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 therotherian mammal with an ossified Meckel’s cartilage in the adult, showing that homoplastic evolution of the DMME occurred in derived therian mammals, besides the known cases of eutherians. 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.

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ys of therian 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 boreocynodontian mammals, the peribrachiosphenic mammals, and therchonothians (1–6). The trechonothian clade of living thersians and spalacotheroids is one of 20 or so Mesozoic mammaliform clades (1, 5).

Spalacotheroids are a basal group of the trechonothian clade (6–10), characterized by the acute triangulation of the molar cusp pattern, a precursor condition to the tribosphenic molars of the common ancestor to marsupials and placentals (1–10). Spalacotheroids have ancestral skeletal features of living thersians (9, 10). Here, we describe an Early Cretaceous spalacotheroid (Figs. 1 and 2) that sheds light on the evolution of the definitive mammalian middle ear (DMME) (11). Whether this key mammalian feature had a singular origin or had evolved multiple times is still being debated (11–16).

Maotherium asiaticus sp. nov. (17) shows a diagnostic pattern of main molar cusps arranged in an almost symmetric triangle, thus also known as symmetrodont molars. The postcanines show an increasingly acute (smaller) angle between cusps B’-A-C from the premolar toward the more posterior molars, a gradient in all symmetrodont molars but most prominently developed in spalacotheroids (6–10). The upper cusp B’ and C are slightly conical, more closely resembling those of zhangheotherians than those of other spalacotheroids. A wear facet is developed along the preparacrista (prevallum) 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 metatarsal. Both suggest a terrestrial habit for Maotherium. That M. sinensis is a terrestrial mammal is also shown by its manual terminal phalanx shape and by phalangeal ratio that can be correlated with the terrestrial ecomorphotypes of extant mammals (23). Zhangheotherium and Maotherium are basal among spalacotheroids, and both had terrestrial habits (9, 10), suggesting that spalacotheroids ancestrally were terrestrial with generalized locomotory function (9, 10, 24).

M. asiaticus has an ossified Meckel’s cartilage. The cartilage has a compressed and tapering anterior limb that is solidly lodged in the

References and Notes
8. See supporting material on Science Online.
15. Maya is distributed through the Autodesk EULA agreement.
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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 postdental trough and a Meckelian groove. The middle ear and its associated postdental rod (partly homologous to the ossified Meckel’s cartilage) are lodged in the postdental trough. A classic problem of Mesozoic mammals is whether the absence of the postdental 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 postdental trough. Thus, the absence of a postdental 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 Yanoconodon has a disarticulated Meckel’s cartilage (12), but the new fossil of Maotherium (HGM 41H-III-0321) shows that the Meckel’s cartilage is 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 anlagen 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 3), but eutriconodonts and spalacotheroids reevolved the middle ear attachment to the mandible; or (ii) DMME was absent in the common ancestor of mono-
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.

Paedomorphosis, 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 similarity of the ossified Meckel’s cartilage in 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, Emx2, 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 and 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 and 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 Chordotheria (1); Superfamily Spalacotherioidea (9, 10); Family Zhegeheotheriidae; Genus Meotherium (10); sp. nov. Meotherium asiaticus. Holotype: Henan Geological Museum, Zhengzhou, China (HGM 41H-III-0321; Figs. 1—41). Full diagnosis in (29). Type model of SCN neurons is referred to Meotherium by apomorphies shared by

Mootherium sinensis (type species): deep precondylar notch of mandible, a deep ectoflexus on upper molars with cusp B; a gradient in increasingly acute angle in triangulation of molar cusps toward the posterior molar, sigmoidal shape of the posterior ventral border of mandible. Differ from M. sinensis in having apomorphies of low central cusp between B’ and C; M. asiaticus has the bital distal stylid process and the promolar lateral process of the metatarsal 5 fall three features absent in M. sinensis). Full diagnosis in (22).
22. Materials and methods are available as supporting material on Science Online.
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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 Hodgkin-Huxley-type model of SCN neurons shows that circadian changes in ionic conductances can account for the circadian variation

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