous matrix metalloproteinases in connective tissue, including bone [Bidwell et al., 2001 and references therein]. Overlapping the Nmp4/CIZ AT-hook domain is an SH3-binding domain that mediates the interaction of these proteins with $p130^{cas}$ [Nakamoto et al., 2000]. Whether $p130^{cas}$ translocates to the osteoblast nucleus with some Nmp4/CIZ isoforms is unknown at present, but a recent study indicates the nuclear co-localization of $p130^{cas}$, cell adhesion kinase β, and Hic-5 within nuclear speckles [Aoto et al., 2002]. Hic-5 is a LIM domain protein that primarily localizes to focal adhesions but translocates to the nuclear matrix and binds to DNA elements that are strikingly similar to the Nmp4/CIZ consensus element [Nishiya et al., 1998; Yang et al., 2000].

SUMMARY AND FUTURE DIRECTIONS

In the proposed model for skeletal mechanotransduction, the load-induced deformation of bone and the subsequent rush of fluid through the canalicular space deforms the bone cell membrane and applies strain to the tissue matrix. This drives changes in SA-CAT channel(s) protein conformation which, in turn, mediates mobilization of $[Ca^{2+}]_i$-dependent kinase cascades, including c-Src activity. The solid-state and diffusion-controlled signaling pathways integrate with the tissue matrix-mediated transfer of mechanical energy to proteins of the adhesion complexes and selected proteins associated with the cyto- and nucleoskeleton. This mechanical to chemical energy conversion drives changes in protein conformation, phosphorylation, and alterations in DNA geometry and mediates the formation and/or mobilization of nascent signaling complexes, including the tyrosine-phosphorylated $p130^{cas}$-Crk complex and the translocation of β-catenin to the bone cell cytoplasm. The formation of mechanosomes via the complexation of $p130^{cas}$ with Nmp4/CIZ and β-catenin with LEF-1/TCF commits the cell to initiating the gene response program to mechanical load. We further propose that these mechanosomes consist of an architectural transcription factor associated with proteins capable of modulating their functional role through allosteric control. Proteins other than Nmp4/CIZ and LEF-1/TCF may function in the capacity of a mechanosome component including zyxin, Hic-5, and numerous others that shuttle between the nucleus and adhesion complexes.

A principal question underlying efforts to elucidate mechanotransduction in bone is the identity of the gene or genes that comprise the SA-CAT channel in osteoblasts. Does the α-ENaC subunit mediate this mechanosensitivity and is this channel regulated, in part, by load-induced release of ATP? What is the specific role of the L-type VSCC in detecting shear-stress mediated deformations in the bone cell membrane? Probes, specific for the mechano-sensitive channel(s) are necessary for meaningful studies on the early events that mediate the detection of the mechanical signal.

The mechanisms by which focal adhesions and adherens junctions regulate nucleocytoplasmic shuttling in bone cells are particularly relevant to understanding mechanotransduction in bone. The identities of the components that comprise the proposed mechanosomes are required for discerning how mechanical information is relayed to the nucleus. More specifically, elucidating the nuclear role of proteins

like β-catenin, and p130^{cas} will provide insights into how mechanical information is processed at the target gene. Do these proteins contribute to the formation and activity of enhanceosomes specifying a response to load?

The mechanistic details underlying tissue matrix-mediated phosphorylation in the context of bone cell mechanotransduction are necessary for understanding how mechanical energy is converted to chemical energy in this system. For example, the use of the α-actinin fragment, or other specific probes directed at the adhesion complex-cytoskeleton interface, to study the role of tissue matrix integrity on c-Src-mediated phosphorylation of p130^{cas} is the type of approach that will elucidate the transfer of energy in response to load.

Ultimately, any mechanotransduction model for bone must be tested in vivo, using probes targeted for specific proteins instead of the typical pharmacological agents currently employed in these kinds of studies. The use of conditional knockout mouse models, in conjunction with the use of biomechanical outcome measures, will be powerful experimental preparations for studying the skeleton's response to load. Another important question is whether the proposed mechanisms for sensing and responding to load allow the cell to distinguish the kind of strain (compression, tension, and/or shear) and its vector within the context of the complex topological surface of bone. Data addressing these issues will elucidate the molecular basis for bone adaptation.

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