The transcription factor nuclear factor of activated T cells (NFAT), first shown to be important in the T cell, is now recognized as playing a central role in osteoblasts (15). This transcription factor is constitutively expressed in osteoblasts. They then introduced calcineurin into MC3T3-E1 osteoblastic cells as a TAT-calciineurin A fusion protein (to allow the transduction of the full-length protein). Expression of the fusion protein directed the enhanced expression of Runx-2 (a transcription factor critical for commitment to an osteogenic lineage) (23). In the most recent study, Winslow et al. (17) generated mice that express a constitutively nucleus-localized NFATc1 variant, NFATc1nuc. Under the control of a tetracycline transactivator, the variant was expressed in thymus, spleen, lymphocytes, and bone. Expression of the variant in bone cells was exclusively in osteoblasts, and the bone phenotype was characterized by an increase in osteoblast number and markedly increased bone volume. Histologic analysis revealed that the bone in the mutant mice had a less organized appearance than normal bone tissue, suggesting rapid ossification. Serum osteocalcin and serum alkaline phosphatase markers of osteoblast activity, were more than doubled. The increased bone mass was an early event, observed at embryonic day 16.5 (E16.5). Investigation of the possible mechanisms showed that the expressions of Wnt4 and Frzr2, members of the Wnt family whose signals can regulate bone mass (24, 25) were increased, while the expressions of Wnt inhibitors Dkk1 and Sfrp2 were decreased. Other factors known to be involved in osteoblast proliferation and differentiation, including the transcription factor Runx2, were unaffected in mice expressing the mutation, and there was no defect in mineralization. Comparison of the findings in the calcineurin and NFAT studies reveals a difference in the observed responses: Runx2 expression was elevated with calcineurin treatment but not with NFATc1. Whether this represents a calcineurin effect that is independent of NFATc1 or reflects other divergent aspects of the models remains to be investigated.

In addition to the effects on osteoblasts, the NFATc1nuc mutant mice in the Winslow study (17) were noted to have increased osteoclast number and increases in markers of osteoclast activity and bone resorption. However, there was no increase in bone loss.
expression of receptor activator of NF-κB ligand (RANKL), a cytokine produced by osteoblasts that activates osteoclast differentiation from monocyte cell line precursors and promotes fusion and survival of osteoclasts (26) or its physiological antagonist osteoprotegerin (OPG). The study presented evidence for increased expression of several monocyte chemoattractants in the NFATc1nuc mutant mice, and NFAT1 was shown to increase the chemokine CCL8. The osteoclastogenic effect of CCL8 appeared to be an indirect effect, as the authors stated that the direct addition of CCL8 did not elicit osteoclastogenesis in an in vitro model.

Effects of the NFAT pathways on bone are of particular clinical interest because of bone side effects of cyclosporine. When cyclosporine was used clinically as an immunosuppressant to prevent tissue rejection after organ transplantation, the treatment was associated with bone loss (27, 28). These clinical findings of bone loss with cyclosporine treatment were paradoxical because early studies showed that cyclosporine inhibits the resorption elicited by multiple factors in organ cultures of fetal limb bones (29) and neonatal calvaria (30). Consistent with the organ culture results on cyclosporine, recent studies established that expression of NFATc1 in precursor cells led to their differentiation into osteoclasts (31, 32). The results from the Koga et al. and Winslow et al. studies indicating that NFATc1 expression leads to osteoblast differentiation (16, 17), and the findings of Sun et al. (20) showing that calcineurin expression promotes the expression of bone differentiation markers suggest that the effects of cyclosporine to interfere with bone anabolic responses by blocking NFAT activation in osteoblasts could override the effects of the drug to inhibit osteoclast differentiation, with the result being a net loss of bone mass. Appealing as this explanation is, it is unfortunately inconsistent with the observations that the bone loss associated with cyclosporine is associated with high bone turnover (27, 28). The in vivo situation is complicated by multiple factors, and the mechanism of the bone loss is still unclear.

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Figure 1. Findings from three recent studies on effects of the calcineurin-NFAT pathway in osteoblasts. A. Overexpression of calcineurin increases the expression of the bone differentiation markers alkaline phosphatase, osteocalcin, bone sialoprotein (BSP), and Runx-2 (20). B. Expression of NFATc1 increases the gene expression from the collagen 1a1 promoter and also increases the number of bone nodules (i.e., NFATc1 expression promotes bone formation) (16). NFATc1 forms a complex with the transcription factor Osterix. C. When a nuclear NFATc1 mutant (NFATc1nuc) is expressed in mice, expression of the mutant in bone is observed only in osteoblasts (17). There was concordant osteoblast proliferation, increased expression of Wnt and Proliferin, and decreased secreted frizzled-related protein 2 (SFRP2) and the Wnt antagonist dickkopf2 (DKK2). The expression of chemokine CCL8 was increased and osteoclastogenesis—whereby the fusion of precursor cells (not shown) to form mature multinucleated osteoclasts—was stimulated. Blue cells represent osteoblasts; the red cells represent a mature osteoclast.
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