together to help modulate metabolic flux.

Phylogeny is also an integral part of the interpretation of any coevolutionary system, such as host-parasite or large-term symbiotic interactions. For instance, in the coevolution of a group of insects and their host plants, the plants evolve chemical defenses against insects, and the insects evolve resistance to the defenses. Just as the Red Queen in Lewis Carroll's *Alice Through the Looking Glass* had to keep running just to stay in the same place, so too do the plants and insects have to keep evolving new defenses or ways of coping with the new defenses just to stay even in the evolutionary race against each other (*9*). However, since the universe of possible defensive compounds is limited, many different plants may evolve similar chemical defenses, so much parallelism and convergence is expected in the plants' defensive systems. Are the insects that feed on the plants more likely to track a lineage of plants through time as it evolves new defenses, or will they "cheat" in the race by switching to a related host plant that contains chemical compounds to which they are already adapted? Becerra (*10*) asked this question of a group of beetles that specialize on the strongly aromatic plants of the genus *Bursera*. If the beetles coevolve with the plants as the plants evolve new chemical defenses, then the phylogeny of the beetles would be expected to match the phylogeny of the plants. On the other hand, if the beetles switch hosts to take advantage of their existing resistance to particular chemical defenses, then the beetle phylogeny would be expected to show a closer match to the plants' chemistry than to their phylogeny. Becerra found significant congruence between the beetle phylogeny and the plant chemistry, but not between the beetle phylogeny and the plant phylogeny. Thus, it appears that the beetles would rather switch than fight when it comes to coping with their host plants.

These few examples sample only a small range of the recent applications of phylogenetic analyses. Phylogenetic analyses have become increasingly important in studies of human diseases: for epidemiological investigations (*11*), for identifying and characterizing newly discovered pathogens (*12*), and for identifying and tracking natural reservoirs of zoonotic diseases (*13*). Recently, phylogenetic analyses have been found admissible as evidence in a criminal court case involving an alleged purposeful viral transmission (*14*). Phylogenetic analysis of molecular sequences is also one of the principal interpretive tools for understanding the organization and evolution of genes and genomes (*15*). Behavioral ecologists have used phylogeny to reconstruct and study the evolution of behaviors (*16*). At the same time, phylogeny has solidified its more traditional role as the criterion for organizing and classifying life (*17*). One can only wonder if Darwin and Haeckel would have ever believed that the fruits of their ideas would come to all of this.

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CELL BIOLOGY

Journey Across the Osteoclast

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Despite its persistence after death, the living vertebrate skeleton is a dynamic enterprise. Bone is continuously forming and being resorbed, starting in the embryo and continuing throughout adult life (*1*). This process is accomplished by precise coordination of two cell types: osteoblasts, which deposit the calcified bone matrix, and osteoclasts, which resorb it. Osteoclasts are large, multinucleated cells that are derived from the same hematopoietic precursor as macrophages (*2*). As in most animal cells, the osteoclast plasma membrane is divided into multiple domains (*3*). One of these, the ruffled border, faces the bone surface (see figure, left panel) and is surrounded by a sealing zone, which forms a tight seal against the bone surface. At the ruffled border, the osteoclast secretes acid and lysosomal enzymes that digest the mineral and protein components of the underlying bone (*4*). A leakproof seal is required to maintain the low pH in the compartment next to the bone, but this presents a disposal problem for the cell—how to remove the soluble degradation products of bone? Now in this issue, Nesbitt and Horton on page 266 (*5*) and Salo *et al.* on page 270 (*6*) show that degraded bone proteins and inorganic matrix components are transcytosed in vesicles to the free surface of the osteoclast opposite the ruffled border and released.

The best-known examples of polarized membrane domains are the apical and basolateral surfaces of epithelial cells (see figure, right panel) (*7*). Proteins reach these surfaces by two pathways. Newly made proteins can travel from the trans-Golgi network directly to the apical or basolateral surface. Alternatively, proteins can reach one surface, generally the basolateral, and then be endocytosed and transcytosed to the opposite surface. Transcytosis is found universally in all epithelial cells examined to date and in some epithelial cells is the only pathway for apical delivery of proteins.

It was once thought that the osteoclast's ruffled border corresponds to the apical plasma membrane domain of epithelial cells and that the free surface is the basolateral

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domain. The situation, however, is more complex. The influenza virus hemagglutinin protein is a standard marker of the apical surface of epithelial cells, and vesicular stomatitis virus G protein, a marker of the basolateral surface (*7*). When expressed in osteoclasts, hemagglutinin is restricted to the central part of the free surface, which is morphologically distinct from the remaining peripheral portion of the free surface, to which the G protein is confined (*3*). The free surface itself is thereby divided into separate "apical" and "basolateral" domains, even though it lacks the tight junctions that divide these domains in epithelial cells. The transcytosed bits of bone digested in the acid compartment under the cell are directed toward the analog of the apical domain, where they are released (*5*, *6*).

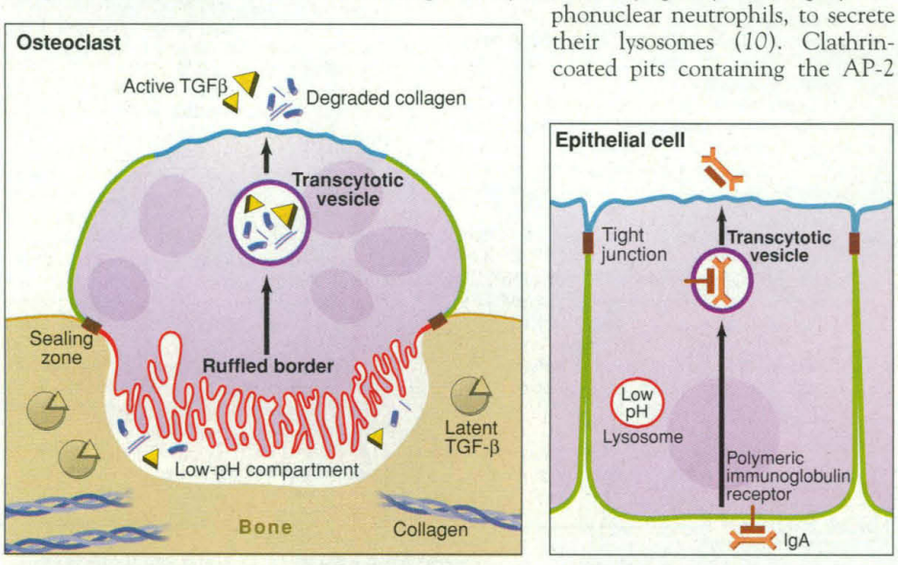
A few years ago, the discovery of this specialization of the osteoclast free surface would have been completely unexpected. Then, apical and basolateral polarity were thought to occur primarily in epithelial cells, including epithelial-derived neurons, which have homologous axonal and somatodendritic domains. But it is now clear that this principle of cellular organization appears more universally in different guises. For example, macrophages, which are closely related to osteoclasts, lack the clearly defined plasma membrane domains characteristic of the osteoclast. Nevertheless, like osteoclasts, macrophages form sealed compartments and endocytose material at one side of the cell and deposit it at the opposite side (*8*).

The ruffled border of the osteoclast is not homologous to either surface of an epithelial cell, but rather more closely resembles a lysosome (or perhaps a late endosome), as it is the site of secretion of lysosomal hydrolases and of acid by the lysosomal proton pump (*9*). The ruffled border can therefore be thought of as a giant extracellular lysosome. This may be related to the ability of certain other hematopoietically derived cells, such as cytotoxic T lymphocytes and polymorphonuclear neutrophils, to secrete their lysosomes (*10*). Clathrin-coated pits containing the AP-2 clathrin adapter protein were previously thought to assemble only at the plasma membrane, but they have recently been found on lysosomes, where they might be involved in forming vesicles that bud off from the lysosome (*11*). This may be similar to the endocytosis of degradation products at the ruffled border. Studying transcytosis by osteoclasts should help us understand movement of material from lysosomes back to the cell surface. This poorly understood process is important for antigen presentation by several cell types including osteoclast-related macrophages.

Release of the transcytosed material from the osteoclast's version of the apical domain is analogous to release from the apical domain of epithelial cells, and so the molecules underlying the process may also be similar. Candidate receptors that could be used by the osteoclast to transport bone matrix proteins include αvβ3 and α2β1 integrins, which bind denatured and native collagens (*12*) and could mediate transcytosis of collagen fragments. Transcytosis in epithelial cells uses NSF (*N*-ethylmaleimide–sensitive factor) and t-SNAREs (target membrane-soluble NSF attachment protein receptors) (*13*). Two t-SNAREs, syntaxins 2 and 3, are found on the epithelial apical surface (*14*); presumably, the pairing of one of these with the correct v-SNARE (vesicle SNARE) ensures accurate targeting of transcytotic vesicles. Osteoclasts likely also use SNAREs for membrane trafficking.

Transcytosis is more than just a disposal pathway. It is likely also an essential part of the regulatory system that balances the destruction of bone by osteoclasts with its rebuilding by osteoblasts. Transforming growth factor–β (TGF-β) and other members of the TGF-β superfamily, such as bone morphogenetic proteins, are important regulators of bone morphogenesis and remodeling (*1*, *15*), although their specific actions are not well understood. TGF-β is stored in a latent form bound to bone matrix, until it is released during oseoclastic bone resorption (*16*). Although the acid environment of the subosteoclast compartment could activate latent TGF-β derived from the bone matrix, its ultimate appearance in the neighboring extracellular space and action on other cells would require its transcytosis. This active TGF-β could then couple bone degradation to the deposition of new bone by osteoblasts.

Bone resorption and deposition are deranged in a variety of disease states. The most important of these is osteoporosis, a pandemic disease in postmenopausal Caucasian women and elderly men, which results from a relative excess of bone resorption over deposition, leading to weak, easily broken bones. Transcytosis is a highly regulated process in epithelial cells (*17*); transcytosis in osteoclasts is likely also to be highly regulated, offering a potential target for therapies aimed at controlling the excess resorption of bone in osteoporosis.



Transcytosis: a common practice. Membrane domains and trafficking pathways in an osteoclast (left) and an epithelial cell (right). Shown are homologous apical surfaces (blue), basolateral surfaces (green), the ruffled border and lysosomal membrane (red), and transcytotic vesicles (purple). The osteoclast can transcytose fragments of degraded collagen, and perhaps also TGF-β. The epithelial cell transcytoses certain membrane proteins to its apical surface, some with bound ligands, such as immunoglobulin A (IgA) bound to the polymeric immunoglobulin receptor (*17*). At the apical surface, the extracellular, ligand-binding domain of this receptor and the IgA are cleaved off and released.

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