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Short Communications

Evolution of inner-ear auditory apparatus in the frog

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The morphology of typical anuran amphibian papillae is thoroughly distinct from that of urodeles. However, the morphological discontinuity lies not between the frogs and the salamanders, but between the most primitive living frog, *Ascaphus truei*, and the more derived anurans. Three features distinguishing the papillae of more derived anurans from that of *Ascaphus* apparently provide peripheral tonotopy in the former. The adaptive significance of a fourth feature, kinociliary bulbs, is not clear.

Auditory sensitivity in the frogs and toads (the Anurans) has been attributed in part to a specialized inner-ear organ, the amphibian papilla[1,6–8]. Beginning with the discoglossids, generally accepted to be among the most primitive of anurans, and progressing to the ranids and their relatives, generally accepted to be among the most derived[4,24,32], one finds that the anuran amphibian papilla consistently exhibits 4 morphological features not found in the amphibian papilla of the salamanders. (1) The typical anuran amphibian papilla consists of two patches of sensory epithelium, each innervated by a separate branchlet of the VIIIth nerve and each having two populations of sensory (hair) cells, oppositely polarized[13–16]; the urodele (salamander) amphibian papilla consists of a single patch[21,22], with a single pair of oppositely-polarized hair-cell populations (Fig. 1). (2) The amphibian periotic canal contacts the posterior end of the typical anuran amphibian papillar chamber (through the contact membrane); in the urodeles it contacts the medial side of the chamber[1,9,21,22]. (3) Hanging from the typical anuran amphibian papilla, the tectorium (an acellular, mucopolysaccharide structure) is thick where it is adjacent to the rostral patch of sensory epithelium and thin where it is adjacent to the caudal patch[8,13,30,35]; in urodeles it is intermediate in thickness over the entire papilla. (4) In the typical anuran amphibian papilla, the single cilium (kinocilium) of the hair cell has a bulb at its distal end (Fig. 2); the kinocilium of the urodele amphibian-papillar hair cell has no bulb.

Recently we extended the morphological observations to the tailed frog (*Ascaphus truei*), one of four living anuran species generally accepted to be even more

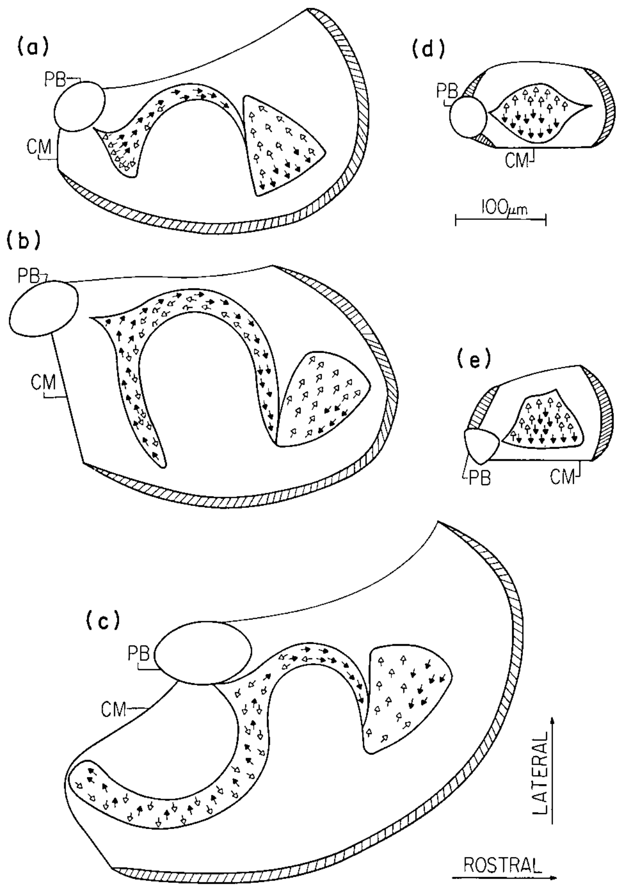

Fig. 1. Outlines of 5 amphibian papillar chambers transected horizontally and viewed from below, all drawn to the same scale; (d) corresponds to the salamander *Ambystoma maculatum*; the other 4 are from anurans: (a) from the discoglossid *Bombina orientalis*; (b) from the pelobatid *Scaphiopus couchi*; (c) from the hyperoliid *Kassina senegalensis*; and (e) from the tailed frog, *Ascaphus truei*. PB = transected branchlet of cranial nerve VIII; CM = contact membrane separating papillar chamber from amphibian periotic canal (which contacts directly the middle-ear apparatus when it is present). Thick border with cross-hatching represents transected labyrinthine wall; thin, lateral-most line corresponds to opening of papillar chamber to fluid-filled space shared by saccule and other inner-ear organs. Within the outlines of the sensory epithelia are arrows indicating hair-cell polarization patterns. Each arrow represents many hair cells and is drawn parallel to the axes of bilateral symmetry of those cells, pointing toward the sides on which the kinocilia reside.

primitive than the discoglossids[4,24,32]. With respect to all 4 features distinguishing the urodele amphibian papilla from that of the anurans, we found that the amphibian papilla of the tailed frog looks like that of a salamander rather than that of a frog. This observation places a discontinuity in amphibian papilla morphology squarely within the Order Anura and thus raises interesting questions regarding the evolution of the auditory periphery in the frogs and toads.

Our comparative morphological studies of the amphibian papilla have been carried out on 72 anuran species, distributed over 12 families, and 8 urodele species, distributed over 4 families. Scanning electron microscopy (SEM) was used for determination of hair-cell surface topography (e.g. polarization, presence or absence of kinociliary bulb) and of overall papillar geometry. Phase-contrast light microscopy was used for determination of the geometry of the tectorium. Tissue was prepared for SEM in the standard way[13,25], first being fixed with buffered glutaraldehyde, then postfixed with buffered osmium tetroxide solution, dissected, dehydrated through a graded ethanol/water series, transferred into liquid carbondioxide in a pressure bomb, critical-point dried, mounted, evaporatively coated with gold, and finally viewed in the SEM. Immediately after postfixation, the tectorium was removed and placed in a pool of buffer solution in a depression slide. The whole tectorium then was viewed in the fully hydrated state under the phase-contrast light microscope.

The key results of the studies are summarized in the first paragraph and in Figs. 1 and 2. In all of the species examined, the kinocilium morphology and the hair-cell polarization patterns were determined for the first time. In most of the anuran species, the overall papillar and tectorial geometries were determined for the first time, as were the tectorial geometries in the urodele species. In the other anuran species, the observations simply reconfirmed the results of previous studies with respect to papillar and tectorial geometries[1,9,21,30,35]. The typical, overall geometry of the urodele amphibian papilla already was well established by previous studies[21,22,30].

The significances of our new observations can be considered conveniently in the framework of the following evolutionary scheme, recently proposed by Lombard and Bolt[23]:

$$A \rightarrow a \rightarrow a' \qquad (1)$$

where state A corresponds to the presence of a macula neglecta and the absence of an amphibian papilla; state a corresponds to the presence of both a macula neglecta and an amphibian papilla; and state a' corresponds to the absence of a macula neglecta and the presence of an amphibian papilla. State A occurs in fish, a in caecilians, and a' in urodeles and anurans. Lombard and Bolt conclude that state a' was derived from state a, which in turn was derived from state A. Our new observations suggest that state a' should be divided into two substates, a'_1 (corresponding to the presence of an amphibian papilla in typical urodele form) and a'_2 (corresponding to the presence of an amphibian papilla in typical anuran form), and that state a'_2 was derived from state a'_1. Thus the evolutionary scheme proposed by Lombard and Bolt would become

$$A \rightarrow a \rightarrow a'_1 \rightarrow a'_2 \qquad (2)$$

Two arguments in favor of this modified scheme can be drawn directly from our comparative studies. The first is based on *congruence*: state a'_1 was found in the species previously judged on the basis of other criteria to be the most primitive of the living anurans[4,24,32], while state a'_2 was found in all of the more derived anuran species examined to date, suggesting that state a'_2 itself is derived relative to state a'_1. The second argument is based on *complexity*: the two-patch papilla of state a'_2 appears to be more complex than the one-patch papilla of state a'_1, also suggesting that state a'_2 is derived relative to state a'_1. A third argument follows from a previous study of hair-

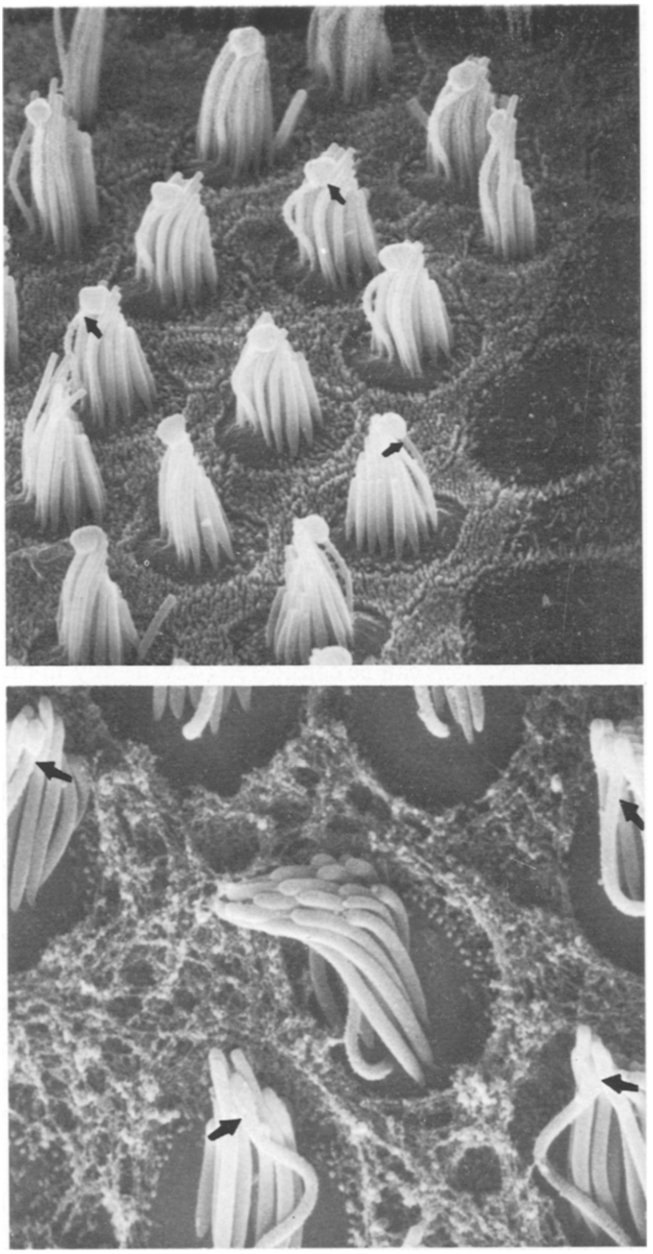

Fig. 2. Hair-cell surfaces viewed with the scanning electron microscope. Top: hair cells from the amphibian papilla of the green tree frog, *Hyla cinerea*, with bulbed kinocilia (indicated on 3 of the hair cells by arrows) typical of anurans (width of micrograph = 19 μm). Bottom: hair cells from the amphibian papilla of *Ascaphus truei*, the only frog among seventy-two species studied to date that exhibited unbulbed kinocilia (arrows) throughout the amphibian papilla (width of micrograph = 9.4 μm).

cell morphogenesis in *Rana catesbeiana*[18,19], and is based on *ontogeny*: all hair cells in that frog initially are formed without kinociliary bulbs; the bulbs develop gradually on certain hair cells as they mature, suggesting that the bulbed kinocilium is derived relative to the unbulbed kinocilium and further supporting the proposition that state a'_2 is derived relative to state a'_1.

Although state a'_2 may be derived relative to state a'_1, that would not necessarily imply that state a'_2 was derived *from* state a'_1. It is possible that states a'_1 and a'_2 were derived separately, perhaps both directly from state a. The occurrence of state a'_1 in *Ascaphus truei* then could be explained in two ways: either *Ascaphus* itself was derived with the urodeles and separately from the other anurans, or state a'_1 in *Ascaphus* came about through convergence. Present evidence overwhelmingly disfavors the separate-derivation proposition[34]. In favor of the convergence proposition, one might argue that state a'_1 is strongly linked to the absence of tympanum and middle ear, which is shared by the urodeles and *Ascaphus*[21,23,33] and may be a derived condition in the latter[5]. However, other anurans (several discoglossids and pipids, one bufonid and one microhylid) lacking tympanum and middle ear were examined in our comparative studies and all exhibited typical anuran amphibian papillae (i.e. state a'_2). Therefore, state a'_1 is not an obligatory concomitant of the absence of tympanum and middle ear. This leaves the convergence proposition exposed to the full force of the congruence argument of the previous paragraph. If we accept convergence, we must accept as coincidental the observation that among all 72 anuran species examined so far, only the single most primitive species exhibited that convergence. If we reject the convergence proposition, then in its place we are left with the proposition that the detailed similarity between the urodele amphibian papilla and the *Ascaphus* amphibian papilla is the result of the existence at some time of a common frog-salamander ancestor, a notion currently in favor but still debated[12,23,26,31]. If we reject convergence *and* accept the proposition that state a'_2 is derived relative to state a'_1, then we are left with the modified evolutionary scheme of expression[2].

The implication of this scheme is initial derivation of anurans with their amphibian papillae in state a'_1 and subsequent evolution to state a'_2. This leads to the question of the selective advantage for anurans of state a'_2 over state a'_1. From recent physiological studies it now seems clear that the combination of the two patches with separate innervation, a tectorium with spatially graded bulk (and, presumably, correspondingly graded mass), and a contact membrane situated at the caudal end of the papillar chamber rather than along its entire medial margin, provides a sorting of frequency sensitivity (i.e. tonotopic organization)[2,15–17]. With its single patch, *Ascaphus truei* exhibits virtually the same range of frequency sensitivity (100–600 Hz) as do many of the anurans with two-patch papillae (Capranica, personal communication). Extension of the range beyond 600 Hz appears to be concomitant with caudal prolongation of the caudal-most patch and to occur in the more recently derived anurans, such as *Kassina senegalensis* (see Fig. 1c)[6,15,16]. Therefore, the evolution from one patch to two patches apparently provided tonotopic organization but no immediate extension of frequency range; and we are left with the possibility that tonotopic organization itself provided the immediate selective advantage for 3 of the 4 character traits associated with state a'_2. A clue to the advantage of tonotopic organization may be provided by the fact that *Ascaphus truei* is mute, whereas beginning with the discoglossids, the more derived anurans possess vocal repertoires[34]. In the ranids, at least, peripheral frequency sorting has been shown to play an important role in the discrimination of vocalizations[7]. Therefore, the ultimate selective advantage of those three traits of state a'_2 may be that provided by the availability of vocal communication (mating calls, warning calls, and the like). Multiple patches of sensory epithelium also have arisen in an auditory organ in elasmobranchs[3] and in an otolithic organ of unknown function in teleosts[29]. To date there is no direct evidence concerning the selective advantages of the multiple-patch state in these cases.

The significance of the fourth trait characterizing state a'_2, namely the kinociliary bulb, also is not clear. Concomitantly with its appearance in the amphibian papilla in the anurans, it also appears in the hair cells of the central fields of the lagena and sacculus and in the hair cells of the medial edge of the basilar papilla. From both TEM and SEM we have convincing evidence that it provides a focussed mechanical linkage between the stereociliary array and the tectorium[10,11,13]. The selective advantage of such a linkage presently is a matter of speculation, one possibility being enhancement of sensitivity to acoustical or seismic vibrations by provision of a more direct mechanical coupling between the hair cell and the acellular structure (otoconial membrane or tectorium) adjacent to it. Kinociliary bulbs occur in certain reptiles[25] and some but not all mammals[20,37], but not in fish[27,28] nor in the one species of caecilian (*Dermophis mexicanus*) that we have examined to date. Since kinociliary bulbs are present in anurans and in selected amniotes, they apparently have arisen more than once, suggesting that their selective advantage, whatever it may be, is compelling under certain circumstances for terrestrial vertebrates.

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