

Suction feeding by a tiny predatory tadpole

This amphibian shoots its mouth forwards in a fish-like manner to suck in its prey.

Pipid tadpoles of the African genus *Hymenochirus* are not only among the smallest free-swimming, feeding vertebrates, but are also predatory suction feeders¹ — unlike other tadpoles, which typically ingest a suspension of organic particles. Here we use a high-speed video system to study details of the feeding mechanics of *Hymenochirus boettgeri* tadpoles and find that they first track individual prey organisms visually, and chase and then capture them by mouth using suction. This feeding mechanism is unique among frogs and is strikingly convergent with that used by teleost fishes.

Frogs of the genus *Hymenochirus* are unique in that both adults and tadpoles are predatory suction feeders^{1,2}. In most species of frog, adults use their jaws, tongue or forelimbs to capture prey², and tadpoles are usually suspension-feeding detritivores³ that use scraping mouthparts to create the suspension. This suspension is pumped into the mouth by rhythmic movements of the hyobranchial apparatus (throat skeleton)⁴, particles are trapped in the branchial basket, and water exits through the gill slits or a single spiracle^{3–5}.

Hymenochirus tadpoles, however, are morphologically divergent, lack a filter apparatus and scraping mouthparts, and have huge, frontally orientated eyes¹. Also, they are obligate air breathers and do not pulse-pump to irrigate the gills like most other tadpoles. Unaided visual observation indicates that they are predatory carnivores¹, but their minute size (the body length is less than 1 mm at first feeding) and rapid movements make the details of their feeding mechanics difficult to determine.

We used a high-speed video system (1,000 Hz) with a powerful macro lens that enabled us to follow the feeding behaviour and mechanics of *H. boettgeri* tadpoles (2–3 mm body length; Gosner stage 26). Our recordings reveal that they target each prey item visually, pursue it, then capture it by extension of a tubular mouth during an explosive buccal expansion (Fig. 1a, b; for movie, see supplementary information). Tadpoles complete their mouth extension within 2 ms and engulf the prey within 4 ms; prey travel into the mouth at 0.6 m s^{-1} . Buccal expansion is completed within 7 ms.

Comparably sized larval teleosts^{6,7} are slower feeders than these tadpoles, taking 4–12 ms to engulf their prey, which enter the mouth at $0.03\text{--}0.3 \text{ m s}^{-1}$. We calculated a Reynolds number of 300 for prey capture in tadpoles (from prey velocity and tadpole mouth diameter) compared with 5–70 for larval teleosts^{6,7}. The higher Reynolds number indicates that, although *Hymenochirus* is about the same size as larval teleosts, it is faster and better at overcoming the viscous drag that typically confronts small aquatic organisms⁸.

We compared our video images of moving *Hymenochirus* with preserved specimens and found that the tadpole's suction action is generated by a combination of hyobranchial movements (ceratohyal depression, basibranchial retraction) and cranial elevation; downward rotation of the lower jaw unfurls the soft tissues that comprise the extensible mouth (Fig. 1c).

After prey capture, the tadpoles expel water slowly (over 200 ms) through the paired gill slits of the reduced and simplified branchial basket¹ by raising the ceratohyals and basibranchial and lowering the head to their resting positions. The Reynolds number drops to 50 during water expulsion (Fig. 1a), and is lower for smaller *Hymenochirus* tadpoles when they begin feeding. Viscosity may thus be more problematic during water expulsion, as has been proposed for larval teleosts⁹.

The feeding mechanism of *Hymenochirus* is remarkably like that of teleosts, which also suction-feed by using a combination of rapid mouth protrusion, hyobranchial depression and cranial elevation, followed by slower water expulsion through the gill slits^{6,7,9}. *Hymenochirus* and teleosts also share a hydrodynamically advantageous round mouth opening¹⁰.

Rapid mouth protrusion confers several benefits — it decreases the distance to the prey, accelerates flow through the mouth, restricts flow to the area in front of the mouth, and reduces the momentum (mass \times velocity) that must be imparted to the water^{1,9}. Suction feeders that have non-protrusible mouths, such as some first-feeding larval fish⁷, must suck in more water than animals that have protrusible mouths. They therefore generate more momentum and suck themselves forwards^{6,9}. *Hymenochirus* imparts little momentum to the water, resulting in only a slight forward movement of the tadpole's body (Fig. 1a).

Miniaturized *Hymenochirus* tadpoles begin feeding at a smaller size than larval teleosts, which are universally suction

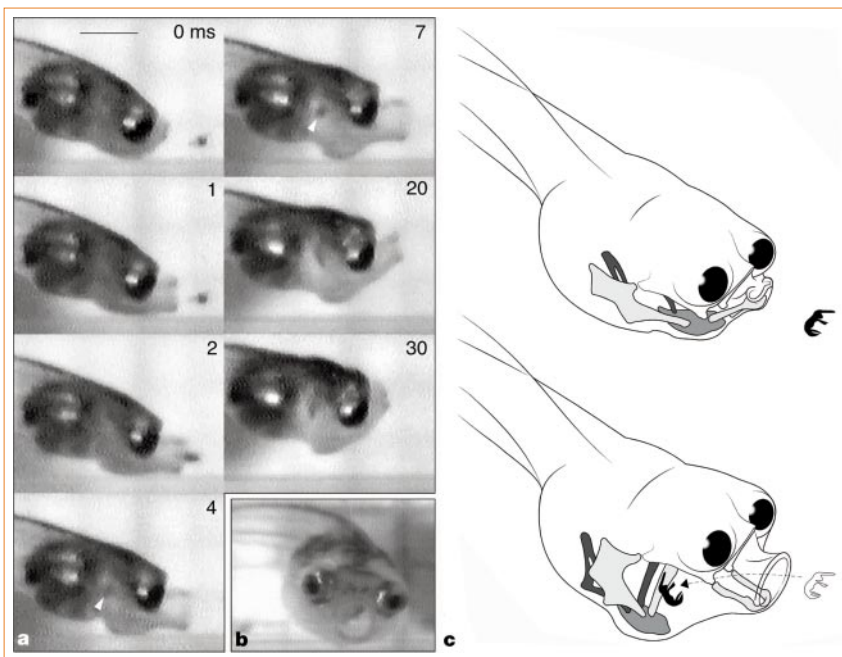


Figure 1 Morphology and function of suction feeding in the tadpole *Hymenochirus boettgeri* (body length, 2.6 mm). **a**, High-speed video sequence of feeding, showing hyobranchial depression, mouth extension and cranial elevation. Prey is engulfed within 4 ms of the commencement of mouth opening. White arrowhead in frame 4 indicates location of prey (the brine shrimp at the nauplius stage) in the pharynx. Scale bar, 1 mm. **b**, 'Yawning' tadpole, showing the hydrodynamically favourable round mouth aperture and the large, frontally orientated eyes. **c**, Dynamics of the tadpole's feeding apparatus, showing the mouth extension and hyobranchial movements that generate suction to ingest prey. Meckel's cartilage (lower jaw) and the ceratohyals are shown in light grey, the copula (basibranchial) is shown in medium grey, and the ceratobranchials are shown in dark grey.

feeders^{10,11}. What makes *Hymenochirus* so unusual is not just its size or feeding mode, but also that it is phylogenetically nested in a group of obligate suspension feeders^{3,12} and has independently evolved suction-feeding mechanics that are highly convergent with those of teleosts.

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Supplementary information accompanies this communication on Nature's website.

Competing financial interests: declared none.

Purinergic receptors

An ATP-gated ion channel at the cell nucleus

Transcriptional activity inside the nucleus of eukaryotic cells is regulated by ions such as calcium that need to be transported across the nuclear membrane. Here we show that an ion channel spanning the nuclear envelope between the cytoplasm and the nucleus could be regulated by an ATP-binding receptor of the P2X₇ subtype. Activation of this nuclear P2X₇ receptor by ATP in the cytoplasm may be a mechanism by which cellular activity can be coupled to changes in gene expression.

P2X₇ receptors are members of a family of ATP-binding receptors that are permeable to calcium. Originally thought to be absent from neurons¹, the P2X₇-receptor subunit P2X₇R has been shown to be targeted to excitatory but not inhibitory terminals, and yet it is absent from plasma membranes of the cell body^{2,3}.

To determine whether inhibitory neurons also express the receptor, we used *in situ* hybridization of the rat hippocampus to detect messenger RNA encoding P2X₇R. A positive signal was seen in the cytoplasm of all neurons in the cell-body layer³, 90% of which are excitatory cells⁴. In contrast to the localization of the P2X₇R protein to the terminals of only excitatory neurons, however, P2X₇R mRNA was also present in cells containing immunoreactivity for the potassium-channel subunit Kv3.1b, which identifies a subset of inhibitory neurons in the hippocampus⁵ (Fig. 1a, b).

The mismatch between the expression of P2X₇R mRNA and its protein in inhibitory neurons was explained when we used different antisera to stain the intra-

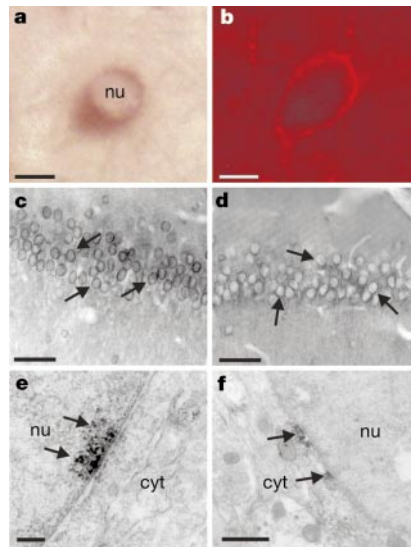


Figure 1 An ATP-gated ion channel spans the nuclear envelope. **a**, Messenger RNA encoding P2X₇R (visualized with alkaline phosphatase²) is present in the cytoplasm of all neurons in the hippocampus, and is shown in one neuron (nu, nucleus) adjacent to the cell-body layer; **b**, this neuron is identified as inhibitory by the presence of predominantly membrane-bound red Kv3.1b immunofluorescence (antibody against the K⁺-channel subunit Kv3.1b from Alomone Labs). **c**, **d**, Localization by light microscopy of P2X₇R protein: 'intracellular' (**c**) and 'extracellular' (**d**) epitopes are seen adjacent to the nuclear membrane (arrows) in all neurons in the cell-body layer. **e**, **f**, Immuno-electron microscopy of P2X₇R, seen here spanning the nuclear envelope, with **e**, its 'intracellular' portion adjacent to the nuclear side of the nuclear envelope (arrows), and **f**, its 'extracellular' portion facing the cytoplasm (cyt; arrows). Scale bars: **a**, **b**, 5 μm; **c**, **d**, 50 μm; **e**, **f**, 0.5 μm.

cellular (specific to amino-acid residues 576–595; 1:1,000 dilution; refs 2, 6) and extracellular (specific to amino acids 60–323; 1:100 dilution; ref. 6) portions of P2X₇R and found staining adjacent to the nuclear envelope in 100% of hippocampal

neurons (Fig. 1c, d).

The protein must span the nuclear envelope because the antisera against the two different epitopes labelled the cytoplasmic and inner surfaces of the nuclear membrane, respectively, with the ATP-binding site being in the 'extracellular' portion facing the cytoplasm (Fig. 1e, f). This finding still leaves unanswered the question of how P2X₇R is transported selectively to the presynaptic terminal in only excitatory neurons but to the nuclear envelope in all neurons.

Insertion of P2X₇R into the nuclear envelope is consistent with patch-clamp studies on nuclei showing that ATP binding maintains the open state of non-selective cation channels⁷ as well as inducing a macroscopic current⁸. The properties of P2X₇Rs correlate with these nuclear channels as they exhibit little or no desensitization⁹, they are activated by ATP (the 50% effective concentration is about 100 μM (ref. 9), which is well within the 5–10-mM range of cytoplasmic ATP), and they form a large pore upon prolonged activation⁹ (which may correspond to the ATP-induced macroscopic current in the nuclear membrane⁸ and the increase in envelope permeability induced by ATP binding⁷).

Moreover, the ATP-gated nuclear channels^{7,8} and the P2X₇R⁹ are both permeable to Ca²⁺ ions, and changes in nuclear Ca²⁺ concentration are linked to changes in the transcription of several genes implicated in neuronal plasticity (principally by regulating the activity of the transcriptional co-activator protein CBP)¹⁰. The presence of P2X₇R in the nuclear envelope of phenotypically heterogeneous neurons and of ATP-gated channels in nuclei of diverse cell types (such as *Xenopus* oocytes and mouse liver cells) therefore has wide implications as it provides a means of regulating nuclear Ca²⁺ concentration in response to cytoplasmic activity.

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Competing financial interests: declared none.