Ipk2p, and overexpression of either *MCM1* or *ARG80* rescues some of the phenotypes (*6, 8*). This argument is contradicted by the finding that yeast mutants lacking Plc1p or with defective Ipk2p kinase activity fail to use arginine and yet they contain normal amounts of Mcm1p and Arg80p (as measured by ArgR-MCM1 complex formation in vitro). This indicates that the ability of Ipk2p to stabilize Mcm1p or Arg80p is not sufficient for its regulation of transcription (*2*). A trivial resolution of this conflict is that deletion of Plc1p, or Ipk2p kinase mutations slightly decrease the abundance of Mcm1p and Arg80p so that they still bind to DNA in vitro but not in vivo, where chromatin interferes with DNA-protein interactions. A yet more interesting possibility is that Ipk2p may not only stabilize Mcm1p and Arg80p but may also facilitate their binding to chromatin. It should be noted that both effects may be bypassed by overexpression of *MCM1* or *ARG80*. This speculation is inspired by the observation that the inositol lipid PIP_2 (which regulates actin) and perhaps inositol phosphates as well, stimulate binding of the actin-containing, chromatin-remodeling BAF complex to chromatin in mammalian cells (*10*). Yeast probably have a similar actin-containing complex called ARI (*11*), which raises the possibility that inositol phosphates regulate chromatin remodeling to assist in the assembly of the ArgR-MCM1 transcription complex in vivo. Alternatively, IP_4 may directly bind to and regulate components of the general transcription machinery or the ArgR-MCM1 complex. This mechanism is reminiscent of an earlier observation that a low-activity form of human DNA polymerase α hydrolyzes PIP to IP, which in turn stimulates polymerase activity through a direct physical interaction (*12*).

Extracellular stimuli are capable of regulating the nuclear inositol signaling pathway. Insulin-like growth factor (IGF), when bound to its receptor in the plasma membrane, rapidly activates nuclear PLC-β1 in tissue culture, and an antisense RNA against PLC-β1 completely abolishes the mitogenic effect of IGF (*4*). In yeast, stress enhances IP_6 levels (*13*), which may increase the export of certain mRNAs that when translated into proteins counteract the stressful stimulus (*4*). External stimuli probably influence the nucleus in the same way that they affect the cytoplasm, that is, by controlling the localization or posttranslational modification of rate-limiting enzymes such as Plc1p. But there may well be unpredictable and exciting nuclear-specific variations on this theme.

Finally, the recent discoveries of York and co-workers also fuel speculation that other potential inositol lipid kinases such as FRAP/Tor may also regulate gene expression. Indeed, the yeast Tor proteins directly modulate transcription in response to multiple nutrient-sensitive signaling pathways, including the nitrogen discrimination pathway that also comes into play in arginine metabolism (*14*). Although current evidence indicates that Tor is a protein kinase, its putative lipid kinase activity is perhaps functionally important because the growth defect of certain Tor mutants can be corrected by overexpression of *PLC1* (*15*). It may be worthwhile investigating whether other members of the FRAP/Tor family, including the DNA repair proteins DNA-KC and ATM, regulate transcription. It is beginning to look as if the complexity of the inositol cycle in the cytoplasm is mirrored in the nucleus.

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PERSPECTIVES: EVOLUTIONARY BIOLOGY

Limbless Tetrapods and Snakes with Legs

Harry W. Greene and David Cundall

Evolution of an elongated body form and a reduction in limb size is observed in organisms as diverse as salamanders, weasels, and whales. Among the lizards, a serpentine (snakelike) body form has evolved dozens of times. Not surprisingly, the biomechanical, adaptive, and morphogenetic mechanisms that underlie this panoply of limbless and near-limbless tetrapods have long fascinated biologists (*1*, *2*). Now, some controversial and stunningly well-preserved ancient fossils from the Cretaceous sediments of Ein Yabrud in the Middle East are lifting the veil of mystery that surrounds the origins of snakes. In particular, the newest and best preserved Middle Eastern fossil, reported by Tchernov *et al.* (*3*) on page 2010 of this issue, seems set to lay to rest the notion that snakes started off life in the sea. The investigators place their fossil with its well-developed hindlimbs in a new taxon called *Haasiophis* and demonstrate that this Cretaceous serpent more closely resembles living terrestrial snakes, such as pythons and boas, than it does other extinct marine reptiles, such as mosasaurs. Their work underscores the need for precise nomenclature and explicit logic when attempting to infer the natural history of extinct organisms.

Although some pythons and other relatively primitive snakes have tiny, clawlike hindlimbs, used during courtship and in combat between males, limblessness has long been viewed as the essence of being a serpent (*2*). Recently, Caldwell, Lee, and their colleagues claimed that a Cretaceous fossil snake with obvious hindlimbs (also from Ein Yabrud) called *Pachyrhachis problematicus*, is the oldest known snake (*4*–*7*). They argue that mosasaurs (extinct marine lizards with limbs adapted as fins) represent "a crucial intermediate stage" in the evolution of modern snakes and that ancestral snakes had limbs and were aquatic. Their ideas contrast dramatically with the long-held view that snakes evolved from small, terrestrial lizards or even burrowing lizards by an increasing reduction in limb size (*2*, *8*).

Crown-clade snakes (suborder Serpentes) include the most recent common ancestor of blindsnakes (Scolecophidia) and other extant snakes (Alethinophidia), and all of its fossil and living descendants (see the figure, top of next page) (*8*). Caldwell and Lee (*4*) used several characteristics to classify *Pachyrhachis* as both "the most primitive snake" and the closest relative of animals traditionally called snakes (*5*). They showed their drawings and reconstructions of *Pachyrhachis* to a number of nonscientists, who use "snake" in its vernacular sense, and all identified *Pachyrhachis* as a snake rather than a lizard. Many people, however, apply the term "snake" to creatures as diverse as

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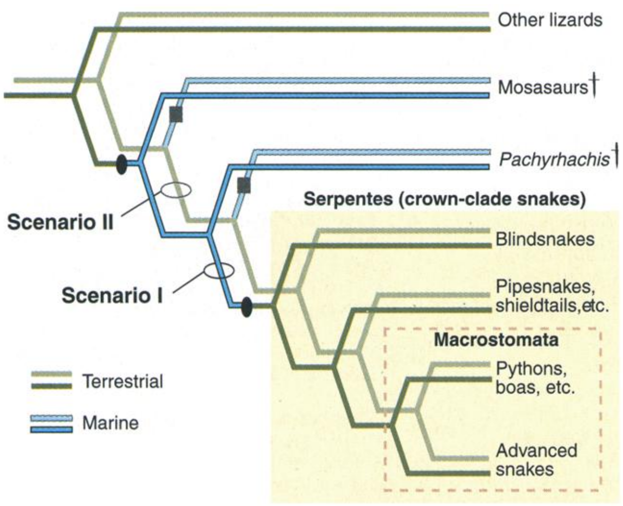


A serpentine classification. The crown-clade snakes (yellow shaded square) traditionally include blindsnakes, all other extant snakes, their most recent common ancestor, and all of its descendants. This is a stable classification anchored to living organisms whose "snakiness" is not in doubt. On this basis, fossils are considered snakes if they fall within that group phylogenetically, and not snakes if they don't. A more arbitrary approach considers some lizardlike fossils that are more primitive than crown-clade serpents to be snakes. In this simplified version of Caldwell and Lee's phylogenetic tree (*4*), blocks and ovals mark equally likely transitions between terrestrial (green) and marine (blue) environments. In Scenario I, the common ancestor of mosasaurs (marine reptiles) and snakes is marine, some of its descendants later returning to land to become the ancestor of crown-clade snakes. In Scenario II, the ancestors of mosasaurs and of *Pachyrhachis* enter marine environments independently.

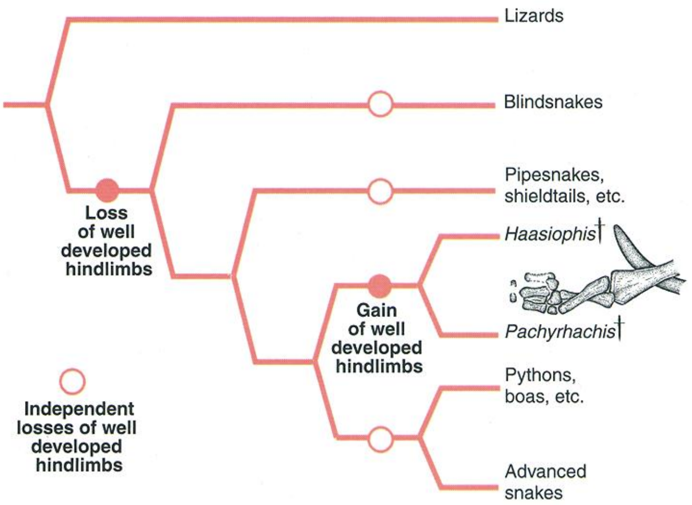
horsehair worms and elongate reptiles (for example, locals call a Mexican worm lizard with forelimbs *culebrita con manitos*, "little snake with little hands"). More importantly, Caldwell and Lee dodge the crucial question of the point on the phylogenetic tree stem connecting mosasaurs and *Pachyrhachis* where a fossil becomes more snakelike than lizardlike. They arbitrarily redefine "snake" such that *Pachyrhachis* must be one, then they contrast their evolutionary scenario with the traditional version, which is based on a narrower definition of the word snake.

Although they later clarified this point (*6*, *7*), Caldwell and Lee in their original paper (*4*) failed to note that a marine or a terrestrial origin for snakes would be equally parsimonious because each would require two evolutionary transitions (see the figure, top). In fact, a terrestrial-to-marine transition seems more likely (*9*) as it is a common theme among tetrapods—for example, modern whales evolved from a terrestrial ancestor. Even if extinct intermediates between mosasaurs and snakes were aquatic, all basal living snakes (that is, snakes that branched off early on the phylogenetic tree) are relatively small (maximum length ~1 m) and inhabit soil, litter, or wet tropical muck. Parsimony implies that the most recent common ancestor of modern snakes was also terrestrial (*2*, *8*). The popular press understandably has failed to catch the subtlety in defining "snake" and in counting evolutionary innovations, and the notion of a marine ancestry for snakes has rapidly gained acceptance among both biologists and the general public (*10*).

Building on their study of *Pachyrhachis*, Lee *et al.* (*6*) state that there are no intermediates between lizards with their relatively inflexible skulls and snakes with their highly mobile skulls. They then argue that the supposed jaw mobility of marine mosasaur lizards is a transitional step toward the enhanced gape of snakes. This surprising claim is contradicted by the biology of living snakes and by the phylogenetic analysis reported by Tchernov and coworkers (*3*). Blindsnakes, pipesnakes, and other basal crown-clade serpents generally do have mobile intramandibular joints (and thus superficially resemble some mosasaurs), but they also retain certain primitive gape constraints, feeding functions, and behavioral ecologies that are not found in more advanced snake groups (*11*, *12*). Most lizards eat large numbers of insects, each item weighing <1% of their own mass; blindsnakes retain that primitive diet. In contrast, shield-tailed snakes feed on earthworms, and pipesnakes tackle eels and other slender but heavy vertebrates (*2*). Unlike more primitive lineages, many macrostomatans (boas, pythons, and advanced snakes) ingest bulkier prey amounting to 100% or more of their own mass (*2*, *13*). Thus, in most respects, the greatly enhanced gape ascribed to "snakes" by Lee *et al.* (*6*, *7*) actually characterizes only that last subgroup of Serpentes; more primitive snakes exhibit feeding biologies intermediate between those of lizards and those of macrostomatans (*2*, *8*, *14*).

More detailed studies, highlighted by the analysis of Tchernov *et al.* (*3*), show that flawed morphological descriptions have led to erroneous conclusions about the phylogenetic position and evolutionary significance of *Pachyrhachis*. The skull and teeth of both *Pachyrhachis* and *Haasiophis* have many features in common with those of macrostomatans, whereas similarities with the skull and teeth of mosasaurs are superficial and convergent (*3*, *15*). The *Haasiophis* fossil together with *Pachyrhachis* can be more robustly interpreted as falling well within the crown-clade snakes rather than having diverged before its origin. One *Pachyrhachis* had the remains of a fossil fish in its stomach (*7*). This is consistent with fossil snakes being closely related to Macrostomata (*13*) because living boas also prey on bulky items (*2*). The fossil evidence supports the notion that *Haasiophis* and *Pachyrhachis* are more closely related to terrestrial Macrostomata than to marine mosasaurs (*3*, *15*).

The *Haasiophis* fossil described by Tchernov *et al.* (*3*) has bigger hindlimbs than *Pachyrhachis*, and tiny digits (see the figure, bottom). The presence of well-developed hindlimbs in both fossils implies that substantial reduction and even complete limb loss might have repeatedly occurred among pythons, boas, and more basal snakes. This alternative is plausible because external hindlimbs and pelvic elements vary mo-



Reinventing hindlimbs. Extreme reduction of hindlimbs in the ancestor of all snakes and their reappearance in the ancestor of *Pachyrhachis* and *Haasiophis* represents only two evolutionary transitions (filled circles). Alternatively, independent reduction of hindlimbs to vestiges or their absence in blindsnakes, pipesnakes, and Macrostomata suggest that their ancestors independently lost hindlimbs three times (open circles).

saically among and even within living snake species. External hindlimbs may be present as clawlike vestiges in both sexes or in males only, or may be absent; pelvic elements vary from none to three among basal snake taxa (*11*, *16*).

Conversely, well-developed hindlimbs in *Haasiophis* and *Pachyrhachis* might reflect a second evolutionary origin of the tetrapod hindlimb (see the figure, bottom of previous page). Thus, although the Tchernov fossil seems to have bones that closely resemble the femur, tibia and fibula, tarsals, and tiny toes of other tetrapods, they may not be strictly homologous. This surprising conclusion is bolstered by recent evidence for the regulation of vestigial leg morphogenesis in living pythons by the hox family of genes (*10*). In terms of evolutionary biology, those fossil hindlimbs are neomorphs (nonhomologs) because tetrapod hindlimbs are not found in the common ancestor of *Haasiophis*, *Pachyrhachis* and other snakes. However, they could be "latent homologs" (*17*) if genes that regulate hindlimb development (such as hox genes) persisted in those legless ancestors.

However much controversy the beautifully preserved skulls and bones described by Lee *et al.* (*4–7*) and Tchernov *et al.* (*3*) continue to generate, they are sure to figure prominently in our growing understanding of snake evolution. These Cretaceous Middle Eastern fossils also significantly broaden our understanding of the spectrum of possible relationships between body elongation and limb reduction in snakes (*1*, *10*, *11*). An unusual combination of hindlimb and skull features in these extinct forms, together with the considerable morphological variation among living taxa, and the proven applicability of molecular genetics, confirm that snakes will prove particularly valuable for integrative studies of development and evolution.

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PERSPECTIVES: MOLECULAR SPECTROSCOPY

H_3^+—an Ion with Many Talents

Benjamin J. McCall and Takeshi Oka

The H_3^+ ion plays an important role in diverse fields from chemistry to astronomy (see the figure). Not only does this most fundamental of molecular ions serve as a benchmark for quantum chemists, it was recently discovered both in molecular clouds (*1*) and in the diffuse interstellar medium (*2*), and it provides a tool for characterizing Jupiter's atmosphere from afar. At a recent discussion meeting in London (*3*), chemists, physicists, and astronomers came together to take stock of what is known about H_3^+ and take a glimpse into its future.

The H_3^+ molecular ion consists of three protons bound by two electrons and can be thought of as a hydrogen molecule (H_2) with an extra proton attached (H^+). This ion is the dominant positively charged ion in molecular hydrogen plasmas and was first identified in 1911 by J. J. Thomson, using an early form of mass spectrometry (*4*). Because H_3^+ lacks a stable electronic excited state (necessary for electronic spectroscopy) and a permanent dipole moment (necessary for rotational spectroscopy), the only spectroscopic probe of this ion is its infrared rotation-vibration spectrum, which was first observed in the laboratory in 1980 (*5*).

In the two decades since this initial spectroscopic observation, over 600 spectral lines of H_3^+ in low-energy ro-vibrational states have been detected. Using state-of-the-art computers, theoretical spectroscopists are now able to reproduce this laboratory spectrum with high accuracy from first principles and provide predictions of new lines to help guide laboratory work. Because H_3^+ is the simplest polyatomic molecule, these calculations for H_3^+ serve as a benchmark for calculations on other polyatomic molecules, such as water. In contrast to the low-energy spectrum, theorists have not yet been able to assign any of the over 27,000 spectral lines in the H_3^+ near-dissociation spectrum (*6*). If the sensitivity of the low-energy experiments can be substantially increased so that higher energy bands can be studied, and if the near-dissociation experiments can reach lower energies using visible lasers, the two techniques may eventually converge, leading to a complete theoretical understanding of this ion.

A collage of images related to H_3^+. In the laboratory, H_3^+ is produced in plasma tubes. The laboratory spectrum of the ν_2 vibrational mode (from A. R. W. McKellar) can be understood in terms of the H_3^+ potential energy surface. The emission of H_3^+ from Jupiter (from J. E. P. Connerney, shown here overlaid on a visible image) allows remote probing of the jovian aurora. H_3^+ also plays a key role in the chemistry of molecular clouds, such as the one in the upper left (imaged by the Hubble Space Telescope), and has been spectroscopically detected in absorption from sources such as NGC 2024 IRS 2 near the Horsehead nebula.

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A controversy surrounds the recombination of H_3^+ with electrons (*7*), the dominant destruction mechanism in some plasmas. In the past three decades, laboratory measurements of this recombination rate have differed by four orders of magnitude. The situation has improved, but discrepancies between different experiments remain, and the rate is still uncertain to within a factor of 10. To make matters worse, the best theoretical estimates of the recombination rate are

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