THY-1 and *ALP-1* loci are found on the long arm of Chr 11; it is therefore reasonable to suggest this chromosome as a potential site for a diabetogenic locus analogous to *Idd-2*.

REFERENCES AND NOTES

1. S. Makino *et al.*, *Exp. Anim.* **29**, 1 (1980).
2. S. Makino *et al.*, *ibid.* **35**, 501 (1986).
3. H. Nakajima *et al.*, *Immunol. Lett.* **12**, 91 (1986).
4. S. Makino *et al.*, *Exp. Anim.* **34**, 425 (1985).
5. M. Hattori *et al.*, *Science* **231**, 733 (1986).
6. M. Prochazka *et al.*, *Mouse News Lett.* **75**, 32 (1986).
7. E. H. Leiter *et al.*, in *Immunology of Diabetes*, G. D. Mollnar and M. A. Jaworski, Eds. (Elsevier Int. Congr. Series 717, Amsterdam, 1986), pp. 29–38.
8. C. E. Hildebrand, F. J. Gonzalez, C. A. Kozak, D. W. Nebert, *Biochem. Biophys. Res. Comm.* **130**, 396 (1985); D. W. Nebert kindly provided the plasmid pP_3450FL.
9. R. A. Jackson, Joslin Diabetes Center, Boston, MA, unpublished data.
10. Rules for Nomenclature of Genes, in *Mouse News Lett.* **72**, 2 (1985).
11. H. Acha-Orbea and H. O. McDevitt, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 2435 (1987).
12. R. D. Guttmann, E. Colle, T. Seemayer, *J. Immunol.* **130**, 1732 (1983).
13. E. M. Eicher, B. A. Taylor, S. C. Leighton, J. E. Womack, *Mol. Gen. Genet.* **177**, 571 (1980).
14. T. Shiroishi, T. Sagai, K. Moriwaki, *Microbiol. Immunol.* **25**, 1327 (1981).

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Modern Turtle Origins: The Oldest Known Cryptodire

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The discovery of a turtle in the Early Jurassic (185 million years before present) Kayenta Formation of northeastern Arizona provides significant evidence about the origin of modern turtles. This new taxon possesses many of the primitive features expected in the hypothetical common ancestor of pleurodires and cryptodires, the two groups of modern turtles. It is identified as the oldest known cryptodire because of the presence of a distinctive cryptodiran jaw mechanism consisting of a trochlea over the otic chamber that redirects the line of action of the adductor muscle. Aquatic habits appear to have developed very early in turtle evolution. *Kayentachelys* extends the known record of cryptodires back at least 45 million years and documents a very early stage in the evolution of modern turtles.

THE EVOLUTIONARY HISTORY OF turtles extends at least to Late Triassic time (200 million years before present), but the fossil record of their early diversification is incomplete. A substantial structural and temporal hiatus exists between the most primitive known form, *Proganochelys quenstedti* of the Late Triassic (middle Keuper of Germany) (*1*), and turtles with essentially modern features, which first appeared in the Late Jurassic (140 million years before present) (*2*). *Proganochelys* has a shell and is hypothesized as the sister group of all other turtles (*3*), but the skull has relatively few chelonian features. There are no characters of *Proganochelys* that indicate that this genus belongs to either the cryptodires or the pleurodires, the two groups of modern turtles. Both cryptodires and pleurodires possess distinctive specializations of the skull and postcranial skeleton consistent with the interpretation that they are monophyletic, and together they are united in a single monophyletic taxon, the Casichelydia (*4, 5*). The discovery of an Early Jurassic (185 million years before present) cryptodire (Fig. 1) extends the known history of the cryptodires back more than 45 million years and documents an intermediate stage in the evolution of modern turtles; the skull and shell structure of this new form represents an appropriate ancestral morphotype for all Cryptodira.

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The systematics:

Order Testudines
  Gigaorder Casichelydia
    Megaorder Cryptodira
      Family Kayentachelyidae, new
        Kayentachelys, new genus

Type species: *Kayentachelys aprix*, new species.

Known distribution: Early Jurassic, Arizona, United States.

Etymology: Kayenta, for the Kayenta Formation; *chelys*, Greek for turtle.

Diagnosis: As for species.

Kayentachelys aprix, new species

Type specimen: Museum of Northern Arizona V1558.

Locality: Gold Spring, Adeii Eechii Cliffs, Coconino County, Arizona (35°45′35″N, 111°04′51″W).

Horizon: Silty facies of the Kayenta Formation, Early Jurassic.

Etymology: *aprix*, Greek for tight, in reference to the fused basicranial articulation.

Referred specimens: MNA V1559-V1570; V2664. MCZ 8914-8917.

Diagnosis: A combination of primitive and advanced characters (*6*). Primitive amniote characters: pterygoid teeth, interpterygoid vacuity; prootic exposed ventrally. Primitive chelonian characters: nine costals; epiplastron with dorsal process. Derived casichelydian characters: antrum postoticum; fused basipterygoid articulation; 11 peripheral bones. Derived cryptodiran characters: processus trochlearis oticum; processes pterygoideus externus projecting posteriorly with a flat, vertical plate (*7*).

Turtles have often been cited as examples of "living fossils," a group that is structurally conservative throughout its history. In fact, however, this viewpoint is erroneous. Although all turtles appear superficially similar because they have a shell, there have been fundamental cranial and postcranial changes during their history. Cryptodires and pleurodires (Fig. 2), the two groups of living turtles, have independently evolved different trochlear mechanisms that redirect the main tendon of the jaw adductor muscle (*8*). As a result, the adductor, which has expanded posteriorly relative to the jaw joint, maintains a vertical line of action during jaw closure. In cryptodires the trochlea is formed by a thickened protuberance on the anterodorsal portion of the prootic and quadrate, whereas in pleurodires the trochlea is a lateral process of the pterygoid. *Kayentachelys* has the cryptodire trochlear condition. Another characteristic of cryptodires is an extensive fusion of the palatoquadrate and neurocranium that encloses the middle ear. Primitive amniotes and *Proganochelys* lack these advanced characters of the ear region and also have an open basipterygoid articulation. *Kayentachelys* represents an intermediate stage in that it has a fused basipterygoid articulation but lacks the distinctive cryptodiran posteroventral floor of the middle ear, exposing the prootic in ventral view. *Kayentachelys* also retains features that are primitive for turtles such as palatal teeth, an interpterygoid vacuity, a dorsal process on the epiplastron, and a ninth costal bone in the carapace. *Kayentachelys* thus shows that cryptodires evolved their distinctive trochlear pattern early in

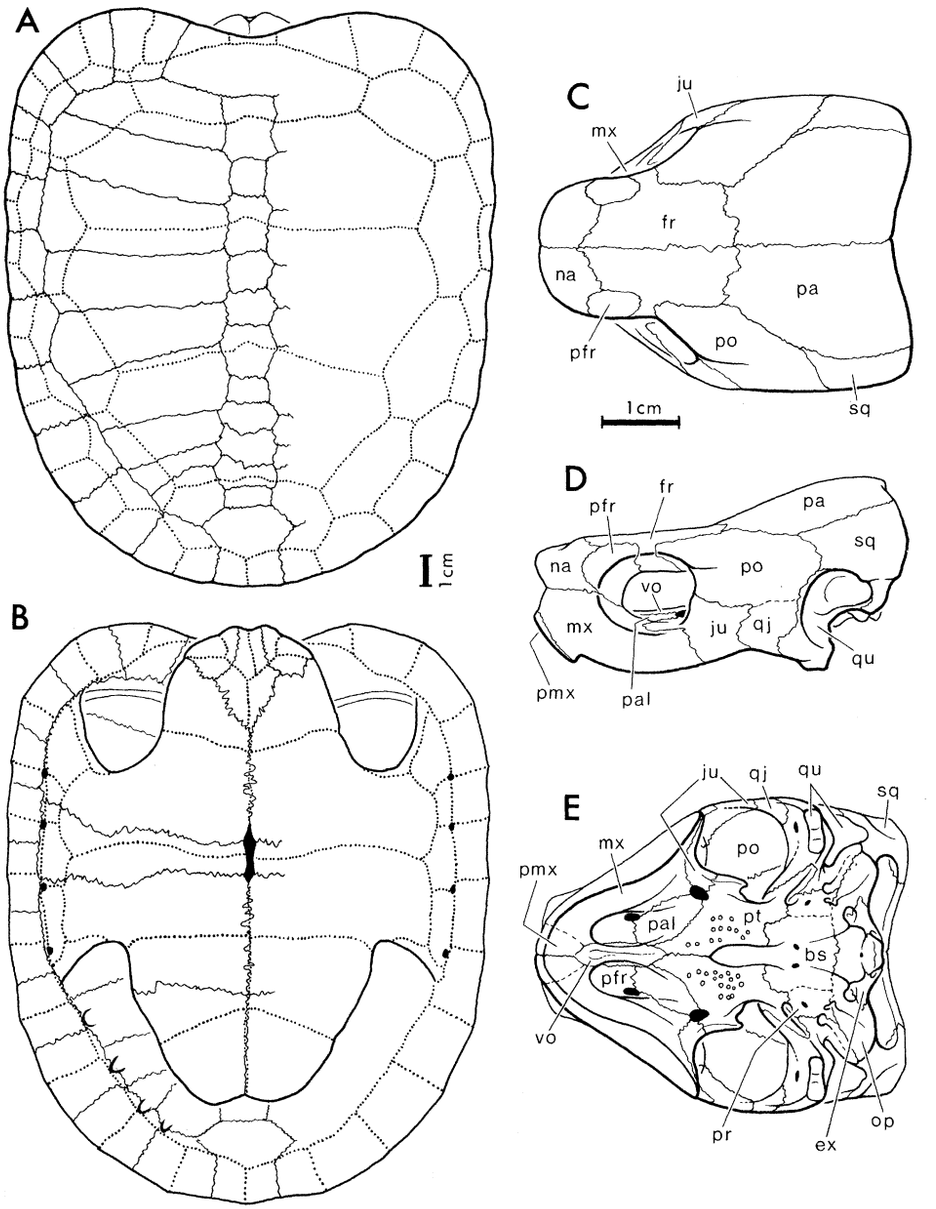

Fig. 1. Reconstruction of the skull and shell of *Kayentachelys aprix*, n. gen. et sp., based on MNA V1558, MCZ 8917. (**A**) Dorsal view of shell: bones are solid and scales dotted. (**B**) Ventral view of shell: bones are solid and scales dotted. (**C**) Dorsal view of skull. (**D**) Lateral view of skull. (**E**) Ventral view of skull. Abbreviations: bs, basisphenoid; ex, exoccipital; fr, frontal; ju, jugal; mx, maxilla; na, nasal; op, opisthotic; pa, parietal; pal, palatine; pfr, prefrontal; pmx, premaxilla; po, postorbital; pr, prootic; pt, pterygoid; qj, quadratojugal; qu, quadrate; sq, squamosal; vo, vomer.

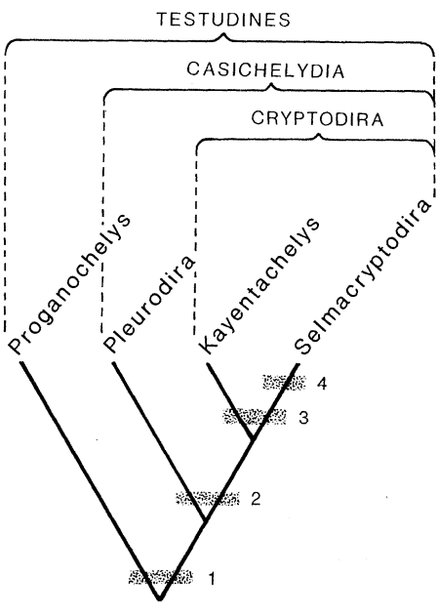

Fig. 2. A cladogram showing the relationships of *Kayentachelys* to other turtles. The shared derived characters for each of the numbered groups are: 1, Testudines: bony shell with costal and peripheral bones sutured together forming a disc-like carapace; plastron incorporating clavicles and interclavicles (*4, 5*). 2, Casichelydia: basipterygoid articulation fused, vomer single, middle ear with lateral wall, lacrimal bone and duct absent (*3–5*). 3, Cryptodira: processus trochlearis oticum present, processus pterygoideus externus projecting posteriorly with a flat, vertical plate. 4, Selmacryptodira: middle ear with ossified floor formed by a posteromedial process of the pterygoid preventing ventral exposure of the prootic (*10*). Derived characters for the Pleurodira may be found in (*4, 5*).

their history before acquiring many other features characteristic of modern representatives of the group.

Kayentachelys is the earliest known turtle to exhibit a shell that has all the features usually associated with an aquatic habitat. The sharp, tapered edges along the carapace margin, the apparently moderately low-domed shell, the expansion of the plastral lobes (restricting the ventral rather than lateral excursion of the limbs), and the absence of limb armor and coarse sculpturing on the carapace are all features that are often associated with aquatic turtles (*9*). Interpreting the mode of life of *Kayentachelys* can only involve speculation, but it is the oldest turtle with all of these typically aquatic features.

Kayentachelys is another classic fossil that fills a structural and temporal gap. Its greater significance, however, lies in the evidence it provides that much of chelonian evolution took place after the shell was acquired but before the specializations characteristic of modern turtles developed. Although the shell of turtles has been comparatively stable morphologically for 200 million years, giving rise to the popular conception of turtles as "living fossils," the skull, neck, and other structures were initially very similar to the generalized amniote condition and only later evolved diverse and complex specializations. *Kayentachelys* documents the early stages of the evolution of chelonian jaw mechanics, showing that the trochlear apparatus was one of the first specializations acquired by cryptodires.

REFERENCES AND NOTES

1. O. Jaekel, *Paläontol. Z.* **2**, 88 (1918); E. S. Gaffney, *Neues Jahrb. Geol. Paläontol. Abhl.* **170**, 1 (1985).
2. E. S. Gaffney, *Bull. Am. Mus. Nat. Hist.* **157**, 489 (1976); *ibid.* **162**, 91 (1979).
3. ——— and L. J. Meeker, *J. Vert. Paleontol.* **3**, 25 (1983).
4. E. S. Gaffney, *Bull. Am. Mus. Nat. Hist.* **155**, 387 (1975).
5. ———, *Syst. Zool.* **33**, 283 (1984).
6. Additional primitive chelonian characters are foramen posterius canalis carotici interni formed completely by basisphenoid; vertebral scutes much broader than long; pleural scutes longer than broad; four pairs of inframarginals; nine neurals sutured to vertebrae; first thoracic rib extends to peripherals and lies behind the tip of the axillary buttress; epiplastra separated ventrally by rostral process of entoplastron; pelvis not sutured to shell; one pair of mesoplastra meeting in midline; plastral buttress limited to peripherals. Additional derived casichelydian characters are cavum tympani deep and well developed; quadrate with well-developed medial wall for cavum acustico-jugulare; lacrimal bone and duct absent; supratemporal absent; cleithrum absent; four or more pairs of musk duct foramina.

Additional derived cryptodiran characters are ventral process of the prefrontal contacting vomer; supramarginal scutes absent; carapacial caudal notch absent; and 12th marginal scutes in mutual contact.

7. In addition to the presence of a cryptodiran processus trochlearis oticum, *Kayentachelys* also has two other hypothesized cryptodiran synapomorphies. The posteriorly directed processus pterygoideus externus with a vertical plate laterally is absent in *Proganochelys* and occurs only in *Kayentachelys* and other cryptodires. The vomer-prefrontal contact, present in *Kayentachelys* and other cryptodires is apparently absent in *Proganochelys*, although the area is poorly preserved and not definitely determinable.
8. G. H. Schumacher, in *Biology of the Reptilia*, C. Gans and T. Parsons, Eds. (Academic Press, New York, 1973), vol. 4, pp. 101–199.
9. The presence of frogs and other amphibians in the fauna together with the occurrence of limestone lenses and ripple marks at various levels within the formation is corroborative evidence of local aquatic conditions during Kayenta time.
10. The Selmacryptodira (Fig. 2) is a new term for the monophyletic group defined by the pterygoid process flooring the middle ear (*selma*, Greek for floor) and consisting at present of all cryptodires except *Kayentachelys*. For those using the classification of E. S. Gaffney (*5*) this requires the addition of a new category, the Capaxorder (*capax*, Greek for large) to be inserted between megaorder and hyperorder. The resultant higher classification of cryptodires would then be

Megaorder Cryptodira
  Capaxorder Kayentachelydia
    Family Kayentachelyidae
  Capaxorder Selmacryptodira
    Hyperorder Pleurosternoidea
    Hyperorder Daiocryptodira

11. We thank C. R. Schaff, W. W. Amaral, K. K. Smith, K. Padian, T. B. Rowe, J. M. Clarke and other members of the Harvard and Berkeley field teams for collection of specimens; W. W. Amaral, O. Simonis, K. Kishi, and A. Burke for preparation; F. Ippolito for illustration of the shell; and P. Meylan and E. E. Williams for thoughtful criticism and advice. We thank the Navajo Tribal Council and the Coal Mine Mesa Chapter (T. T. Nez, president) for permission to conduct paleontological exploration on Navajo land, and the National Geographic Society for support of the fieldwork. Also supported in part by NSF grants DEB 8002885 and BSR 8314816 to E.S.G. and DEB 78-01327 to F.A.J.

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Reversible Inhibition of Mammary Gland Growth by Transforming Growth Factor–β

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Transforming growth factor–β (TGF-β) can stimulate or inhibit growth of cells in vitro, as well as induce the transformed phenotype. Although widely distributed in animal tissue, the effects of TGF-β in vivo are largely unknown, and a physiological role for the peptide hormone has not been demonstrated. The effect of TGF-β on developing epithelial tissue in situ was studied by using slow-release plastic pellets containing TGF-β to treat developing mouse mammary gland. Powerful inhibition of mammary growth and morphogenesis was observed. This growth-inhibited mammary tissue was histologically normal, and the inhibitory effect was fully reversible. Under the conditions of these experiments, TGF-β displayed many of the characteristics expected of a physiologically active growth-regulatory molecule.

THE PEPTIDE GROWTH FACTOR called transforming growth factor–β (TGF-β) is found in cultured normal cells from connective tissues, various epithelia, and the immune system (*1–4*) and has been shown to inhibit proliferation of primary or secondary cultures of epithelial cells. The cell types affected include endothelium (*5*), bronchial epithelial cells (*6, 7*), mammary cells (*6, 8*), and hepatocytes (*9*). In some cases, inhibition is associated with terminal differentiation (*7*), while in others the inhibition is fully reversible (*10*).

Although the effects of TGF-β on cells and tissues in vivo are poorly understood, the wide distribution of both the peptide and its high-affinity receptors suggests that this peptide has a physiologic role (*3, 11*). The only known effects of TGF-β on tissues are rapid induction of fibrosis and angiogenesis when the factor is administered subcutaneously in wound chambers (*12*) or by injection (*13*). There have been no reports of TGF-β effects in vivo on any epithelial tissue, nor are there any examples of negative control by TGF-β in animals.

The effects of TGF-β on ductal growth in virgin mice were investigated with ethylene vinyl acetate copolymer (EVAc) implants that allow the slow release of bioactive molecules to small zones of the mammary gland (*14*). Implants containing TGF-β were placed in front of the mammary end buds (Fig. 1A, solid arrows), which are the focus for regulatory influences on ductal growth in the glands of the subadult virgin (*15*). End buds are bulbous, highly mitotic, multilayered epithelial structures found only during active growth; the numbers of end buds reliably indicate the rate of enlargement of the ductal tree (*16*). As an end bud approaches the edge of the gland's fatty stroma or is confined by ductal epithelium, forward growth ceases and the bulbous tip shrinks to the size of the duct (Figs. 1 and 2).

Human platelet–derived TGF-β, implanted for 4 days (Fig. 1B), caused the complete inhibition of end buds; a control implant containing bovine serum albumin (BSA) had no effect on the contralateral gland (Fig. 1A). Both treatment and control implants were placed in approximately the same location with respect to the growth zone, and it is clear that ducts in the treated gland did not elongate. TGF-β inhibition was limited to the implanted gland, with no observable perturbation of either the contralateral or ipsilateral glands, indicating that the factor was acting directly on the gland and not through a systemic intermediary. Thus TGF-β, acting locally, can inhibit the growth and morphogenesis of normal mammary epithelial tissue.

The implant contained 543 ng of TGF-β mixed with BSA as a carrier; the latter, as the major protein component of the implant, determined the release kinetics for TGF-β (*17*). From previous experience with the release of peptides from implants of this type (*18*), we estimate the TGF-β concentration in a 1-ml hypothetical volume around the implant to range from 8 ng/hour during the first 24 hours down to 1.3 ng/hour during the next 2 days. This range is comparable to TGF-β concentrations shown to affect cells in vitro (*2, 7, 10*).

Thymidine autoradiography was used to investigate the effects of TGF-β on glandular DNA synthesis. Growing end buds had numerous labeled cells (Fig. 2A), and previous studies have shown similar structures to have labeling indices of 15 to 20% (*19*). TGF-β treatment (Fig. 2C) reduced epithelial DNA synthesis to levels virtually identical with those seen in untreated, growth-quiescent ducts (Fig. 2B and Table 1). Stromal DNA synthesis normally accompanies ductal growth and is reduced in growth-quiescent glands (*20*). Levels of stromal DNA synthesis in TGF-β–treated glands were similar to those seen around terminal ducts from control tissue (Fig. 2) and in both cases were somewhat lower than in stroma around growing end buds (Table 1).

To investigate the reversibility of TGF-β–induced inhibition, we treated glands for 4 days to induce inhibition, after which the implant was surgically removed. Eleven days after TGF-β removal, end bud growth in the treated and contralateral control glands was determined. At 4 days, TGF-β had reduced end bud number by about 75% (Table 2). After the 11-day recovery period, end bud numbers in the treatment and control glands were the same, demonstrating complete recovery from inhibition. The distance that TGF-β–treated ducts grew beyond the implant is also indicative of growth inhibition and its subsequent recision. In 11 days after

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