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Osteocytes, Strain Detection, Bone Modeling and Remodeling

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Summary. One of the characteristic features of mammalian and avian bone is a population of live cells of the osteoblast lineage distributed both on the surface and throughout the matrix. These cells communicate with one another via gap junctions. A number of roles have been proposed for both osteocytes and the lacunar/canalicular labyrinth they occupy. These include arrest of fatigue cracks, mineral exchange, osteocytic osteolysis, renewed remodeling activity after release by resorption, stimulation, and guidance of osteoclastic cutting cones involved in mineral exchange and the repair of microdamage, strain detection, and the control of mechanically related bone modeling/remodeling. The question of whether osteocytes control or influence modeling and remodeling is of major importance. Such influence could be crucial in relation to three importance consequences of remodeling activity: calcium regulation, microdamage repair, and mechanically adaptive control of bone architecture. Mechanically adaptive control of bone architecture requires feedback concerning the relationship between current loading and existing architecture. This feedback is most probably derived from the strain in the matrix. The arrangement of the osteocyte network seems ideally suited to both perceive strain throughout the matrix and to influence adaptive modeling and remodeling in a strain-related manner. The hypothese that osteocytes perform this role has growing experimental support.

Key words: Osteocytes, strain – Modeling/remodeling.

The Osteocyte: Osteoblast Network

As bone matrix is rigid and cannot be expanded from within, bone formation takes place by deposition of new tissue either on existing surfaces or within voids excavated within the existing structure. As the layer of osteoblasts on these surfaces secrete osteoid, some of their number secrete less matrix [1], and, falling behind the advancing osteoid front, become buried within the osteoid seam. As this seam mineralizes, they become entombed within the bone itself. Once entombed, osteocytes are no longer in a position to make a direct structural contribution to the growth or subsequent remodeling of the tissue.

During the process of becoming embedded in the matrix the prospective osteocytes change their shape and generate long processes which make contact with neighboring cells, establishing gap junctions between them [2, 3]. The generation of processes is assymetric, being primarily directed towards the forming surface [4]. Those osteoblasts that secrete less matrix and become osteocytes may therefore be those with which underlying osteocytes have established connections. The consequence of this arrangement is that within the mature bone tissue there is a network of entombed osteocytes that communicate with each other and with osteoblasts on the bone's surface.

Despite the strategic arrangement and location of this connecting network of cells, its function remains obscure. Reasons for this include difficulty in studying osteocytes *in situ*, and the fact that once released from their surrounding matrix *in vitro* they loose their connections with both the matrix and their neighboring cells. Furthermore, they do not divide and so cannot be sustained in propagating cultures. Until recently, they could also not be reliably identified [5].

The colonization of bone tissue by a network of communicating cells appears purposeful and poses the question "What aspect of bone's function requires a network of live communicating cells distributed in a labyrinth of lacunae and canaliculi throughout the bone tissue?" A number of the potential roles of this arrangement do not require all of its features.

The Lacunar and Canalicular Labyrinth

By establishing connections with neighboring cells at the time of osteoid secretion, osteocytes not only establish cell: cell communication but also produce a labyrinth of extracellular spaces through which the bone tissue is perfused. There is no evidence that perfusion of bone tissue contributes to the tissue's immediate mechanical properties but by providing discontinuities that could arrest cracks, the lacunar/canalicular network itself may increase the tissue's fatigue resistance [6]. However, if the purpose of the osteocyte entombment were solely to establish a crack arresting labyrinth, this would not need to be inhabited by live cells. Nevertheless, such live cells are a characteristic of healthy bone. Thus, though fatigue resistance may be enhanced by the lacunar/canalicular labyrinth, providing a crack-arresting network is unlikely to be the osteocytes' primary role. It is more likely that the labyrinth provides the means by which the osteocytes are nourished and by which they may be kept alive. What purpose then is there for live osteocytes?

Release of Osteocytes by Resorption

It has been suggested that osteocytes have a potential role in further new bone formation when liberated by resorption [7, 8]. To provide such a role they would certainly have to be kept alive, but it is difficult to see the advantages of a long

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"sleeping beauty" existence. The number of osteocytes to be liberated would be small compared with the number of freshly recruited osteoblasts already present at times of new bone formation. Elmardi et al. [9] show convincing morphological evidence that instead of liberating osteocytes, osteoclasts engulf and degrade them.

The primary role of live communicating osteocytes should therefore be sought in their normal location within the tissue.

Osteocytic Osteolysis and Mineral Exchange

In the late 1970s, the phenomenon of osteocytic osteolysis was the caused of considerable interest. However, Boyde [10], in a magisterial review refuted the role of osteocytes in either resorption or formation. This was a view supported by Marotti et al. [11]. However, more recently, Alcobendas et al. [12] resurrected the possibility by showing morphological evidence strongly suggestive of lacunar expansion in the vertebrae of hibernating vipers.

Before accepting that osteocytic osteolysis is a normal occurrence in mammals, it would be sensible to await evidence of activity within osteocytes of those enzymes necessarily involved in the processes of bone resorption and formation. To our knowledge, that evidence does not exist although there are morphological indications from the organization of their organelles that osteocytes continued to respond to the effects of PTH, $1,25(OH)_2D$, and disuse [13]. This may not be surprising as osteocytes were once osteoblasts. However, whether they continue to play a meaningful role in calcium regulation is not clear. If they were concerned in this process they could participate, without the need to resorb and form bone, by the extraction and restitution of nonstructural, noncrystalline calcium phosphate aggregate from the matrix immediately adjacent to them. Continued involvement in mineral exchange would require live osteocytes and the labyrinth which they occupy would greatly increase the surface area of bone available for such ion exchange. Boyde considered that at any time there is sufficient mineralizing osteoid as a result of remodeling for all the mineral exchange required, and that it is not necessary to invoke the surface area of the canalicular/lacunar network to account for observed Ca fluxes. However, it would be safer to determine whether osteocytes actually do or do not participate in mineral exchange rather than relying on calculations that they do not need to. If osteocytes are normally involved in significant mineral exchange, then any decline in their activity would constitute an increased requirement for remodeling. Since in the elderly remodeling is usually negatively balanced, any increase is better avoided.

If the requirement for live osteocytes rests on their involvement in mineral exchange, they would be operating primarily under the influence of calcium-regulating hormones to extract or restore mineral in the tissue immediately adjacent to them. Under such control, and working on such a restricted front, there would be no need for them to communicate with one another. However, a feature of the osteocyte/osteoblast network is gap junction communication. Which of the osteocytes' potential roles *in situ* requires such communication?

Repair of Microdamage and Control of Osteonal Resorption

One continuing mystery in the process of bone remodeling is the mechanism by which the osteoclastic cutting cones responsible for the resorptive phase of secondary osteonal remodeling are directed towards their target. Activation of cutting cones is increased as a result of disuse, microdamage caused by fatigue failure, and areas of devitalized bone. In the first case, activation appears to be the result of hormonal stimulation no longer countered by the conservational effect of load bearing [14]. As the osteonal remodeling of disuse is characterized by incomplete infilling of the resorption spaces, the result is a porotic cortex; this is usually accompanied by endosteal resorption. The extent to which this conservational, antiresorptive effect is derived from osteocytes' perception of reduced loading is discussed below. In the case of mineral exchange and disuse osteoporosis, the location and track of the cutting cones may not matter. However, in the case of revitalizing areas of dead tissue and repairing microdamage, accurate targeting is essential.

It has been proposed that the existence of fatigue damage is a major stimulus for the osteonal remodeling which replaces the damaged tissue [15–19]. Such damage does occur in bone exposed to physiological strains [20]. However, the nature of the stimulus to activate and then target osteoclast cutting cones is not clear. Presumably, osteoclasts are activated by cells of the osteoblast lineage on the bone's surfaces [21], and these cells communicate with the osteocyte population throughout the bone tissue. A signal to activate, and perhaps subsequently guide, a cutting cone could therefore come as a positive excitatory signal from osteocytes in the neighborhood of cracks. Alternatively, such a signal could be derived from the failure of osteocytes killed or disabled by damage in their locality to provide a continuing inhibitory signal to prevent osteoclast invasion. This latter interpretation fits better with devitalized bone also providing a stimulus for replacement. However, in areas of adaptation it is not uncommon for woven bone, established early in the adaptive process and containing recently deposited healthy osteocytes, to be selectively replaced by secondary osteons. It is possible, therefore, that osteonal targeting is achieved in response to a positive attracting signal or the absence of an inhibiting signal. Regardless of which process is involved, a communicating network of live cells would be required to transmit the need for remodeling to sites where osteoclasts may be recruited, and to convey the positional data necessary to target the cutting cone to the damaged area. Without some indication from the osteocyte network, either as a positive signal or absence of a negative one, it is difficult to see how osteoclasts, or the surface osteoblasts thought to control them, would have the relevant information for the targeting process.

Though an attractive possibility, there is as yet no direct evidence that osteocytes influence the activation or subsequent targeting of osteoclastic cutting cones. Secondary osteonal remodeling in acellular teleost bone does not seem to require osteocytic direction.

Throughout this consideration of fatigue damage it has been assumed that such damage takes the form of cracks within the matrix that can only be repaired by excision of the damaged tissue and its replacement by a secondary osteon. It is perhaps worth considering the possibility that there are less severe forms of fatigue (or creep and fatigue [22]) failure which may be repaired without such replacement. To our knowledge there is no evidence on the repair of such damage *in vivo*. However, it is a possibility that should be explored. If it exists, and cells are involved, then these will most likely be osteocytes as they are the only cells in the vicinity.

Modeling and Remodeling in Growth and Adaptation

Another role for which a communicating network of cells

seems to be particularly well suited is an appreciation of form and "harmonische einfugung" (loosely translated as harmony of function in structure) [23]. This might be necessary during growth and perhaps repair. The means available to establish an appreciation of form may also be concerned in any assessment of the distribution of mechanical strain throughout the bone structure.

Adaptation of bone architecture in relation to functional loading is a characteristic feature of bone physiology, and in mammals and birds at least, it is the mechanism on which the establishment and maintenance of load-bearing competence depends. In experiments in which the magnitude and/or distribution of normal locomotor strains have been altered by osteotomy of a supporting bone, apparently purposeful adaptive remodeling and renewed modeling occurs [24, 25]. This adaptive response involves the recruitment of new populations of cells without any change in their individual rates of activity [25]. This finding, and the nature of the adaptive response [24], suggest that functional adaptation is not achieved simply through a direct influence of local strain magnitude on the cells responsible for formation and resorption on the bone's surfaces. Rather, it is likely to be controlled by a stimulus derived from an appreciation of strain distribution throughout the matrix.

The Role of Osteocytes in Adaptive Modeling/Remodeling

It is our hypothesis that an important role of the communicating network of osteoblasts and osteocytes is to respond to dynamic strain in the bone tissue, to process this strain-related information, and subsequently to influence modeling/remodeling activity in a strain-related manner in order to establish and maintain structurally appropriate bone architecture.

This hypothesis is neither new nor original to us; however, it is increasingly amenable to study. For it to be substantiated it is necessary to demonstrate that osteocytes show changes in their behavior that are related in an obligatory fashion to both the strain situation in their matrix, and to the strain-related control of the modeling and remodeling processes responsible for mechanically adaptive changes in bone architecture. The data below partially fulfill those criteria.

In bone, it is the mineralized matrix that carries the loads and experiences the strains that those loads engender within it. These strains reflect the relationship between the prevailing bone architecture (including mass, geometry, and material properties) and the loads imposed. Functionally adaptive modeling/remodeling ensures that bone architecture is appropriate for prevailing bone loading. The adaptive process can be most readily regulated by using functional strain as its controlling variable.

There is no evidence that exposure to mechanical strain is necessary for osteocyte metabolism. Live osteocytes may exist in bone tissue that has been immobilized for some time, as evidenced by their presence in the ear ossicles of elderly people. As ear ossicles neither grow nor remodel after the first year of life, this means that osteocytes can survive undisturbed for over 70 years [26]. However, unlike ear ossicles, load-bearing bones constantly need to adapt to their loading environment. If strain is the controlling variable for this adaptive process it must at some stage be transduced into the biological language with which cells communicate and influence each others' behavior.

Mechano-transduction is a property of many cell types [27] and has received considerable attention in bone cells. In general, however, these tests have been carried out in culture where normal cell:cell and cell:matrix relationships are absent, and the cells concerned have been osteoblasts rather than osteocytes [see 28–31]. To our knowledge, Ypey et al. [32] are the only investigators to have studied strain-related behavior in identified osteocytes. There is no evidence on which aspect, or consequence, of strain change *in vivo* provides the strain-related influence on osteocytes, or the mechanism by which strain change normally influences these cells. In addition to direct deformation of the cells themselves, dynamic strain inevitably involves changes in intralacunar pressure, fluid flow through the extracellular spaces, and the electrical potentials that flow of such charged fluid engenders. Any or all of these could directly affect osteocytes in a strain-related manner. However, Ypey et al. suggest that stretch-activated ion channels are not involved.

The evidence of strain-related activity in osteocytes *in situ* is fragmentary but consistent with their proposed role in our hypothesis. The observations related to these instances are outlined below.

The externally loadable adult avian ulna model *in vivo* has been shown that bone mass can be substantially influenced in a peak strain magnitude-related manner by daily interruption of disuse by a short period of dynamic, but not static, loading [33–35]. This influence on bone mass involves prevention of the resorption which normally occurs in the absence of loading, and a strain magnitude-related increase in periosteal and endosteal bone formation. Exposure of the same avian preparation to a single period of such loading transforms the bone's quiescent periosteum to one actively forming bone 5 days later [36]. It can be supposed that the longer term modeling/remodeling response is the cumulative effect of daily exposure to this single stimulus.

In the same avian ulna preparation 5 minutes after a single period of such loading, the number of osteocytes showing activity of the enzyme glucose-6-phosphate dehydrogenase (G6PD) is increased by an amount that is related to the peak strain magnitude in their immediate locality [37]. As this increased G6PD activity is not accompanied by an increase in the activity of glyceraldehyde 3-phosphate dehydrogenase or lactate dehydrogenase, there is probably increased use of the pentose monophosphate shunt without any change in glycolysis. The pentose monophosphate pathway is involved in the synthesis of ribose sugars necessary for the production of RNA.

In rat tibiae subjected *in vivo* to a period of loading likely to be osteogenic, G6PD activity is similarly raised 5 minutes after loading in both osteocytes and surface cells [38]. In surface cells, G6PD activity remains elevated for 24 hours and is accompanied at that time by increased alkaline phosphatase activity (suggesting an osteogenic response at the bone's surface). In osteocytes 24 hours after loading, G6PD levels are restored to nonloaded levels. In osteocytes there is no ALP activity at any time. This is consistent with the absence of any matrix production by these cells.

In the avian model *in vivo* 24 hours after a period of "osteogenic" loading, the number of osteocytes shown by autoradiography to be incorporating ^{3}H-uridine is increased overall by a factor of 6 (from 12 to 72%) [39]; this is consistent with these cells increasing their RNA production. The distribution of cells showing increased ^{3}H uridine was not the same as that showing increased G6PD activity. The greatest increase in ^{3}H uridine uptake was, instead, in the cortex beneath the area of greatest subsequent new bone formation. This suggests possible "signal processing" by the osteocyte population, transforming an initial chemical response directly proportional to the raw strain data to a con-

trolling influence for the subsequent adaptive processes. We hypothesize that this latter stage may involve the production of IGF-II.

Loading 17-day chick tibiae *in vitro* prevents the decline in alkaline phosphatase levels in periosteal cells associated with a period in culture, and increases the expression of the gene for collagen type 1 [40]. This model, therefore, exhibits all the obligatory stages between strain change and enhanced osteogenic modeling activity. As in the adult avian situation *in vivo,* this embryonic avian bone model shows a rapid, strain magnitude-related elevation in G6PD activity in osteocytes. This is followed over the subsequent 24 hours by a strain magnitude-related increase in RNA synthesis, in this case illustrated by an increase in the specific activity of ^{3}H-uridine in extracted RNA [39].

The osteogenic response to a single period of strain change in the adult avian model *in vivo* is substantially modified by a single high dose of indomethacin at the time of loading [42]. In the embryonic chick tibia model *in vitro,* both the strain-related increase in G6PD and RNA are also eliminated by indomethacin [41]. This suggests a prostanoid-dependent step similar to that observed *in vivo,* and indicates that this step in the modeling/remodeling response occurs prior to the increase in G6PD activity.

Mechanical loading of perfused cores of adult canine cancellous bone also shows loading-related increases in osteocytes' G6PD activity and RNA production [43, 44]. This model also demonstrates transient loading-related production of PGE and PGI_2 released into the medium. Immunocytochemistry confirms that the PGE is produced in surface lining cells but that the PGI_2 is present in both surface cells and osteocytes.

Perfusion of these cancellous cores with exogenous PGE_2 and PGI_2 shows that both prostanoids increase G6PD activity in both types of cell but that whereas PGI_2 quantitatively mimics the loading-related increase in RNA production there is no such response to PGE_2. This suggests that bone strain elicits more than one response in osteoblasts and osteocytes, both of which may play a part in adaptive modeling and remodeling. Surface cells (which may themselves participate in matrix synthesis) are exposed directly to osteoregulatory effects of PGE [45–47] and prostacyclin, as well as any indirect stimulus from influences resulting from prostacyclin production. Osteocytes, which are not involved in matrix synthesis, are exposed only to prostacyclin and the products that it stimulates.

Discussion and Conclusions

The communicating network of osteocytes and surface osteoblasts seems best placed to influence structural bone cell activity in relation to three processes: (1) strain-related adaptive modeling/remodeling, (2) repair of microdamage and revitalization of dead tissue, and (3) mineral exchange.

Strain-Related Adaptive Modeling/Remodeling

The skeleton's structural competence is maintained by means of a functionally adaptive relationship between bone architecture and bone loading. Because the bone's strain situation reflects the relationship between architecture and loading, strain throughout the bone is probably the only functional variable containing all the information necessary to control adaptive modeling/remodeling. It is also the functional variable most likely to be able to influence bone cell behavior. The bone cells best placed to be influenced by bone strain are osteocytes and the surface osteoblasts with which they communicate.

The hypothesis that osteocytes (and surface osteoblasts) are involved in the transduction of strain in bone tissue, and the subsequent influence of adaptive modeling/remodeling, has experimental support. Osteocytes are rapidly responsive to mechanical events in their surrounding tissue in a peak strain magnitude-dependent manner. Their early responses include (in sequence) the production of prostacyclin, increased G6PD activity, and increased RNA synthesis. Exogenous administration of prostacyclin imitates all the strain-related behavior of osteocytes and osteoblasts we have been able to demonstrate, including adaptive new bone formation. Indomethacin at the time of loading eliminates or reduces all these responses.

The involvement of osteocytes in detecting strain, forming an appreciation of strain distribution, and influencing modeling/remodeling would account for the need for a population of life, strain-sensitive cells, distributed throughout the matrix and communicating with one another and with osteoblasts on the surface.

Further investigation needs to be directed to (1) the mechanism(s) by which strain is transduced, (2) the process by which the osteocytes form their collective appreciation of strain distribution throughout a volume of tissue, and (3) the means by which modeling/remodeling activity is influenced in a strain-related manner.

Repair of Microdamage and Revitalisation of Tissue

Repair of intracortical microcracks by secondary osteonal remodeling requires that osteoclasts be activated in response to the presence of damage, and then targeted towards its location. Similar activation and attraction towards areas of dead bone are necessary for revitalization to occur. In either case, the mechanisms by which this is accomplished are unknown but could involve positive attraction, or the absence of continuing inhibition, from osteocytes. Further study is required to examine these possibilities.

The existence of a level of microdamage less than overt crack formation, and capable of repair without osteonal remodeling, should also be investigated. Such repair could involve the agency of osteocytes.

Mineral Exchange

The role of osteocytes in mineral exchange also remains unclear and should be reexamined.

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DISCUSSION

DR. RUBIN: You have demonstrated that by applying an

osteogenic load you get changes in cellular homeostasis in bone. Do you find similar changes when you remove the stimulus? Are the osteocytes involved in regulating the bone resorption response?

DR. LANYON: We haven't done that specifically. In organ culture models, where normal loading is no longer occurring, functional loading will restore alkaline phosphatase activity at the site of subsequent new bone formation. We have not looked at the control of resorption after withdrawing the functional stimulus.

DR. RAISZ: Are prostaglandins involved in the resorption response in your model? Thompson and Rodin found that the early resorption response to tenotomy was blocked by indomethacin.

DR. LANYON: We haven't looked at resorption. The direct action of the prostanoid itself is a complication. I am intrigued that the prostacyclin in this situation actually reproduces the loading-related RNA response. One could postulate that depending on its dose, some unknown strain-related growth factor, acting alone or in combination with other growth factors, could stimulate either bone formation or resorption as part of a dose related response to load.

DR. MAROTTI: Although it is clear that osteocytes can easily control osteoblasts, I don't think they can control osteoclasts. What is your opinion?

DR. LANYON: Tim Chambers' work and that of others does suggest that osteoblasts exert substantial control over osteoclasts. Because of that work, we were able to avoid having to deal with the possibility of osteocytes talking independently both to osteoclasts and to osteoblasts.

DR. LEES: Years ago I came to the conclusion that the limit to the number of osteocyte layers that you could have around an osteon was due to the limit in the transport characteristics of the cell processes which are there. Another conclusion was that as the body became older, the transport characteristics deteriorated somewhat. Do you think that aging of the transport characteristics across the processes, which are vital to the life of the osteocyte and now presumably to components which sense the strain, could contribute to the aging of bone and the problems we are talking about.

DR. LANYON: Yes, if you postulate that a communicating network is necessary for proper function, and that anything that contributes to degradation of communication is going to interfere with the effectiveness of that process, be that functional adaptation or targeting BMUs to particular places in the cortex.