Noncoding RNAs of Trithorax Response Elements Recruit Drosophila Ash1 to Ultrabithorax

Tilman Sanchez-Elsner,1 Dawei Gou,1 Elisabeth Kremmer,2 Frank Sauer1*

Homeotic genes contain cis-regulatory trithorax response elements (TREs) that are targeted by epigenetic activators and transcribed in a tissue-specific manner. We show that the transcripts of three TREs located in the Drosophila homeotic gene Ultrabithorax (Ubx) mediate transcription activation by recruiting the epigenetic regulator Ash1 to the template TREs. TRE transcription coincides with Ubx transcription and recruitment of Ash1 to TREs in Drosophila. The SET domain of Ash1 binds all three TRE transcripts, with each TRE transcript hybridizing with and recruiting Ash1 only to the corresponding TRE in chromatin. Transgenic transcription of TRE transcripts restores Ubx expression in Drosophila cells and tissues that lack endogenous TRE transcripts. Small interfering RNA–induced degradation of TRE transcripts attenuates Ash1 recruitment to TREs and restores Ubx expression, which suggests that noncoding TRE transcripts play an important role in epigenetic activation of gene expression.

The identity of cells in metazoan organisms is established during development and mitotically propagated throughout the entire life cycle. Phylogenetically highly conserved protein families of epigenetic regulators determine the fate of developing cells by establishing and maintaining mitotically stable gene expression programs (1–4). In Drosophila, members of the trithorax group (trxG) of epigenetic regulators maintain active transcription states, whereas members of the Polycomb group (PcG) maintain repressed transcription states (2–4), and Repressors (Enhancer of zeste) proteins (5) are lysine-specific histone methyltransferases (HMTs) and contain a SET domain, the catalytic hallmark motif of HMTs. Methylation of histone H3 (H3-K4) and repression [Lys6 and Lys27 in H3 (H3-K4)] (6–8). Several epigenetic activators [Trx, trithorax–related (Trr)] and repressors (Enhancer of zeste) are lysine-specific histone methyltransferases (HMTs) and contain a SET domain, the catalytic hallmark motif of HMTs. Methylation of lysine residues in histones H3 and H4 has been correlated with epigenetic activation [Lys4 in H3 (H3-K4)] and repression [Lys8 and Lys27 in H3 (H3-K4)] (6–8).

We previously showed that the epigenetic activator “absent small and homeotic discs” (Ash1) promotes transcriptional activation by trimethylating H3-K4, H3-K9, and Lys20 in H4 (H4-K20) (9). Ash1 maintains activated transcription states in larval imaginal discs that give rise to the appendages in the adult fly (10, 11). For example, Ash1 is essential for the expression of the homeotic gene Ultrabithorax (Ubx) in third-leg and haltere imaginal discs, and Ubx expression coincides with Ash1-mediated histone methylation (9–11).

PcG and trxG regulators are recruited to specific chromosomal elements that are present in the cis-regulatory region of target genes (2–4). The same element can act as an activating or a silencing module (4). In the repressed state, the elements represent Polycomb response elements (PREs) and facilitate the recruitment of PcG proteins (2–4). In the activated state, the DNA-elements function as trithorax response elements (TREs) and recruit trxG proteins (3, 4).

Transcription of noncoding RNAs (ncRNAs) from TRE/PRE elements switches silent PREs into TREs, which indicates that TRE/PRE transcription plays an important role in epigenetic activation (12–15). How transcription of TREs culminates in the recruitment of trxG regulators is unknown. Here, we address the question of how epigenetic regulators without known DNA binding capabilities, such as Ash1 (16), recognize and bind target genes in chromatin.

Ubx TREs are transcribed in Drosophila imaginal discs. The coincidence of the tissue-specific transcription and trans-regulatory activity patterns of TREs and trxG proteins, respectively, suggests that not only TRE/PRE transcription but also the resulting ncRNAs might play a role in epigenetic activation (12–15). Here, we analyze the role of ncRNAs transcribed from three Ubx TRE/PREs. The Ubx locus contains a cluster of three characterized TRE/PREs (TRE1 to TRE3) within the boundaries of the chromosomal memory element (CME) bxd that is located 22 kb upstream of the Ubx promoter (Fig. 1A) (17, 18).

To correlate the transcriptional activity of Ubx with bxd transcription in Drosophila, we used rapid amplification of cDNA ends (RACE) to detect bxd transcripts in third-leg discs. Three capped, polyadenylated bxd transcripts transcribed by RNA polymerase II were detected in third-leg and haltere discs (tre1, tre2, tre3) (Fig. 1A) (19).

We next used the reverse transcription polymerase chain reaction (RT-PCR) to determine nonprecipitates detecting the association of Ash1 with Ubx TREs in imaginal discs and S2 cells. Input represents the amount of transcripts or TREs detected in 0.5% of the starting material.

![Fig. 1. Cell type-specific transcription of Ubx TREs.](image-url)
Fig. 2. Recruitment of Ash1 to Ubx TREs in third-leg imaginal discs. (A) PCR analysis of XChIP immunoprecipitates detecting the association of Ash1 and the Ash1 histone methylation pattern at the TREs and promoter of Ubx in wild-type (WT) and ash122 mutant third-leg imaginal discs. In vivo cross-linked chromatin was immunoprecipitated with the indicated antibodies and rat/rabbit antiserum (control). Input represents the amount of TRE-1 detected in 0.5% of the starting material. (B) XChIP analysis as described in (A), except that chromatin was immunoprecipitated with an antibody to dimethylated H3-K9. (C) RT-PCR analysis detecting bx d transcripts in RNA pools isolated from wild-type (WT) and ash122 mutant third-leg discs or in genomic DNA (G).

whether the presence of the three TRE transcripts coincides with Ubx transcription. RNA was isolated from third-leg discs and haltere imaginal discs (haltere discs), which both transcribe Ubx, and from wing imaginal discs (wing discs) and embryonic Drosophila Schneider 2 (S2) cells that do not transcribe Ubx (9–11). Transcripts from Ubx and all three TREs were detected in third-leg and haltere discs, whereas Ubx and TRE transcripts were not detected in S2 cells and wing discs (Fig. 1B) (figs. S1 and S2).

Recruitment of Ash1 to Ubx TREs. To investigate whether Ash1 is recruited to transcriptionally active Ubx TREs, we used in vivo cross-linked chromatin immunoprecipitation (XChIP) to detect Ash1 at the Ubx TREs in third-leg, haltere, and wing discs and in S2 cells, all of which express ash1 (9, 10). Ash1 was detected at all three TREs in third-leg and haltere discs (Fig. 1C). In addition, the characteristic Ash1 histone methylation pattern was detectable in all three TREs and the transcriptionally active Ubx promoter in third-leg discs (Fig. 1C and Fig. 2A). Ash1 was not detected at the TREs of the transcriptionally inactive Ubx locus in wing discs and S2 cells, which do not transcribe TREs (Fig. 1C).

We also compared the recruitment of Ash1 to Ubx in wild-type and homozygous mutant ash122 third-leg discs by XChIP. The ash122 mutant is recessive lethal and expresses a truncated protein that lacks the SET domain and trans-activation activity (10). Ash1 and the characteristic Ash1 histone methylation pattern were detected at the transcriptionally active Ubx locus in wild-type discs but not in ash122 mutant discs (Fig. 2, A and B) (fig. S3); this finding indicates that recruitment of Ash1 and Ash1-mediated histone methylation coincides with activation of Ubx expression in third-leg discs. We monitored TRE transcription in the wild-type and ash122 mutant third-leg discs by RT-PCR. TRE transcripts were detected at comparable levels in wild-type and mutant discs, which indicates that Ash1 is not a major regulator of TRE transcription in imaginal discs (Fig. 2C) (fig. S3).

Ash1 SET domain interacts with TRE transcripts in vitro. The association of Ash1 with TREs in cells producing TRE transcripts suggests that TRE transcription or TRE transcripts nucleate recruitment of Ash1 to Ubx TREs. SET-domain proteins can bind single-stranded RNA and DNA in vitro, and ncRNA has been implicated in protein recruitment in gene dosage compensation (20–24). We used in vitro protein-RNA binding assays to assess whether Ash1 associates with TRE transcripts. Ash1SET, which consists of amino acids 1001 to 1619, retained TRE1(+), TRE2(+), and TRE3(+) but not the H3-K9-specific HMT Medusa (Mdu) (fig. S3A) (fig. S4). In contrast, Ash1, Ash1ΔN, and Mdu did not bind the antisense RNA of the Ubx TREs (Fig. 3A) (fig. S4). Ash1ΔN did not interact with the N-element in tre2 (Fig. 3A), which corresponds to the DNA spacer separating TRE-2 and TRE-3 (Fig. 1A).

In competition experiments, unlabeled TRE transcripts could outcompete the interaction of Ash1 with the corresponding TRE transcript (fig. S5). In contrast, double-stranded TRE transcripts, double-stranded DNA TRE sequences, and DNA-RNA hybrids consisting of TRE transcripts and TREs failed to disrupt the interaction; these findings suggest that Ash1 associates with single-stranded TRE transcripts (fig. S5).

To delineate the RNA-binding motif of Ash1, we investigated the interaction of truncated ash1 proteins with TRE transcripts. In addition to Ash1SET, we tested Ash1ΔN (amino acids 1001 to 2218), which contains the Ash1 SET module, and Ash1N (amino acids 1 to 1001) and Ash1C (amino acids 1619 to 2218), which both lack the SET domain and cysteine-rich regions (Fig. 3B). Ash1ΔN and Ash1SET, but not Ash1N and Ash1C, retained TRE transcripts, indicating that the SET domain of Ash1 binds TRE transcripts in vitro (Fig. 3C).

RNA-dependent recruitment of Ash1 to Ubx TREs in Drosophila. We next used XChIP to investigate whether Ash1 associates with TRE transcripts in vivo. Ash1 coprecipitated with TRE transcripts but not control transcripts from mock-treated chromatin (Fig. 3D) (fig. S6). Ash1 bound TRE transcripts in ribonuclease (RNase) III–treated chromatin, indicating that double-stranded RNA (dsRNA) motifs within TRE transcripts do not mediate the association of TRE transcripts with Ash1 in vivo (Fig. 3D) (fig. S7). In contrast, Ash1 did not interact with TRE transcripts from RNase A– and RNase H–treated chromatin, indicating that single-stranded RNA (ssRNA) is important for the association of Ash1 with TRE transcripts (Fig. 3D) (fig. S7). The disruption of the association between Ash1 and TRE transcripts by RNase H (which degrades DNA-RNA hybrids) in chromatin suggests that TRE transcripts hybridize with DNA in chromatin.

Is the association of Ash1 with TREs dependent on RNA? We used XChIP to compare the interaction of Ash1 and TRE in mock- and RNase-treated chromatin. Antibodies to Ash1 precipitated all three TREs, but not the spacer DNAs (S-2), from mock-treated and RNase III–treated chromatin, indicating that dsRNA does not contribute to the interaction of Ash1 with TREs (Fig. 3E) (fig. S7). In contrast, treating chromatin with RNase H or RNase A alleviated the association of Ash1 with TREs, indicating that the association of Ash1 with the Ubx TREs is RNA-dependent (Fig. 3E) (fig. S7). The disruption of the interaction of Ash1 with TREs in chromatin by RNase H and RNase A raises the possibility that ssRNA motifs in RNA-DNA hybrids play a role in the recruitment of Ash1 to TREs.

To verify that the observed attenuation of Ash1-TRE interactions is based on specific rather than general disruption of protein-DNA interactions in RNase-treated chromatin, we investigated the recruitment of the general transcription factor TFIIID to target genes in mock- and RNase-treated chromatin (25). The TATA-binding protein (TBP) subunit of TFIIID
interacts with the TATA box in eukaryotic promoters (25). PCR detected the interaction of TBP with the promoter of Ubx and string, whose transcription requires TFIID activity (26). TBP interacted with both promoters in mock-treated and RNase A–, RNase H–, and RNase III–treated chromatin, indicating that RNase treatment did not attenuate TBP-promoter interactions and protein-gene interactions in general (Fig. 3F) (fig. S7).

To test whether the detected association of Ash1 with TREs and TRE transcripts occurs in chromatin or is the result of fortuitous interactions generated in chemically cross-linked chromatin, we investigated the association of Ash1 with TRE transcripts and TREs in native chromatin with the use of native chromatin immunoprecipitation (NChIP). Ash1 bound all three TREs and TRE transcripts in mock- and RNase III–treated chromatin but not in RNase H– or RNase A–treated chromatin, indicating that Ash1 coimmunoprecipitates with TREs and TRE transcripts in native chromatin (Fig. 4, A and B) (fig. S8). An association of Ash1 with the N portion of the TRE2(+) transcript, as observed in cross-linked chromatin, was not detectable in native chromatin; this result indicates that, as in vitro, Ash1 binds the RNA corresponding to TRE-2 but not the N region of the TRE2(+) transcript.

Collectively, our data indicate that the recruitment of Ash1 to the TREs of Ubx is mediated by RNA and suggests the existence of a trimeric protein–nucleic acid complex in chromatin, consisting of Ash1, TREs, and TRE transcripts.

Ash1 is detectable at about 150 loci on Drosophila polytene chromosomes (10). To assess whether RNA facilitates Ash1 recruitment to target loci other than Ubx, we compared the interaction of Ash1 with target loci on...
mature and RNase-treated chromosome squashes. Compared to mock-treated chromosomes, RNase treatment attenuated the association of Ash1 with the majority of the target loci (fig. S9). This result suggests that RNA plays an important role in the recruitment of Ash1 to target genes in chromatin.

Ash1 associates with chromatin-bound TRE transcripts. To assess whether TRE transcripts associate with chromatin, we investigated whether Ash1 coprecipitates TRE transcripts from chromatin-free nuclear extract. Ash1 bound TRE transcripts in chromatin but not chromatin-free nuclear extract (Fig. 4C) (fig. S8), indicating that TRE transcripts are preferentially associated with chromatin in the cell.

We used XChIP to determine whether the association of Ash1 with TRE transcripts precedes the recruitment of Ash1 to TREs in chromatin, or vice versa. In vivo cross-linked chromatin was isolated from wild-type and ash122 mutant third-leg discs, sheared, and immunoprecipitated with antibodies to dimethylated H3-K9 present at the TREs of the transcriptionally active and inactive Ubx locus in third-leg discs (Fig. 2, A and B). The antibody to dimethylated H3-K9 coprecipitated with TREs and TRE transcripts from the chromatin of wild-type and ash122 third-leg discs (Fig. 4D) (fig. S10), indicating that TRE transcripts are retained at Ubx TREs before recruitment of Ash1.

TRE transcripts recruit Ash1 in trans. To dissect the role of TRE transcripts in Ubx transcription, we asked whether transiently transcribed TRE transcripts could restore the recruitment of Ash1 to Ubx TREs and Ubx expression in S2 cells, which express Ash1 but lack endogenous TRE transcripts. S2 cells were transiently transfected with plasmids transcribing sense or antisense TRE transcripts (19) (Fig. 5A) (fig. S11). In PCR assays, Ubx transcription was undetectable in S2 cells transiently transcribing antisense TRE transcripts or mdu (Fig. 5, A and B). In contrast, Ubx transcription was activated by one TRE transcript (Fig. 5B) (fig. S11) and cooperatively activated by multiple TRE transcripts (fig. S12).

We next used XChIP to determine whether activation of Ubx transcription by transient TRE transcripts coincides with the recruitment of Ash1 to TREs. In vivo cross-linked chromatin was isolated from wild-type S2 cells and cells transiently transcribing one or multiple TRE transcripts and control RNAs, and it was then immunoprecipitated with antibodies to Ash1 and the Ash1 histone methylation pattern (Fig. 5C). Ash1 was not detected at the TREs of transcriptionally silent Ubx in cells transcribing mdu or antisense TRE RNAs (Fig. 5C). In contrast, Ash1 and the Ash1 histone methylation pattern were detected at the Ubx TREs in cells transcribing TRE1(+) and TRE2(+), and/or TRE3(+) (Fig. 5C) (fig. S13). Each of the three TRE transcripts facilitated the association of Ash1 only with the corresponding template TRE but not with other TREs.

To verify the specificity of the described recruitment, we investigated whether TRE transcripts facilitate recruitment of Ash1 to CMEs containing TREs/PREs and genes other than Ubx. In XChIP assays, Ash1 was not detected at Drosophila genes and the CMEs MCP and Fab7 in S2 cells transcribing TRE1(+), TRE2(+), or TRE3(+) (fig. S14) (12, 13). Thus, TRE transcripts facilitate Ash1 recruitment specifically to the corresponding TRE template DNA.

We used NChIP and XChIP to assess whether transiently transcribed TRE transcripts associate with TREs and Ash1 in chromatin. Native chromatin was isolated from wild-type S2 cells and S2 cells transiently cotranscribing all three sense or antisense TRE transcripts. Ash1 did not associate with TRE transcripts (Fig. 5, D and F) and TREs (Fig. 5, E and G) in cross-linked (Fig. 5, D and E) and native chromatin (Fig. 5, F and G) from S2 cells transcribing mdu. In contrast, Ash1 interacted with TREs and TRE transcripts in S2 cells cotranscribing TRE1(+), TRE2(+), and TRE3(+) (Fig. 5, D to G) (fig. S11).

The association of Ash1 with TREs and TRE transcripts was attenuated by RNase A and RNase H but not RNase III (Fig. 5, D to G) (fig. S11). RNase treatment did not abolish the association of TBP with the Ubx promoter (Fig. 5, E and G). These results indicate that Ash1 associates with TRE transcripts and TREs in vivo and that TRE transcripts mediate the association of Ash1 with TREs in trans.

To test this hypothesis, we used RNA interference (RNAi) to assess whether degradation of TRE transcripts attenuates recruitment of Ash1 to Ubx TREs and Ubx expression in third-leg discs (27). In vitro cultivated third-leg discs were incubated with small interfering RNAs (siRNAs) targeting all three TRE transcripts or with control siRNA. RT-PCR and XChIP assays indicated that siRNA-mediated degradation of TRE transcripts attenuates Ubx transcription and the interaction of Ash1 with TREs (Fig. 6, A and B) (fig. S15).

Next, we used the binary Gal4/UAS system to determine whether ectopic transcription of TRE transcripts restores recruitment of Ash1 to Ubx TREs and Ubx transcription (28). Effector flies carrying a heat-inducible driver (hs70Gal4) were crossed with reporter flies carrying Gal4-dependent reporter genes (UAS-TRE) consisting of Gal4-responsive UAS DNA sites and a promoter driving the transcription of

![Fig. 5](https://www.sciencemag.org/content/311/5762/1121/F5)

**Fig. 5.** TRE transcripts reconstitute the interaction of Ash1 with Ubx TREs and Ubx transcription in S2 cells. (A) PCR analysis detecting TRE transcripts and actin5C transcription in wild-type S2 cells (+) and S2 cells transfected with plasmids transcribing mdu (mock), TRE transcripts [TRE1(+), TRE2(+), TRE3(+)] or antisense TRE transcripts [TRE1(−), TRE2(−), TRE3(−)]. (B) PCR assays as in (A) but detecting Ubx transcription in wild-type and transfected S2 cells. (C) PCR analysis of immunoprecipitates detecting the association of Ash1 with Ubx TREs in S2 cells transcribing mdu (mock) or sense and antisense TRE transcripts. (D and E) RT-PCR and PCR analyses of immunoprecipitates detecting the association of Ash1 with Ubx TRE transcripts (D) and TREs (E) and TBP with the Ubx promoter (Ubx-P) (E) in chromatin from S2 cells transiently cotranscribing TRE1(+), TRE2(+), and TRE3(+). (F and G) RT-PCR (F) and XChIP assays (G) as in (D) and (E), except that native chromatin was used. Transcripts and DNA elements detected in Drosophila genomic DNA are also shown.
sense and antisense TRE transcripts. Heat treatment of second-instar larvae resulted in ectopic transcription of TRE transcripts in all imaginal discs of third-instar larvae. Ectopic transcription of each TRE transcript nucleated ectopic transcription of Ubx in wing imaginal discs (Fig. 6, C and D) (figs. S15 and S16) and facilitated the recruitment of Ash1 to the corresponding Ubx TREs (Fig. 6E). It is noteworthy that ectopic TRE transcription in second-instar larvae caused lethality in pupae. In contrast, ectopic Ubx expression was not observed in discs prepared from heat-treated parental strains and discs transcribing antisense TRE transcripts (Fig. 6C).

Transcription of antisense TRE transcripts attenuated endogenous transcription of Ubx in wing discs isolated from young third-instar larvae, which suggests that ectopic transcription of antisense RNA interferes with the TRE transcript–mediated recruitment of Ash1 to Ubx TREs. In summary, our data provide evidence that noncoding TRE transcripts facilitate activation of Ubx expression by recruiting Ash1 to the Ubx TREs in the fly.

Discussion. Noncoding RNAs play an important role in the recruitment of proteins in several epigenetic phenomena. Recent studies have linked siRNAs to heterochromatin formation and transcriptional silencing of transgenes and transposons (29, 30). siRNAs facilitate the recruitment of HMTs and DNA methyltransferases to chromatin (31, 32). In Schizosaccharomyces pombe, heterochromatic silencing involves the RNA-induced initiator of transcriptional gene silencing complex (RITS), which contains an siRNA component that is essential for the recruitment of RITS to heterochromatin loci (31). The inability of RNase III, the key enzyme of the RNAi machinery, to degrade TRE transcripts into siRNAs and the interaction of Ash1 with full-length TRE transcripts in chromatin strongly argues against the involvement of siRNAs in the described RNA-dependent recruitment of Ash1 to chromatin.

Long ncRNAs are key players in imprinting and gene dosage compensation (22, 27, 33). In Drosophila, gene dosage compensation is achieved by a global twofold up-regulation of transcription from the male X chromosome and depends on the activity of the dosage compensation complex (DCC) that contains male-specific proteins and two ncRNAs, RNA on X 1 (rox1) and RNA on X 2 (rox2) (20). Both RNAs are transcribed by single-copy genes that, as well as several other X chromosome regions, serve as chromatin entry sites for the DCC on paternal X chromosomes (20, 27). Rox1 and Rox2 facilitate the assembly and recruitment of the DCC to chromatin entry sites (20). In mammals, spreading of Xist RNA culminates in X chromosome inactivation (22). Current models propose that the association between ncRNAs and chromatin involves their interaction with proteins, nascent transcripts at template DNA, or the template DNA (27, 34). The observed attenuation of the association between TRE transcripts and TREs by RNase H suggests that TRE transcripts are retained at TREs through hybridization with the corresponding template DNA. Because none of the known DNA repair systems targets DNA-RNA hybrids, RNA-DNA hybrids represent stable molecular entities that, in general, may anchor ncRNAs at corresponding DNA templates in chromatin (35).

The three TRE transcripts of Ubx do not share common sequence motifs. This is not surprising, because the functionally redundant rox RNAs and functionally identical regions in Xist, which are required for chromatin localization and protein recruitment, lack identifiable sequence motifs (27). Because many RNA-protein interactions are facilitated by RNA secondary structures, the interaction of Ash1 with TRE transcripts might be mediated by secondary RNA structures rather than sequence motifs. In addition, the specificity of RNA-protein interactions is often generated by induced-fit mechanisms that involve complex, extensive conformational changes in both proteins and the target RNA generating a specific interaction surface (36, 37).

Rox1 and rox2 RNAs transcribed from autosomes can localize to and mediate gene dosage compensation on the male X chromosome, indicating that the chromatin entry of rox RNAs does not depend on transcription of chromatin entry sites in cis (38). Thus, the association of transiently transcribed TRE transcripts with TREs in S2 cells suggests that TREs function as chromatin entry sites for the corresponding TRE transcripts in trans and cis, and that the transcription and
A Swimming Mammaliaform from the Middle Jurassic and Ecomorphological Diversification of Early Mammals

Qiang Ji,1,3 Zhe-Xi Luo,2,1* Chong-Xi Yuan,3 Alan R. Tabrum2

A docodontan mammaliaform from the Middle Jurassic of China possesses swimming and burrowing skeletal adaptations and some dental features for aquatic feeding. It is the most primitive taxon in the mammalian lineage known to have fur and has a broad, flattened, partly scaly tail analogous to that of modern beavers. We infer that docodontans were semiaquatic, convergent to the modern platypus and many Cenozoic placentals. This fossil demonstrates that some mammaliaforms, or proximal relatives to modern mammals, developed diverse locomotory and feeding adaptations and were ecomorphologically different from the majority of generalized small terrestrial Mesozoic mammalian insectivores.

The Middle Jurassic mammalian diversification gave rise to several emergent clades: basal eutriconodontans, amphitheriid cladotherians, the basal mammalian lineage of shuotheriids, and basal australosphenidans (1–5). These new clades of crown Mammalia coexisted with several mammaliaform lineages (the proximal relatives to modern mammals) (6–8). Docodontans are a Mesozoic mammaliaform lineage that have specialized molars for omnivorous feeding:


*To whom correspondence should be addressed. E-mail: LuoZ@CarnegieMNH.org

Several taxa are known from the Middle Jurassic (1, 9–13). Here, we report on a large docodontan mammaliaform that has some dental features for feeding on aquatic invertebrates and small vertebrates, plus specialized skeletal and soft-tissue features for swimming and burrowing.

Description and comparison. Castorocauda lutrasimilis, gen. et sp. nov. (14), is from the Middle Jurassic Jiujiangshan Formation, dated to be approximately 164 million years ago (15–17). The fauna includes pterosaurs (17, 18), a coelurosaurian dinosaur (19), lissamphibians (20), abundant fossil insects (21), and the conchostracan Euestheria (22). The holotype of C. lutrasimilis (Fig. 1) is represented by a partial skeleton (preserved rostrum-tail length ≥425 mm) with incomplete cranium (preserved length ≥60 mm) but well-preserved mandibles and lower dentition (incisors 4, canine 1, premolars 5, molars 6). Lower molars 3 to 6 have the diagnostic characteristics of docodontans (Fig. 2): anteriorly placed and enlarged lingual cusps g, triangulated crests formed by cusps a-c and a-g, and two partially enclosed basins formed respectively by cusps a, b, and g, and by cusps a, c, and d (9–12). As in all docodontans, the molars were capable of both shearing by the triangulated crests and grinding between the anterior (“pseudotalonid”) basin and the transversely widened upper molars (9–12). Castorocauda is distinctive from other docodontans in having mediolaterally compressed crowns of molars 1 and 2, each with five cusps in straight alignment (23, 24); primary cusp a and posterior cusps c and d are slightly recurved (Fig. 2). These “triconodont-like” anterior molars are plesiomorphic for mammaliaforms (6–8) but nonetheless distinctive among docodontans. They are convergent to those of placental mesochynians and Eocene whales (25). This type of molar with recurved cusps in alignment is hypothesized to be a specialization for feeding on fish and aquatic invertebrates by functional analogy to the teeth of modern pinniped carnivores such as seals.

Castorocauda is preserved with intact middle ear bones (Fig. 2) on the mandible, including the articular (malleus), the surangular, and the angular (ectotympanic). The middle ear bones in anatomical association with the mandible corroborate a previous interpretation of the middle ear in docodontans (26). A concavity on the