The *PCSK9* alleles that provided a direct path to clinical translation confer protection from, rather than susceptibility to, CHD. Identification of protective alleles is limited by several factors: Alleles that cause disease are likely to be more frequent in clinical populations, whereas protective alleles are not and must be ascertained from the general population. Proving genetic association with protection from disease is more difficult than is proving association with increased risk of disease, and is only feasible for alleles present at appreciable frequencies in the study population. Because purifying selection is a powerful barrier to accumulation of deleterious alleles, few mutations with large phenotypic effects are likely to reach the requisite allele frequencies (*10*).

The effectiveness of statin therapy for CHD has been firmly established, yet substantial residual risk of disease remains in treated individuals (>50% in most studies). By contrast, comparable reductions in LDL associated with *PCSK9* mutations result in very low rates of incident CHD. This suggests that the residual risk of CHD in statin-treated individuals is not due to inadequate cholesterol lowering, or to other risk factors independent of LDL, but rather to delayed initiation of cholesterol-lowering intervention. Early intervention that ensures modest but lifelong reduction of plasma LDL may be the best approach to prevent CHD. Decreasing LDL by the requisite magnitude (~30%) can be easily achieved in most individuals by currently available agents. What then is the role of PCSK9 inhibitors?

Cost and convenience are considered the two major obstacles to large-scale use of antibodies against PCSK9. The importance of inconvenience as a barrier to antibody therapy may be overstated. Clinical experience with immunotherapy for allergy relief indicates that many patients will tolerate regular injections for years. The high cost of monoclonal antibodies (typically more than $1000/month) is likely to be a more substantial obstacle, particularly given that one of the most powerful and widely used statins is available in generic form for ~$10/month. Thus, antibody-based anti-PCSK9 therapy is likely to be targeted at individuals at high risk for CHD in the near term, and to those who do not achieve satisfactory LDL concentrations with, or are unable to tolerate, conventional cholesterol-lowering agents. A broader role for PCSK9-based therapy may have to await the development of small-molecule inhibitors.

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PALEONTOLOGY

Feathers Before Flight

Fossil data indicate that feathers and their precursors may have evolved over a much longer span than previously thought.

Julia Clarke

Feathers are branched structures consisting of β-keratin—a rigid protein material formed by pleated β sheets—with a hollow central shaft. They are strikingly different from other forms of vertebrate integument such as scales, skin, and hair. Until recently, evolutionary hypotheses envisioned their origin through elongation of broad, flat scales driven by selection for aerial locomotion such as gliding or flapping flight. Over the course of the past two decades, fossil discoveries, especially from northeast China, have revealed that the early precursors of feathers were filament-like rather than expanded scales and that branched pinnate feathers of modern aspect predate the origin of active flight. The revolution in our understanding of feather evolution continues, driven by rapid fossil discoveries and by new information from the study of extant birds.

One of the most transformative ideas to affect understanding of living birds has been the recognition of their perch within the tree of life on branches crowded with their extinct dinosaurian cousins. This insight came first from comparisons of bones, the most commonly preserved part of a fossil vertebrate. Fossilized soft tissues are only preserved in a few exceptional places (Lagerstätten). The Chinese deposits provide one such unique snapshot, where over a thousand specimens with fine details of soft tissues such as feathers, hair, and skin are preserved in ash-rich lake deposits ranging from the Late Jurassic (~150 million years ago) to the Early Cretaceous (~120 million years ago). Fossils from these deposits have revealed that dinosaurs that were inferred from bone characteristics to be closely related to living birds also share more features of feather structure.

The closest theropod dinosaur relatives of birds have pinnate feathers; more distantly related theropods have simple filaments or bunches of filaments of varying lengths and diameters (*1*, *2*). The latter forms do not fit the hypothesis of flat scales morphing directly into flat feathers. But these hollow filaments or "protofeathers" are similar to structures seen early in feather development; a simple hollow cylindrical sheath arises first in feather ontogeny from the collar of the feather follicle before the barb ridges, linked to the development of its branching shape, form (*3*). Fossil data indicated dramatic shifts from scale to filament, to bunches of filaments, to branched feathers in theropod dinosaurs. In the lineage of dinosaurs including birds, *Tyrannosaurus rex*, and many small raptors, filament- and feather-bearing species were common.

The more recent discoveries of a basal ornithischian dinosaur with a filamentous body covering, and another ornithischian more closely related to *Triceratops* with a bristle-covered tail, force reconsideration of the timing of this transition. These fossils indicate that filamentous structures may be ancestral to dinosaurs (*4*). Filaments called pycnofibers also covered some pterosaurs (*5*). Ornithischian dinosaurs, sauropod dinosaurs, and pterosaurs are on evolutionary branches that split from that of theropod dinosaurs and birds about 230 million years ago in the Triassic. If these structures have the same evolutionary origin, a form of filamentous integumentary structure evolved from scales nearly 100 million years before the locomotor transition that we call the origin of birds (see the figure).

The recent fossil data suggest that key integumentary shifts might be related not

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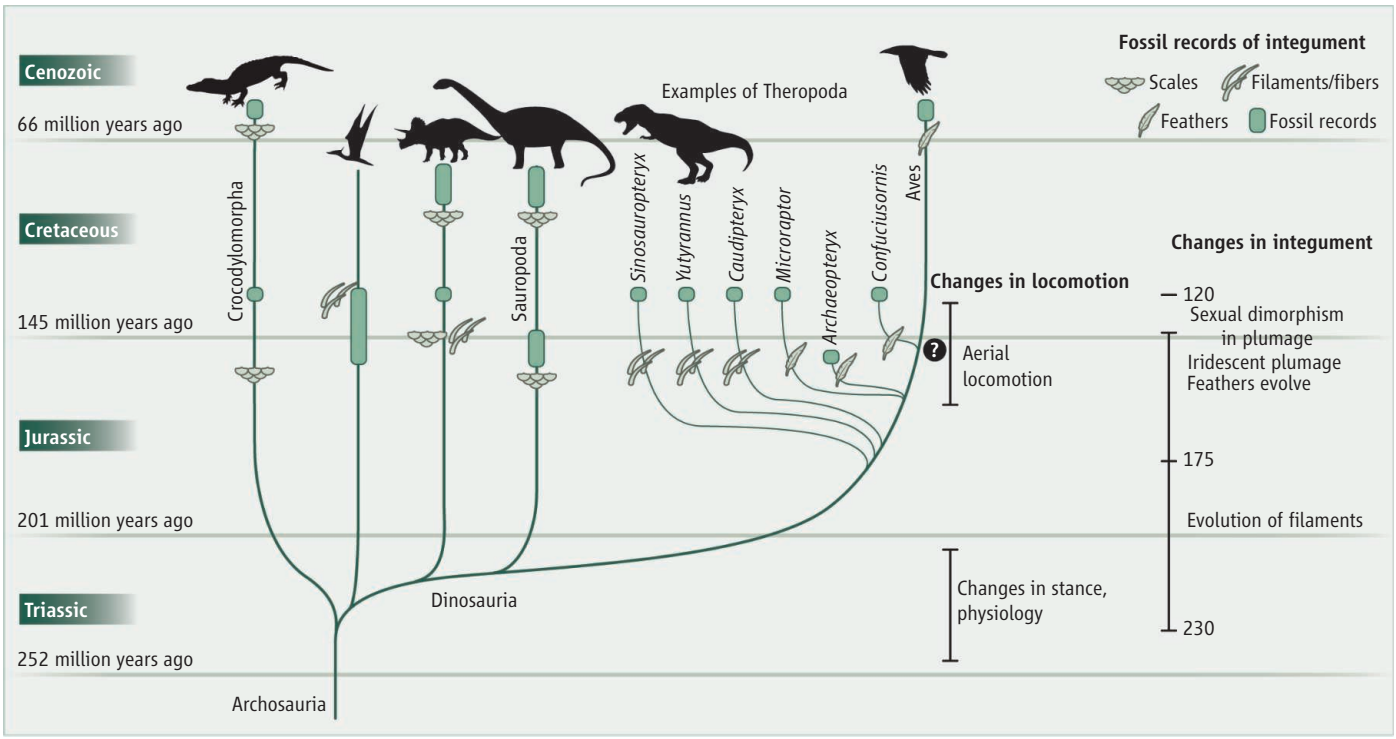

Preserved evidence of archosaurian body covering. The earliest preserved scales, filaments, or feathers are from the late Jurassic; the earliest crown clade bird with feathers is from the Paleocene. Filamentous feather precursors may have originated nearly 100 million years before the origin of flight, but very few fossil deposits sample this period. Sexual dimorphism in plumage and color patterning in Late Jurassic and Early Cretaceous dinosaurs suggest that display functions played a key role in the early evolution of pinnate feathers.

to flight but to innovation in stance, terrestrial gaits (*6*), and life history (*7*) in early archosaurs, which came to dominate terrestrial ecosystems by the end of the Triassic. However, there are unanswered questions. Were there at least three independent and convergent shifts from scales to filaments in Archosauria, with only one of these linked to the origin of feathers and flight? Or was there a single ancient origin of filaments, with subsequent losses in some species and, much later, a second period of novelty seen in the evolution of a branching feather form? Answering these questions is key to understanding the evolution of feathers and other integuments.

These questions send paleontologists back into the field. Early fossils of most major archosaur lineages are known from records in the Late Triassic and Early to Middle Jurassic (~225 to 165 million years ago). However, no dinosaur older than the Late Jurassic has been recovered with preserved integuments (scales or feathers). Early pterosaurs are virtually unknown in the fossil record; their earliest fossils with integuments are also Late Jurassic in age (see the figure). A Late Triassic or Early Jurassic site with fine-scale soft tissue preservation would offer crucial insight into this question. However, very few candidate sites are known.

The fossil snapshots that we do have offer much more insight into the evolution of pinnate feathers seen in living birds. There is no known analog of archosaur filaments in adult living animals, but bird feathers are known to have diverse functions, for example, in flight, display, camouflage, and heat retention. Iridescent feathers, long tail feathers, large color patches, and dimorphism are all linked to sexual selection in living birds. Recent findings suggest that early pinnate feathers also played a role in sexual selection. Investigation of the shape and form of fossilized melanin-containing organelles (melanosomes) has indicated that the forelimb and hind limb feathers of one maniraptoran dinosaur were patterned with large conspicuous patches of white with black spots (*8*). Inference of plumage color in the "four-winged" maniraptoran *Microraptor* yielded evidence of glossy or weakly iridescent feathers (*9*). Long tail feathers are known from many species during the transition to powered flight (*9*–*11*). Finally, evidence of sexual dimorphism in early birds was recently confirmed by recovery of a bone tissue unique to reproductively active female birds in a *Confuciusornis* specimen with feathering; specimens with long tail feathers were males and those with short tails, females (*11*).

Evidence is thus accruing for the function of early pinnate feathers in sexual selection, but there is little consensus on shifts in feather function associated with the evolution of flight. Reconstruction of ancestral conditions for the bird lineage requires consensus on the evolutionary relationships of key species. These species differ in feather shape as well as in their organization and layering on the forelimb and hind limb (*12*, *13*). Whether observed differences can presently speak to a gliding or flapping origin for flight is debated. Species with elongate feathers or a "wing" on the hind limb show characters consistent with a form of aerial locomotion but not one seen in living birds (*14*). At the same time, continued research indicates a broader variety of locomotor functions for forelimb feathering in living birds other than powered flight; young living birds flap short pinnate feathers on the forelimb, increasing traction to climb highly inclined surfaces (*15*). Although historically, feathers were firmly linked to flapping flight, the evolution of their early locomotor function in climbing, taking off, turning, landing, gliding, or flapping is a key outstanding question.

The evolution of feathers is now seen as one part of a broader story concerning the origin of novel integumentary structures in archosaurs, although data on the early parts of this story are very limited. New data multiply the set of questions we must ask about

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the locomotor transition that we call the evolution of flight. Model-based approaches are needed to explore the varieties of aerial and nonaerial locomotor strategies that extinct dinosaurs may have employed. These must take into account not only the diverse locomotor strategies in living birds but also potential differences in feather properties, shape, and plumage organization.

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IMMUNOLOGY

Crowdsourcing Immunity

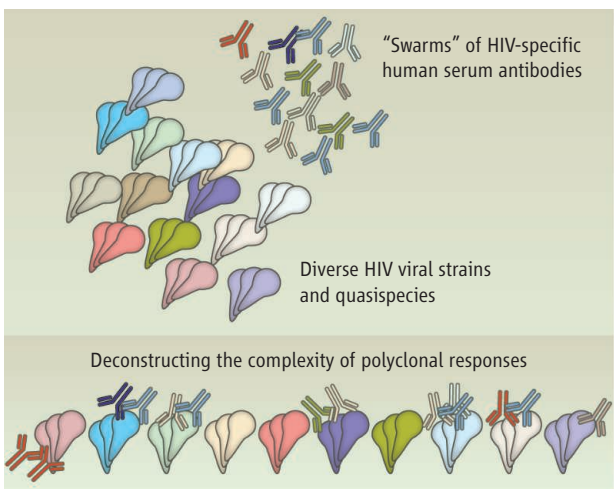
Computational methods reveal the nature and diversity of antibodies in HIV-infected individuals.

James E. Crowe Jr.

Many viral pathogens exhibit a dramatic variability in the sequence and structure of surface proteins that are targets of the humoral immune system, allowing them to evade inhibition by neutralizing antibodies. This constant shape-shifting generates swarms of viruses in infected hosts that are often termed "quasispecies," especially for agents such as HIV that cause chronic infections. On page 751 of this issue, Georgiev *et al*. (*1*) show that humans use complex and varied swarms of their own to respond to virus infection. Previously, it had been technically challenging to study the complex mixtures of antibodies found in serum in order to understand the clonal components of natural immune responses. These investigators address this with an approach that marries large-scale bioassays with computer algorithms.

Antibody engineers have recently made substantial progress in developing technologies for isolating monoclonal antibodies to viral pathogens such as influenza and HIV; these antibodies hold promise as biological drugs for passive immunization or treatment (*2*–*5*). However, it is not clear that the relatively specific recognition motifs of monoclonal antibodies can accommodate the diverse antigenic landscape in the swarms of viruses present in infected patients or populations. Fortunately, mammalian immune systems fight viral quasispecies with repertoires containing diverse variants of antibodies (*6*, *7*), so the alternative strategy of active vaccination to induce a broad repertoire of potent antiviral antibodies is promising.

Monoclonal antibody studies provide snapshots of the immune response, but what is also needed is a comprehensive method for the study of the global response of the antibody repertoire. Furthermore, the antibodies in serum, which derive principally from long-lived plasma cells in the bone marrow, may differ in specificity from the antibodies encoded in circulating memory B cells that are most often used for monoclonal antibody studies (*8*). Thus, methods for analyzing the clones in polyclonal serum are needed. The work of Georgiev *et al*. shows that patterns of recognition of complex mixtures of antibodies in human serum can be deconstructed by comparing their reactivity to a diversity of HIV field strains (see the figure). This simple approach could allow us to define the molecular and structural basis for neutralizing polyclonal antibody responses in great detail, thereby facilitating the rapid evaluation of the immunogenicity of experimental HIV vaccines.



Deconstructing the recognition pattern of complex mixtures of polyclonal antibodies. Probing the specificities of serum antibodies in HIV-infected subjects for reactivity to particular diverse field strains allows for the identification of the dominant antigenic sites recognized on the gp120/gp41 protective antigens by that individual.

There are several surprising and informative features of these new results. First, a relatively small panel of viruses (fewer than three dozen) reveals the pattern of antigenic recognition by polyclonal HIV immune serum. This finding provides a new technique for evaluating the quality and breadth of the antibody response to new experimental HIV vaccines, but moreover suggests that the variability in HIV field strains is tractable. Second, the results reveal different patterns of recognition among individuals. This finding is interesting because it has long been a mystery how viruses can use one or a few amino acid changes in a single epitope to escape the neutralization activity of polyclonal antibody responses directed to a large number of antigenic sites. Influenza virus antigenic drift is a classic example of this phenomenon. The results shown by Georgiev *et al*. with HIV suggest that each individual has a private experience characterized by dominant recognition of one or a few, but not all, major antigenic sites.

If the variability of viral glycoproteins such as the HIV envelope is infinite, then universal immunization against such pathogens will never be possible. Fortunately, viral variability is large but ultimately constrained. The HIV envelope in diverse strains exhibits a large degree of sequence variation and also length polymorphism and differences in predicted N-linked glycosylation sites. However, the scale of information on the observed variability is manageable. With contempo-

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