Pathological bone loss always reflects enhanced net osteoclastic activity. Recognition and binding of the receptor activator of NF-κB (RANK) by RANK ligand (RANKL) is the key osteoclastogenic event, and the signaling cascades induced by this reaction therefore contain potential anti-osteoporosis therapeutic targets. A study reported in this issue of the JCI documents that a pivotal component of RANKL/RANK-mediated osteoclast recruitment involves sequential induction of the transcription factors c-Jun and nuclear factor of activated T cells 2 (see the related article beginning on page 475).

What is an osteoclast?
Osteoporosis always represents an imbalance in favor of osteoclast-mediated bone resorption relative to the bone-forming capacity of osteoblasts. In some conditions of accelerated skeletal loss, such as Paget disease, osteoclasts are greatly enlarged, which enhances the individual cell’s resorptive activity. Most osteopoietic disorders, however, develop as a consequence of accelerated bone degradation due to increased osteoclast number. Thus, inhibition of osteoclast formation by estrogen supplementation, or osteoclast function by bisphosphonate administration, has been the most effective means of arresting pathological bone loss. However, both forms of therapy are not without drawbacks, and new anti-resorptive targets are rapidly emerging.

In this issue of the JCI, Ikeda et al. elegantly show that the JNK-1–activated c-Jun signaling pathway is key to osteoclastic bone resorption (1). The authors find that mice bearing a dominant-negative osteoclast-specific c-Jun transgene have impaired osteoclastogenesis and, because of failed bone resorption, develop increased bone mass in the form of osteopetrosis. Importantly, the failure of dominant-negative c-Jun transgenic mice to generate osteoclasts is due to arrested activation and expression of members of the nuclear factor of activated T cells (NFAT) family of transcription factors in osteoclast precursors.

The osteoclast needs TRAF6, Fos, and Jun
The importance of activator protein-1 (AP-1) transcription factors, specifically dimers of the Fos and Jun families of proteins, in the osteoclastogenic process was first documented in the laboratory of Erwin Wagner, wherein c-Fos knockout mice were shown to be osteopetrotic due to a complete absence of osteoclasts (11). Consequently, the discovery of RANKL as the key osteoclastogenic cytokine prompted interest in the mechanisms by which RANK activation regulates AP-1 transcription factors.

Just why RANKL is unique among TNF superfamily members in its capacity to induce osteoclast differentiation is still unresolved but probably involves its interaction with TNF receptor–associated factor 6 (TRAF6) (12, 13). Other RANKL-stimulated intracellular signaling molecules essential to the osteoclast phenotype include the p50/p52 NF-κB subunits and the PI3K/AKT axis. Similarly, the MAPKs extracellular regulated kinases 1 and 2 and p38 are required for osteoclast differentiation or function.

Association of RANKL and TRAF6 activates all key events involving AP-1–mediated transcription of osteoclast specific genes. Expression of the c-Fos gene in 393T cells requires TRAF6, but whether the same holds true in osteoclasts is unresolved.

In the other hand, c-Jun is clearly RANKL

Steven L. Teitelbaum
Department of Pathology, Washington University School of Medicine, St. Louis, Missouri, USA.

Nonstandard abbreviations used: activator protein-1 (AP-1); MAPK kinase 7 (MK7); nuclear factor of activated T cells (NFAT); receptor activator of NF-κB (RANK); RANK ligand (RANKL); TNF receptor–associated factor 6 (TRAF6).

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activated via TRAF6 by a process involving JNK1 but not JNK2 (14). c-Jun is not the only member of its family to regulate osteoclastogenesis, as Jun-B–deficient mice also have arrested bone resorption (15).

When transcriptionally active, c-Jun associates with members of the Fos family of transcription factors, a number of which – such as Fra-1, itself a c-Fos target – may substitute for c-Fos in the osteoclastogenic process (16). Although c-Jun also homodimerizes, the fact that c-Jun overexpression does not rescue the c-Fos−/− osteopetrotic phenotype indicates that c-Jun is necessary, but not sufficient, to transactivate osteoclast specific genes.

NFAT and AP-1

In 2002 Takayanagi et al. identified NFAT2 by genome-wide screening as the predominant gene activated in osteoclast precursors under the influence of RANKL and documented the transcription factor’s essential role in osteoclastogenesis (17). RANK occupancy mobilizes intracellular calcium, a requisite for calcineurin-mediated NFAT activation (Figure 1). Moreover, RANKL not only induces the transcription factor’s expression but facilitates its nuclear translocation, where NFAT binds to its DNA response element via a ternary complex with AP-1 proteins, including Fos/Jun, to transactivate target genes (18). Thus, it is not surprising, in retrospect, that RANKL-induced osteoclastogenesis involves partnering of Fos/Jun with NFAT2. The fact that Ikeda et al. (1) found that expression of NFAT2 itself is NFAT1/Fos/Jun–dependent is in keeping with the presence of NFAT and AP-1 response elements in the NFAT2 promoter (19). Reflecting its role as an essential RANKL-activated signaling molecule, NFAT2, when overexpressed in wild-type macrophages, prompts osteoclast differentiation in the absence of the osteoclastogenic cytokine (17). RANKL-stimulated gene transcription is, therefore, a reflection of NFAT partnering with Fos/Jun.

Thus, Ikeda et al. (1) provide evidence of important components of RANK-mediated osteoclastogenesis specifically as it pertains to the AP-1 transcription complex. Clearly, TRAF6 is an essential player in RANKL-mediated c-Jun and NFAT activation and, perhaps, c-Fos expression. NFAT2/Fos/Jun is a critical osteoclastogenic complex, and deletion of any of the three arrests osteoclast formation. The pivotal role that Ikeda et al. document for c-Jun in osteoclast formation is in keeping with the fact that the anti–bone resorptive effects of estrogen are substantially mediated by c-Jun repression (20). Given that RANKL expression is also enhanced in estrogen-deficient women (21), RANKL → TRAF6 → MAPK kinase 7 (MKK7) → JNK1 → Jun → NFAT signaling is likely pivotal to the pathogenesis of postmenopausal osteoporosis, and inhibition of any of the components will theoretically arrest accelerated bone resorption (Figure 1). The therapeutic challenge is how to specifically target these intracellular signaling molecules in osteoclasts.

Address correspondence to: Steven L. Teitelbaum, Department of Pathology and Immunology, Washington University School of Medicine, Campus Box 8118, 660 South Euclid Avenue, St. Louis, Missouri 63110, USA. Phone: (314) 454-8463; Fax: (314) 454-5505; E-mail: teitelbs@path.wustl.edu.

Figure 1

The AP-1/NFAT transcription complex mediates osteoclast precursor differentiation. Osteoclastogenesis is initiated by RANKL occupying RANK on the surface of osteoclast precursors. Subsequent recruitment of TRAF6 initiates the 3 depicted signaling cascades, in addition to other pathways not shown here. Phosphorylation (P) activates c-Jun in an MKK7/JNK-1–dependent manner, and NFAT1 is activated by dephosphorylation via calcium-mediated induction of calcineurin. RANKL/RANK also induces c-Fos expression by an incompletely understood mechanism. NFAT1 partners with the AP-1 proteins of the Fos/Jun families to transactivate the NFAT2 gene, the product of which forms a similar ternary transcription complex on osteoclastic genes eventuating in appearance of the mature osteoclast phenotype.
AMP-activated protein kinase: the guardian of cardiac energy status

D. Grahame Hardie

Division of Molecular Physiology, Faculty of Life Sciences, Wellcome Trust Biocentre, University of Dundee, Dundee, United Kingdom.

Several years ago it was proposed that the AMP-activated protein kinase cascade might protect cells against stresses that deplete cellular ATP. Young et al. have now directly tested this by studying the effects of ischemia and reperfusion in perfused hearts from mice expressing a dominant-negative mutant that suppresses the kinase activity in cardiac muscle (see the related article beginning on page 495). Compared with control hearts, the mutant hearts showed clear evidence for increased necrotic damage and increased apoptosis. These findings may have implications for the treatment of ischemic heart disease.

AMP-activated protein kinase (AMPK) is the downstream component of a protein kinase cascade that is highly conserved in all eukaryotic cells (1). AMPK is activated by the rising cellular AMP that (due to the action of adenylyl kinase) always accompanies a fall in the cellular ATP/ADP ratio, and this activation is antagonized by high concentrations of ATP (Figure 1). Downstream targets and processes regulated by the kinase are being identified on a regular basis (Figure 2).

In general, AMPK switches off ATP-consuming processes such as biosynthetic pathways, while switching on catabolic processes that generate ATP, including cellular uptake of glucose (2) and fatty acids (3) and increased fatty acid oxidation (4) in the heart.

The first evidence that AMPK was activated by metabolic stresses appeared 13 years ago, when my group found that AMPK was activated by ATP depletion caused by incubation of isolated rat hepatocytes with high fructose (5), while Witters et al. (6) reported that it was activated by various metabolic poisons in hepatoma cells. We proposed at the time

Nonstandard abbreviations used: 5-aminoimidazole-4-carboxamide riboside monophosphate (ZMP); AMP-activated protein kinase (AMPK).

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Figure 1
Role of AMPK in regulating energy balance at the single-cell level. The way in which the AMPK system controls the balance between ATP consumption (e.g., by biosynthesis, cell growth, or muscle contraction) and ATP production via catabolism is illustrated. If the rate of ATP consumption exceeds its rate of production, ADP will tend to rise and be converted to AMP by the enzyme adenylyl kinase. The rise in level of the activating ligand AMP, coupled with the fall in level of the inhibitory nucleotide ATP, activates AMPK, which then switches off ATP-consuming processes and switches on catabolism in an attempt to redress the balance.