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Evolutionary Development of the Middle Ear in Mesozoic Therian Mammals

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The definitive mammalian middle ear (DMME) is defined by the loss of embryonic Meckel’s cartilage and disconnection of the middle ear from the mandible in adults. It is a major feature distinguishing living mammals from nonmammalian vertebrates. We report a Cretaceous tretochthinian mammal with an ossified Meckel’s cartilage and the adult, showing that homoplasic evolution of the DMME occurred in derived therian mammals, besides the known cases of eutriconodonts. The mandible with ossified Meckel’s cartilage appears to be paedomorphic. Reabsorption of embryonic Meckel’s cartilage to disconnect the ear ossicles from the mandible is patterned by a network of genes and signaling pathways. This fossil suggests that developmental heterochrony and gene patterning are major mechanisms in homplastic evolution of the DMME.

References and Notes
8. See supporting material on Science Online.
15. Maya is distributed through the Autodesk EULA agreement.
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Supporting Online Material
www.sciencemag.org/cgi/content/full/326/5950/275/DC1 Materials and Methods Tables S1 and S2 Fig. S1 References 15 June 2009; accepted 21 August 2009 10.1126/science.1177734

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terian mammals of the Mesozoic are relatives to modern marsupials and placentals that make up 99% of living mammals today. The marsupial-placental clade, also known as living Theria, is successively nested within the boreosphenidan mammals, the peritrichobosphenian mammals, and the tretochthinian (1–6). The tretochthinian clade of living therians and spalacotheriods is one of 20 or so Mesozoic mammaliaform clades (1, 5).

Spalacotheriods are a basal group of the tretochthinian clade (6–10), characterized by the acute triangulation of the molar cusps' pattern,

B' and C are slightly conical, more closely resembling those of zhangheotheriods than those of other spalacotheriods. A wear facet is developed along the preparacrista (prevalum) between cusp A (paracone) and cusp B' on M1 and M2, which erupted first in the molar series. However, the facet is not yet developed on the more posterior molars that would erupt later (6, 7, 9). Thus, the match of upper and lower wear facets occurred after eruption and after substantial occlusal contact of the upper-lower molars. The triangulated shearing surfaces on the molars suggest that M. asiaticus was an insectivorous mammal.

M. asiaticus was a generalized terrestrial mammal. It represented a common ecomorphotype and lifestyle among a wide range of ecomorphotypes of Mesozoic mammals (18–21). M. asiaticus is estimated to have a total body length from 150 mm to 155 mm and to weigh between 72 (scaling from its 28.5-mm mandibular length) and 83 g (scaling from skull length of 36.5 mm) [details in (22)]. In the hind-foot digit ray III of M. asiaticus and M. sinensis, the intermediate phalanx is short relative to the proximal phalanx; the proximal and intermediate phalanges are short relative to the metatarsals. Both suggest a terrestrial habitat for Maotherium. That M. sinensis is a terrestrial mammal is also shown by its manual terminal phalax shape and by phalanged ratio that can be correlated with the terrestrial ecomorphotypes of extant mammals (23). Zhangheotherium and Maotherium are basal among spalacotheriods, and both had terrestrial habits (9, 10), suggesting that spalacotheriods ancestrally were terrestrial with generalized locomotory function (9, 10, 24).

M. asiaticus has an ossified Meckel’s cartilage. The cartilage is a compressed and tapering anterior limb that is solidly lodged in the
Meckelian groove of the mandible (Fig. 2, A and C). The cartilage is curved at mid-length, and its posterior limb is twisted relative to the anterior limb so the posterior end diverges away from the mandible (Fig. 2A, red and blue arrows). Its preserved part in *Maotherium* is more gracile than the Meckel's cartilage in situ with connection to the mandible in *Repenomamus* (12). It is identical to the Meckel's cartilage preserved in *Gobiconodon* (13) and the ossified Meckel's cartilage in *Yanoconodon*, which is connected to the ectotympanic and malleus (15). Thus, we infer that *Maotherium* has a similar, ossified connection between the mandible and the middle ear (Fig. 3C) as in eutriconodonts.

Basal mammaliaforms have both a postdentary trough and a Meckelian groove. The middle ear and its associated postdentary rod (partly homologous to the ossified Meckel's cartilage) are lodged in the postdentary trough. A classic problem of Mesozoic mammals is whether the absence of the postdentary trough would mean the loss of the connection between the middle ear and the mandible (14, 16). In *Maotherium*, the middle ear is connected by Meckel's cartilage to the mandible without a postdentary trough. Thus, the absence of a postdentary trough cannot exclude the possibility that the middle ear was still attached to the mandible. It adds to the evidence that Meckel's cartilage is evolutionarily labile within the spalacotheroid clade.

Because the Meckelian groove is present in the Early Cretaceous spalacotheroids *Maotherium* and *Zhangheotherium* but absent in the derived spalacotheroids of the Late Cretaceous (6), the adult retention of Meckel's cartilage occurred only in Early Cretaceous taxa of this clade and was likely lost in its Late Cretaceous members that show no trace of Meckel’s groove (6).

*Maotherium* corroborates an earlier observation that its related genus *Zhangheotherium* has a disarticulated Meckel's cartilage (12), but the new fossil of *Maotherium* (HGM 41H-III-0321) shows the Meckel's cartilage connected to the mandible in spalacotheroids and adds new anatomical information. Compared with *Yanoconodon*, the Meckel’s cartilage in *M. asiaticus* indicates that the middle ear is oriented at an angle to the mandible (Figs. 2C and 3). The mid-length curvature of Meckel’s cartilage made it feasible for its anterior limb to be nestled in the Meckel’s groove on the mandible, whereas its posterior limb was separated mediolaterally from the mandible (Fig. 2, A and C). From the curvature of Meckel’s cartilage, it can be inferred that the ectotympanic ring and the malleus manubrium should be oriented obliquely to the vertical coronoid-angular part of the mandible (Fig. 2) (15). Observation of *Maotherium* and *Yanoconodon* suggests that, before its disconnection from the mandible, the ancestral middle ear was already mediolaterally separated from the mandible (12, 15, 25).

DMME in extant mammals is accomplished by two ontogenetic steps (25–27): first, a mediolateral separation of the middle ear anlage from
the mandible in embryonic stages (seen in extant monotremes and placentals); second, a loss of connection to the mandible by reabsorption of Meckel’s cartilage in fetal stages. In *Maotherium* and eutriconodonts, the mediolateral separation of the middle ear from the mandible had already occurred. However, Meckel’s cartilage is ossified, and its reabsorption never happened, resulting in retention of a middle ear connection to the mandible otherwise seen only in the embryonic or fetal stage of extant mammals.

The middle ear of *Maotherium* and eutriconodonts shows a paedomorphic resemblance to the embryonic pattern of modern monotremes and placentals in which the middle ear is mediolaterally separated from the mandible but still connected via Meckel’s cartilage to the mandible (25–27). All that is necessary for adult eutriconodonts and spalacotheroids to retain this paedomorphic mandibular ear connection is a relatively earlier timing in ossification of the Meckel’s cartilage. The homoplastic separation of the middle ear from the mandible in Mesozoic mammals is correlated with ontogenetic heterochrony.

By parsimony of all characters (1–6, 15), *Maotherium* and its spalacotheroid clade are more closely related to living therians than to multituberculates and eutriconodonts, all within Mammalia (Fig. 3) (22). *Maotherium* is similar to eutriconodonts in having the middle ear connected to the mandible in adults (Fig. 3 right side, DMME absent) but conspicuously different from living therians, multituberculates (22, 28), and modern monotremes (and possibly *Hadrocodium*), in which the middle ear is separated from the mandible in adults (Fig. 3 left side, DMME present). The separation of the middle ear in mammalian phylogeny may have occurred by two alternative evolutionary scenarios: (i) DMME was present in the common ancestor of Mammalia (Fig. 3, node 1), but eutriconodonts and spalacotheroids reevolved the middle ear attachment to the mandible; or (ii) DMME was absent in the common ancestor of mono-

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**Fig. 2.** Dentition and mandible of *Maotherium asiaticus* (HGM 41H-III-0321; holotype) and interpretation of its middle ear. (A) The preserved Meckel’s cartilage (ossified) and its attachment to the mandible (stereophotos). (B) Upper postcanines (P1 to M5) (stereophotos). (C) Interpretive restoration of the middle ear (dashed line) and its attachment to the mandible [as in (A)]. Red arrow, mid-length curvature of Meckel’s cartilage (identical to eutriconodonts); blue arrow, mediolateral separation; the ectotympanic and malleus are conjectural, based on the middle ears of multituberculates and Yanoconodon. (D) Upper premolar and molars [oblique view, as in (B)]. (E) Mandible (reconstruction, lateral view).

**Fig. 3.** Homoplastic evolution of the mammalian middle ear by paedomorphic retention of ossified Meckel’s cartilage among extant clades of mammaliaforms. Right, ancestral conditions of ossified Meckel’s element in premammalian mammaliaforms, and in paedomorphic retention of ossified Meckel’s cartilage in extinct clades of Mammalia. Left, living mammal and mammaliaform clades achieved the reabsorption of embryonic Meckel’s cartilage and the DMME. (A and B) Extant therian *Monodelphis*: mandible [(A), medial view] and middle ear [(B) medial view]. (C and D) Extinct multituberculate mammal *Lambdopsalis*: absence of Meckel’s cartilage on the mandible [(C) medial view] and separated middle ear ossicles [(D) ventral view] in adult [modified from (28)]. (E) Extant monotreme *Ornithorhynchus*: reabsorption of embryonic Meckel’s cartilage and separation of middle ear from the mandible (top, medial view; bottom, ventral view) in adult. (F) Mammaliaform *Hadrocodium*: mandible (medial view) without Meckel’s groove for middle ear attachment. (G) Partial restoration of the spalacotheroid *Maotherium asiaticus*: ossified Meckel’s cartilage connecting the middle ear to mandible (medial view); hypothetical restoration of ear ossicle based on other Mesozoic mammals. (H) The eutriconodont *Yanoconodon* (and gobiconodontids): ossified Meckel’s cartilage connecting middle ear to the mandible but ossicles partially (mediolaterally) separated from the mandible. (I) Mammaliaform *Morganucodon*: ossified Meckel’s elements and the middle ear in the postdentaly trough and Meckel’s groove of the mandible. (Tree simplified from the phylogeny in [22]); phylogenetic tree nodes 1 and 3 to 6 as labeled; node 2 (*Hadrocodium*) represents an unnamed mammaliaform clade.
tremes, eutriconodonts, and the living therians, and this ancestral feature is retained in eutriconodonts and spalacotheroids; but DMME evolved in extant monotremes (14) and in multituberculates for a second time, and then again for a third time in marsupials and placentals.

Paedomorphism, or retention of fetal or juvenile characteristics of ancestors and/or relatives through developmental heterochrony, is a common phenomenon in vertebrate evolution. Scenario i gains support from the paedomorphic common phenomenon in vertebrate evolution.

For a second time, and then again for a third time

and this ancestral feature is retained in eutriconodonts, eutriconodonts and spalacotheroids to that of extant mammalian embryos (15). The premature ossification of Meckel’s cartilage represents a simple developmental change in timing (heterochrony), for which a genetic mechanism has been established (29, 30).

A mutant genetic and signaling network that can result in a premature ossification of Meckel’s cartilage in mammalian embryogenesis has been characterized by recent genetic studies. Meckel’s cartilage derives from cranial neural crest cells; it serves as scaffolding for development of mandibular and middle ear elements. Normal development of the Meckel’s cartilage and its derivatives in vertebrates requires a wide range of structural and homeobox genes, such as Bapx1, Gsc, Enmx2, Sox9, and Type II Coll., which are expressed in the mammalian middle ear structure [reviewed by (30)]. Morphogenesis of Meckel’s cartilage also requires a variety of growth factors, some of which are ubiquitous in mammalian development. This complex signaling network for Meckel’s normal development includes transforming growth factors-β (Tgf-β) (29), connective tissue growth factors (Ctgf) (29), fibroblast growth factor (Fgf) (30), epidermal growth factor (Egf) (31), and bone morphogenetic proteins (Bmp), among others.

In normal chondrogenesis of Meckel’s cartilage of Mus (wild type), the signaling pathway from Tgf-β (upstream) to Ctgf (downstream) stimulates the proliferation, and inhibits the terminal differentiation, of chondrocytes (29). Mutant Tgfbr2–/–;Wnt1-Cre genes (a mutant of Tgf-β) accelerate chondrocyte proliferation and cause ossification of Meckel’s cartilage in mutant Mus (29). The phenotype of ossified Meckel’s cartilage in Tgfbr2–/–;Wnt1-Cre mutant mice is similar to the fossilized and (prematurely) ossified Meckel’s cartilage in the spalacotheroids (Fig. 2) and eutriconodonts (Fig. 3). This suggests that some similar developmental pathway had underlined the ossification of Meckel’s cartilage in extinct Mesozoic mammals.

Among living mammals, an ossified Meckel’s cartilage occurs only in certain mammal mice; the cartilage is retained only in pathological cases among humans. However, ossified Meckel’s cartilage evolved at least twice in Mesozoic spalacotheroids and eutriconodonts. The absence of ossified Meckel’s cartilage in the adult in extant monotremes, marsupials, and placentals represents a more-canalized development of the middle ear for these living lineages, in contrast to a much more labile evolutionary development of middle ear features, made possible by a greater diversity of about 20 Mesozoic mammaliform clades (1, 5).

References and Notes
17. Systematics: Class Mammalia; Clade Theria (U); Superfamily Spalacotherioidea (9, 10); Family Zasigiatheriidae; Genus Moaetherium (10); sp. nov. Moaetherium asiaticum. Holotype: Henan Geological Museum, Zhengzhou, China (HGM 411-H1-0321; Figs. 1 and 2), an almost complete skull, and most of the postcranial skeleton. Eology: asistasi, Latin for the Asiatic provenance of zasigiatheroids. Locality and Age: Lujianzhuang Locality (120°54′E, 36°48′N), Henan, China, the Yixian Formation. The site dated to be 123.2 ± 1.0 million years old (27). Diagnosis: I3, C1, P1, M5; I3, I3, c1, p1, m6. M. asiaticum is referred to Mautheriom by apomorphies shared by Moaetherium sinensis (type species): deep pectoral foramen of mandible, a deep ectoecus on upper molars with cusp B; a gradient in increasingly acute angle in trigulation of molar cusps toward the posterior molar, sigmoidal shape of the posterior ventral border of mandible. Differs from M. sinensis in having apomorphies of low central cusp between B’ and C; M. asiaticus has the tubal distal stylid process and the promotoral process of the metatarsal 5 (all features absent in M. sinensis). Full diagnosis in (27).
22. Materials and methods are available as supporting material on Science Online.
32. We thank G. Cui for fossil preparation; M. R. Dawson for improving the manuscript; and R. Asher, Y. Chai, and A. Tucker for discussion. Support from the 973 Project of Ministry of Science and Technology of China (Q.J.); from Institute of Geology of Chinese Academy of Geological Sciences (Beijing (C.-X.Y.)), from NSF (USA), National Natural Science Foundation (China), and National Geographic Society (Z.-L.Z.); and from Henan Provincial Government (to Z.-L.Z. and L.X.).

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References
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Daily Electrical Silencing in the Mammalian Circadian Clock

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Neurons in the brain’s suprachiasmatic nuclei (SCNs), which control the timing of daily rhythms, are thought to encode time of day by changing their firing frequency, with high rates during the day and lower rates at night. Some SCN neurons express a key clock gene, period 1 (per1). We found that during the day, neurons containing per1 sustain an electrically excited state and do not fire, whereas non-per1 neurons show the previously reported daily variation in firing activity. Using a combined experimental and theoretical approach, we explain how ionic currents lead to the unusual electrophysiological behaviors of per1 cells, which unlike other mammalian brain cells can survive and function at depolarized states.

In mammals, behavior and physiology are regulated on a daily basis by the brain’s master circadian (~24-hour) clock in the suprachiasmatic nuclei (SCNs). The period 1 (per1) gene is a key component of the molecular mechanism of this clock (1); its expression in the SCN peaks during the day and is low at night, and can be used as a marker of clock-containing SCN neurons and their circadian phase (2, 3). SCN neurons are also thought to express time of day by changing their firing frequency, with high rates during the day and lower rates at night (4–6). A fundamental question in circadian biology is how the intracellular molecular circadian clock regulates the electrophysiology of SCN neurons. A Hodkin-Huxley-type model of SCN neurons shows that circadian changes in ionic conductances can account for the circadian variation