

# The transformation of sound stimuli into electrical signals

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## I Introduction

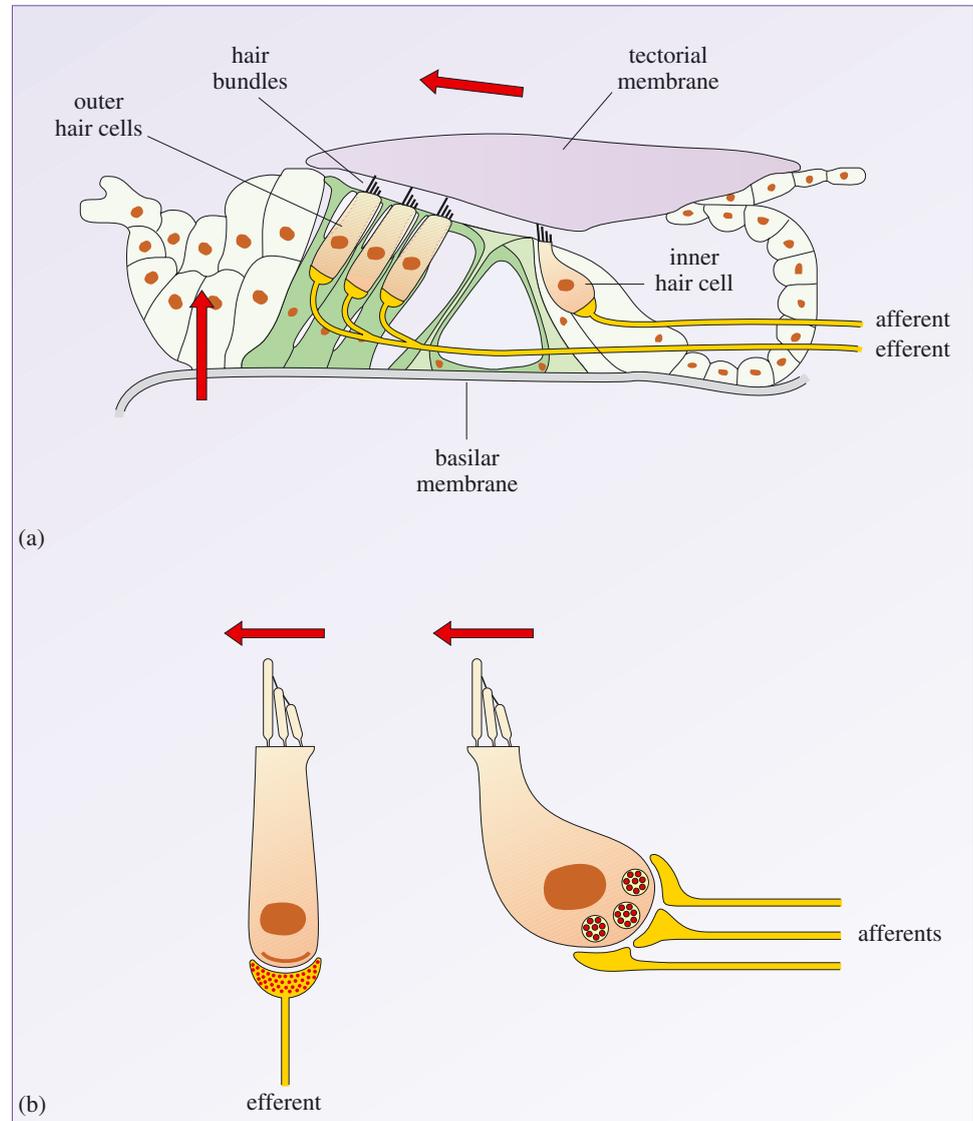
Our sense of hearing depends on the correct performance of about 15 000 hair cells in each cochlea that serve as an interface between the mechanical vibrations of speech, music and other sounds and the electrical signals of the brain. Hair cells can detect vibrations of atomic dimensions down to 0.2 nm, and can respond more than 20 000 times a second. These cells cannot regenerate, so their atrophy in old age or destruction by loud sounds or drugs like streptomycin causes permanent deafness. Loss of sensation is often restricted to higher frequencies, and is linked to degeneration of hair cells near one end of the cochlea. This chapter explains how acoustic stimuli are detected by the hair cells and converted into a stream of electrical impulses that are relayed to the brain. It also describes the mechanisms by which each cell is affiliated with certain input frequencies.

## 2 Hair cells and their mode of stimulation

Hair cells are the sensory receptors for all components of the inner ear, including the organ of hearing, the cochlea, and those of balance – three semicircular canals, a saccule and utricle (see Chapter 7, *The vestibular system*). In each of these the hair cells are tightly anchored to non-sensory supporting cells in an epithelial sheet. In the cochlea, this sheet of cells, known as the organ of Corti, rests on a freely moveable basilar membrane (Figure 1a overleaf). The mechanically sensitive **hair bundles** project above the epithelial surface and the majority contact a gelatinous flap known as the tectorial membrane. The hair cells have no axons, but make synaptic contact with the endings of **afferent** and **efferent nerve fibres** that travel in the VIIIth cranial nerve (Figure 1b). The afferent fibres, bipolar neurons with cell bodies in the spiral ganglion, transmit the auditory message to the brain, whereas the efferent axons send signals back to the cochlea from the brain to influence hair cell sensitivity. The cochleas of mammals (and birds) contain two types of sensory cell, outer hair cells and inner hair cells, which have different function and innervation. While the majority of afferents emanate from the inner hair cells, only the outer hair cells receive synaptic contact with efferent fibres.

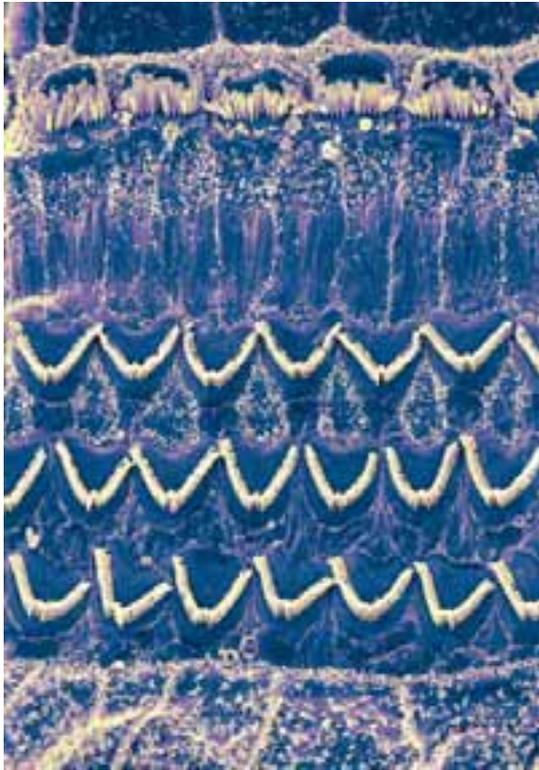
Sound stimuli reaching the cochlea produce vibrations of the basilar and tectorial membranes. The relative movements of the two membranes excite the hair cells by bending their hair bundles relative to the rigid epithelial surface. The mechanically sensitive hair bundle is composed of about 50 to 100 modified microvilli called stereocilia (Figures 2 and 3 overleaf). These are cylindrical projections, measuring a few tenths of a micron in diameter and 1 to 10  $\mu\text{m}$  in height, which taper at the bottom where they fuse to the cell body. Each stereocilium is packed with actin filaments running along its length, but only a small fraction of the filaments traverse the narrow bottom to be anchored in the cytoskeleton of the cell body. Actin is a specialized protein also found in muscle tissue. The reduced number of actin filaments at the stereociliary base makes it the weakest mechanical point about which the stereocilium bends. The hair bundle superficially resembles the bristles of a hairbrush but when deflected its motion differs from the brush in several important

**Figure 1** The mammalian cochlea contains two types of sensory cell embedded in a matrix of supporting cells. A single row of inner hair cells contacts auditory afferent fibres, whereas three rows of outer hair cells receive synaptic contact from efferent nerves from the brain. Sound stimuli vibrate the basilar membrane causing to-and-fro motion of the sensory hair bundles relative to the overlying tectorial membrane. The red arrows show excitatory motion that bends the hair bundles towards their taller edge.



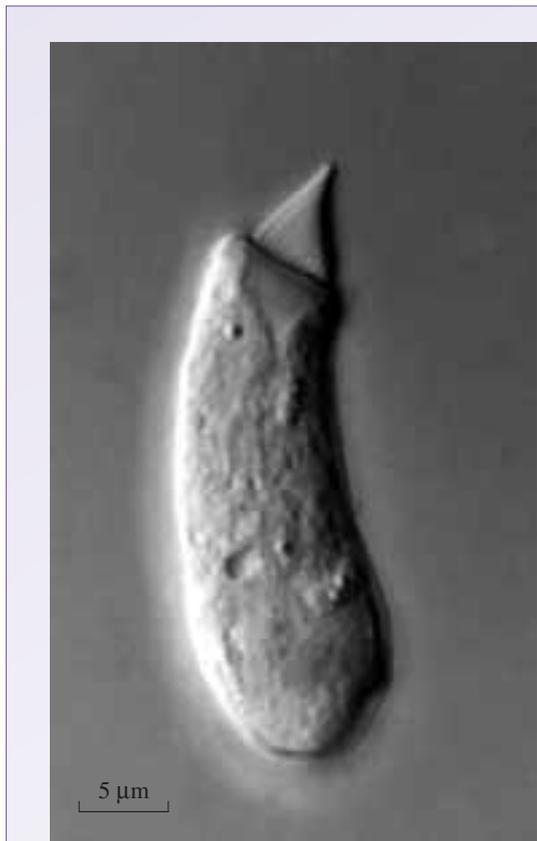
respects. Firstly, the stereocilia do not flex but behave as stiff rods pivoting around their base. Secondly, because the stereocilia are interconnected by fine extracellular strands they all move together when displaced.

An important feature of the hair bundle is its asymmetric bevelled form, reflecting the progressive increase in height of sequential ranks of stereocilia across the bundle (Figure 3). As a consequence, the hair bundle is morphologically polarized with an axis of symmetry bisecting it from the shortest to tallest edge. Only those movements of the bundle along the axis of symmetry (towards or away from the tallest stereociliary rank) are detected, whereas movements at right angles to this axis are not. In the cochlea, all hair bundles are oriented so that they sense only transverse motion of the overlying tectorial membrane (Figure 1). In the vestibular organs and in the developing cochlea, the hair bundle also possesses a single kinocilium. The role of the kinocilium is unknown, but it is not required for transduction and disappears in the adult cochlea.

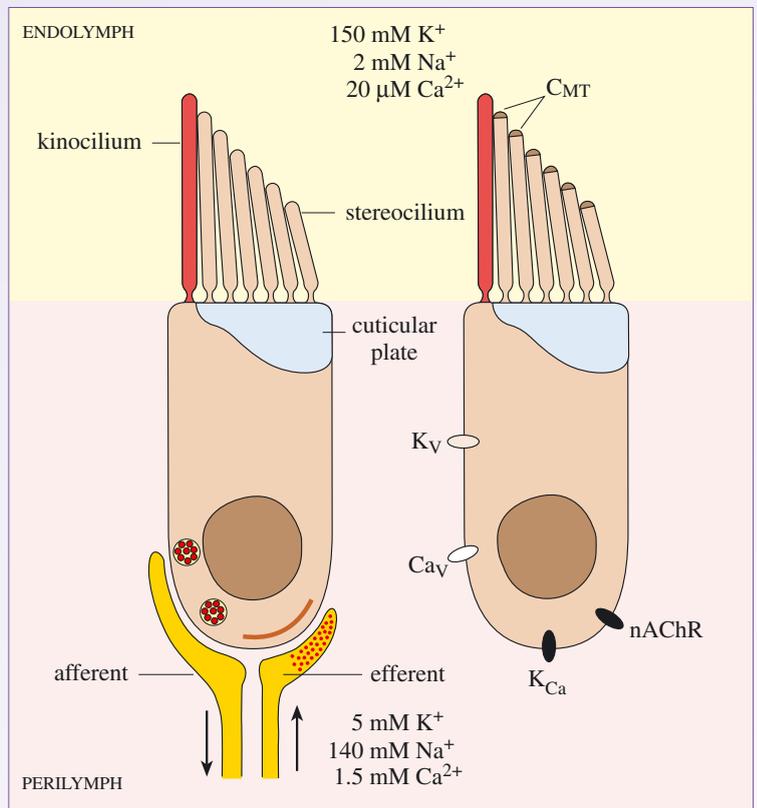


**Figure 2** A scanning electron micrograph of the surface of the organ of Corti shows three rows of V-shaped outer hair cell hair bundles at the bottom and a single row of inner hair cell bundles at the top. The hair cells are excited by deflections of the hair bundles towards the point of the 'V'.

▼ **Figure 3** (a) A hair cell from the turtle cochlea. (b) The hair cell layer separates fluids of different ionic composition, the  $K^+$ -rich endolymph enveloping the hair bundles and the perilymph, which contains higher concentrations of  $Na^+$  and  $Ca^{2+}$ . Displacements of the hair bundle activate mechanosensitive channels ( $C_{MT}$ ) allowing influx of cations. Synaptic transmission depends on the influx of calcium ions through voltage-dependent calcium channels ( $Ca_V$ ) to liberate neurotransmitter. An efferent axon releases acetylcholine, which binds to the nAChR receptor on the hair cell. Most hair cells also possess potassium channels activated by changes in membrane voltage ( $K_V$ ) or intracellular calcium ( $K_{Ca}$ ).



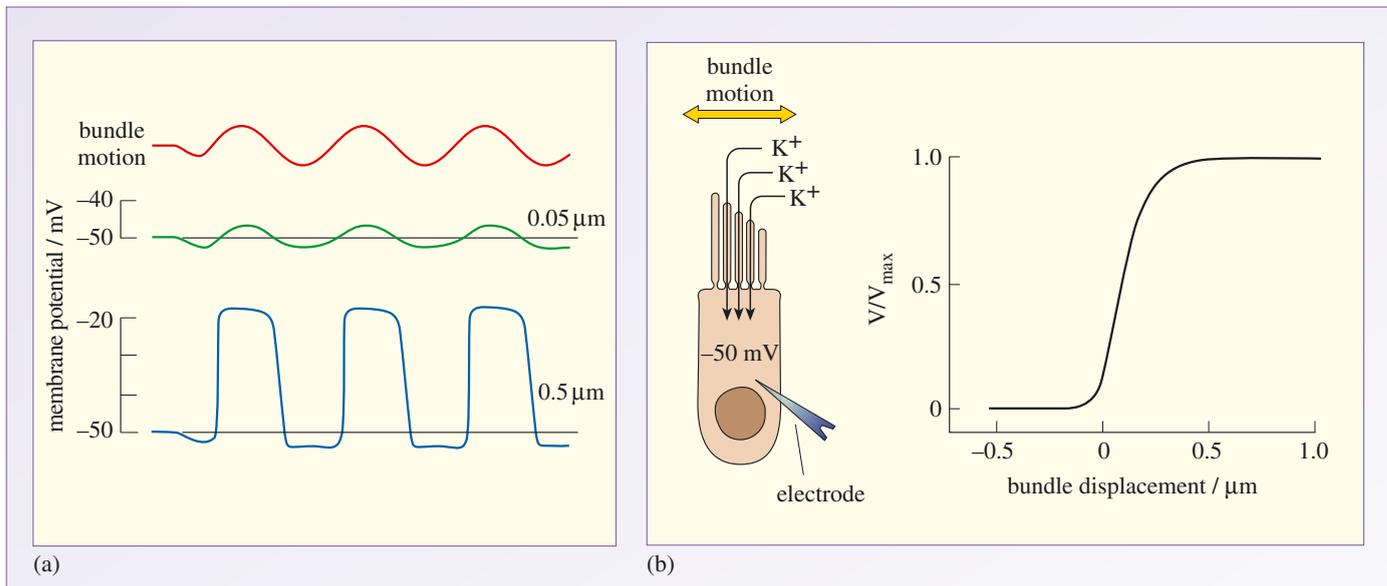
(a)



(b)

### 3 Mechano-electrical transduction and generation of the receptor potential

Mechano-electrical transduction refers to the conversion of the mechanical stimulus into an electrical signal called the **receptor potential**. The mechanism underlying transduction has been studied using isolated preparations of hair cell epithelia, where the electrical response of the hair cells can be recorded while manipulating the hair bundle. Because of the fragility of the mammalian cochlea, much evidence about mechano-electrical transduction comes from experiments on hair cells of sub-mammalian vertebrates like frogs and turtles (Figure 3a). In those preparations, rotation of the bundle towards its taller edge was first shown to open specialized **mechanosensitive ion channels**. This allows entry of small positively charged ions, depolarizing the hair cell from its resting potential of approximately  $-50$  mV (Figure 4). A small proportion (about 10%) of the mechanosensitive channels are normally open at rest, and rotation of the bundle towards its shorter edge closes those channels already open and hyperpolarizes the cell. Thus sinusoidal motion of the hair bundle about its resting position, as might be produced by a pure tone, generates a receptor potential oscillating around rest. As the magnitude of the stimulus is increased, the receptor potential grows in size and eventually saturates, reaching the greatest amplitude for hair bundle displacements of only  $0.5$   $\mu\text{m}$ . The maximal stimulus transduced is therefore comparable to the diameter of a stereocilium. At auditory threshold, the smallest displacement of the hair bundle detectable is 1000 times less than this, and is estimated to produce a receptor potential of  $0.1$  mV. At threshold, the hair bundle motion is comparable to the size of a hydrogen atom, testifying to the extraordinary sensitivity of the mechanotransduction process.



**Figure 4** (a) Receptor potentials generated by sinusoidal excursions of the hair bundle for a low-level ( $0.05$   $\mu\text{m}$ ) and a high-level ( $0.5$   $\mu\text{m}$ ) stimulus. On the positive part of the stimulus cycle, the bundle moves towards its taller edge, opening mechanosensitive channels, which depolarizes the cell. On the negative half of the cycle, the bundle is deflected towards its shorter edge, closing channels and hyperpolarizing the cell. For low-level stimuli the response is sinusoidal, for higher-level stimuli it becomes square. (b) A plot of the receptor potential against hair bundle displacement is asymmetric about the hair bundle's resting position. The receptor potential,  $V$ , is scaled to a  $V_{\text{max}}$ , of  $35$  mV.

Hair cells do not themselves generate action potentials since they lack the requisite voltage-dependent sodium channels present in the axon; action potentials are first produced in the afferent nerve fibres for signalling to the brain. However, the performance of the hair cells, the encoding and transmission of the auditory message, depends on the operation of several other kinds of ion channels (Figure 3b). Motion of the hair bundle is transduced into changes in membrane potential by the mechanosensitive channels. The receptor potential is then shaped by the operation of several types of potassium channel that are activated by depolarization or a rise in intracellular calcium. Besides determining the resting potential, potassium channels may also modify the time course and amplitude of the receptor potential. In sub-mammalian vertebrates, **calcium-activated potassium channels** generate oscillations in the membrane potential and play a role in the hair cell's frequency tuning. Finally, release of neurotransmitter at the synapse is triggered by calcium influx through voltage-dependent calcium channels clustered around the base of the hair cell beneath the synaptic endings.

The mechanosensitive channels, like other ion channels, are thought to be protein pores spanning the plasma membrane. These channels, when opened by bundle deflection, allow the passage of small monovalent ions like sodium and potassium. The mechanosensitive channels are also permeable to calcium ions, which traverses them even more readily than potassium. The protein pore may therefore be lined with negative charges for calcium ions to interact with in transit. However, some larger positively-charged molecules can occlude the open channel thereby blocking the ionic flux. Blocking compounds of medical importance are the aminoglycoside antibiotics like streptomycin. Prolonged exposure to these antibiotics can cause deafness due to destruction of the cochlear hair cells. This pathological effect develops over the course of several days, and may require the antibiotic to gain access to the hair cell interior and interfere with the cell's energy supply from the mitochondria.

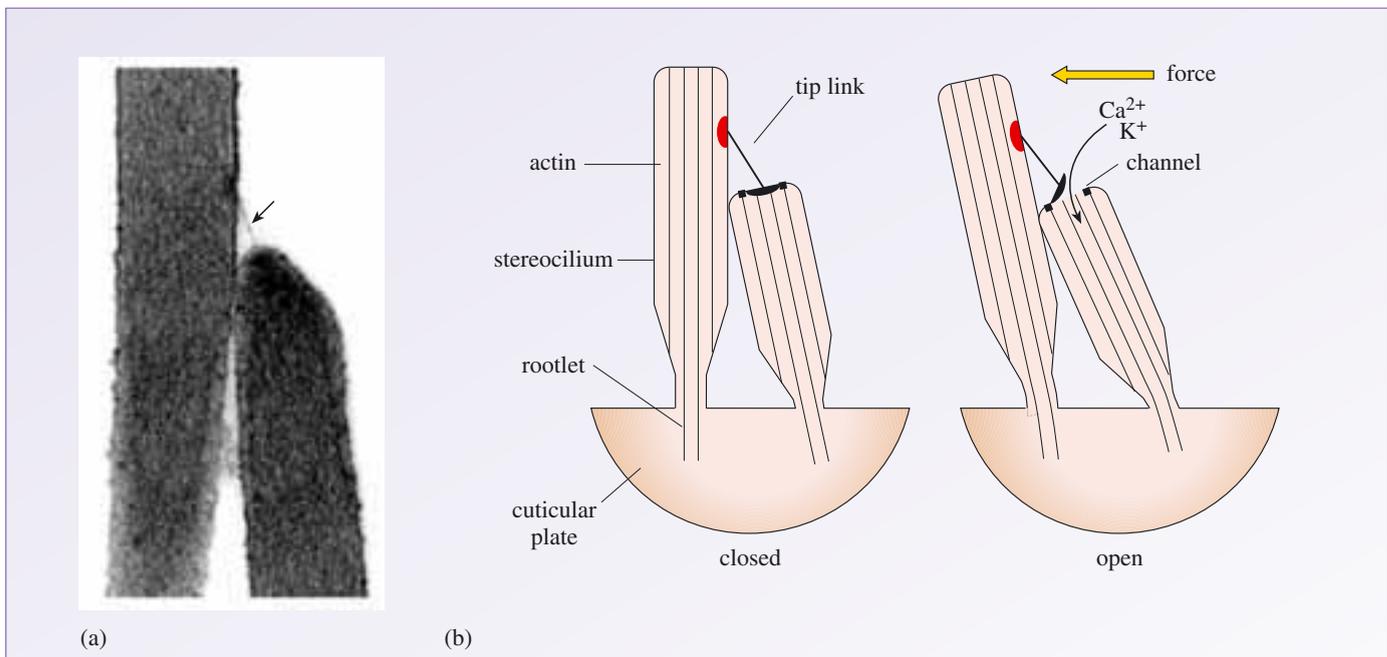
To understand the flow of ions through mechanosensitive channels in the intact cochlea, it is necessary to take into account the hair bundle's ionic environment. The layer of hair cells and supporting cells forms a tight epithelium separating two extracellular fluids of quite different ionic composition (Figure 3b). The base of the hair cells and the afferent nerve dendrites are bathed in perilymph, which has a composition the same as extracellular fluids elsewhere in the body. Perilymph has a normal sodium concentration in order to sustain action potentials in the afferent axons. In contrast, the hair bundles are immersed in an unusual solution known as endolymph, which is rich in potassium. Thus potassium will be the major ion flowing through the mechanosensitive channels *in vivo*. Because the concentration of potassium in endolymph is similar to its concentration in intracellular fluid, potassium ions will be forced through the mechanosensitive channels by the potential difference across the hair bundle membrane. This will be the sum of the negative resting potential of  $-50$  mV and an endocochlear potential of  $80$  mV that exists between the endolymph bathing the hair bundle and the perilymph. The **endocochlear potential** increases the driving force on potassium influx and enhances the sensitivity of mechano-electrical transduction.

## 4 The site of transduction

The coupling of motion of the hair bundle to opening of the mechanosensitive channels must be rapid if the cells are to encode the waveform of the sound stimulus. Human hearing extends to frequencies near  $20\,000$  Hz, where each cycle of

the sound pressure waveform lasts only  $50\ \mu\text{s}$ ! Such rapid temporal performance is incompatible with a mechanism that involves intermediate metabolism of second messengers, such as the cyclic nucleotides that mediate transduction in the sensory receptors for vision and smell. It is more likely that deformation of the hair bundle stretches mechanical linkages that directly open the channel (Figure 5). This is referred to as the ‘bath-plug mechanism’, where pulling on the chain removes a plug from the channel permitting ions to enter the cell. To establish this mechanism, it is necessary to define the location of the plug and identify the chain. Several pieces of evidence suggest that the mechanosensitive channels are concentrated towards the tops of the stereocilia. For example, it has been demonstrated (by filling the hair cell with a dye whose fluorescence changes after binding calcium ions) that deflection of the hair bundle causes an initial rise in intracellular calcium near the stereociliary tips. The additional calcium ions are assumed to have entered via the mechanosensitive channels. It has also been proposed that the channels are stimulated by **tip links**, fine extracellular filaments that pass from the tips of the shorter stereocilia to the sides of the taller row in front (Figure 5). Such tip links are visible in high-resolution electron micrographs where they appear to run approximately parallel to the bundle’s plane of symmetry.

The ‘tip link’ hypothesis is attractive because it explains the directionality of transduction and the ubiquitous gradation of stereociliary heights in hair bundles. Thus rotation of the bundle towards its taller edge will result in a vertical shear between adjacent rows of stereocilia, and will stretch the tip links to open the channels. Conversely, rotation towards the shorter edge will slacken the links and close the channels. It has not yet been possible to identify the molecular composition of the mechanosensitive channels or the tip links. Part of the difficulty stems from the small amount of protein available, which is an obstacle to biochemical purification

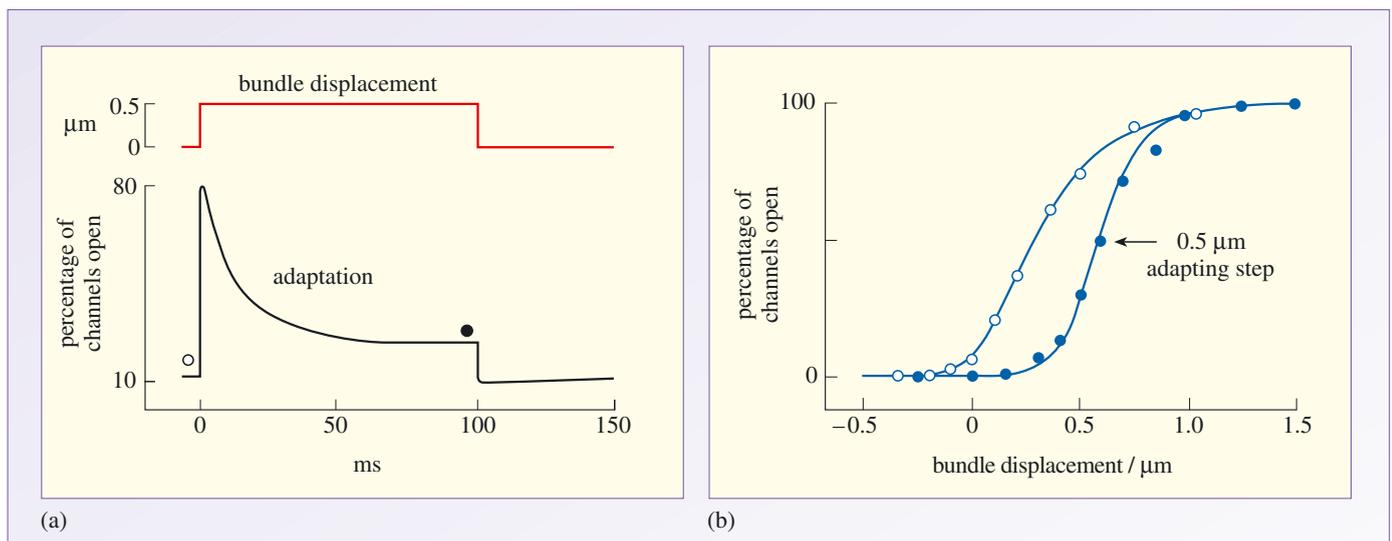


**Figure 5** (a) Transmission electron micrograph of a pair of stereocilia showing the tip link (arrow) proposed to deliver force to the mechanosensitive channels. (b) The stereocilia are filled with actin filaments that flex where their rootlets enter the actin meshwork of the cuticular plate. The mechanosensitive channel may be located at the tip of the stereocilium and opened by force transmitted via the tip link.

of the channel. For example, it has been estimated that there are only a few hundred channels per hair bundle, or just a few per stereocilium. Characterization of the mechanosensitive channel is an important goal of cochlear research because the channel may be a prime site for hair cell malfunction in certain kinds of deafness.

## 5 Adaptation

The **activation curve** for the mechanosensitive channels, the relationship between the number of channels open and bundle displacement, is S-shaped with about 10% of channels being activated when the bundle is unperturbed. At this bundle position, the activation curve has the steepest slope so that the cell is maximally sensitive for the small perturbations. To preserve high sensitivity in the face of drifts in bundle position, an **adaptation** mechanism exists to keep the channels within a narrow operating range (Figure 6). For a maintained deflection of the hair bundle, the channels first open but then close again over a time period of 1 to 100 ms, depending on the conditions. Such adaptation does not represent inactivation of the transducer channels, but rather a shift of the activation curve to a new working point. This may be a way of ensuring that the sensitivity is always maximal close to the resting position of the bundle. Adaptation is mediated by calcium ions that enter the cell through the mechanosensitive channels to reset their mechanical sensitivity.



**Figure 6** (a) During a prolonged displacement of the hair bundle, the mechanosensitive channels first open (open circle) but then close again (filled circle) in a process of adaptation. (b) The activation curve for the channels was determined from a set of brief displacements of the bundle presented prior to (open circles) or at the end of (filled circles) the adapting step. The results show that adaptation is due to a shift in the activation curve.

Two mechanisms have been suggested to account for adaptation. In one mechanism, calcium binds directly to the mechanosensitive channels and closes them. This process can occur very rapidly, within a millisecond, which may be important for the channel's operation at high frequencies. A slower mechanism may regulate the mechanical input to the channels by moving the upper attachment of the tip link along the side of the stereocilium. It has been proposed that this motion is driven by the climbing and slipping of myosin along the actin backbone of the stereocilium. Myosin is a specialized protein that interacts with actin to produce muscle

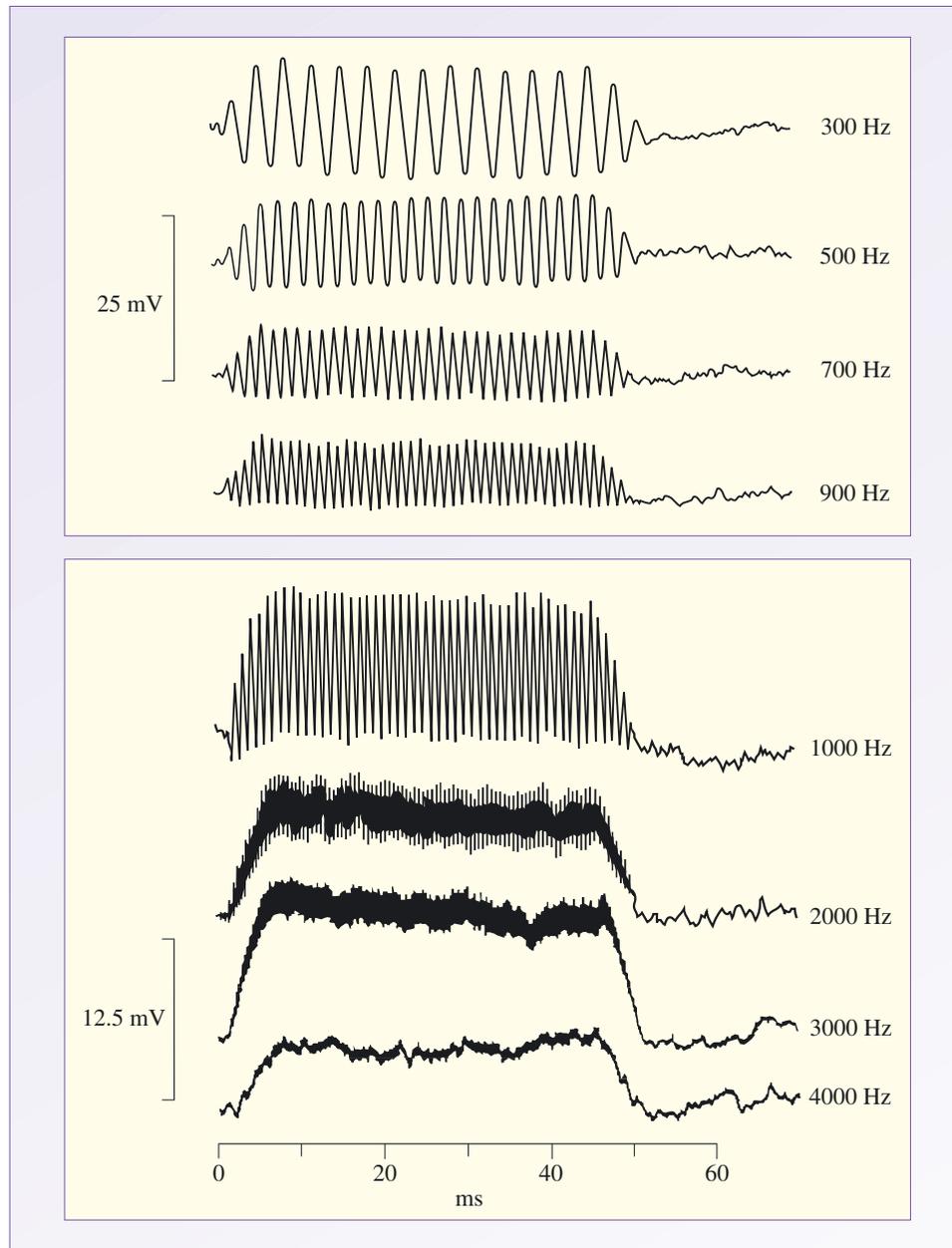
contraction or cell movements. Calcium entering the stereocilia through the mechanosensitive channels is thought to control both types of adaptation. For such a control mechanism, it is important for the mechanosensitive channels to have a high permeability to calcium. Because calcium ions easily traverse the mechanosensitive channels, sufficient calcium can enter the cell to promote adaptation even at the low calcium concentration in endolymph.

## 6 The afferent synapse and the auditory nerve response

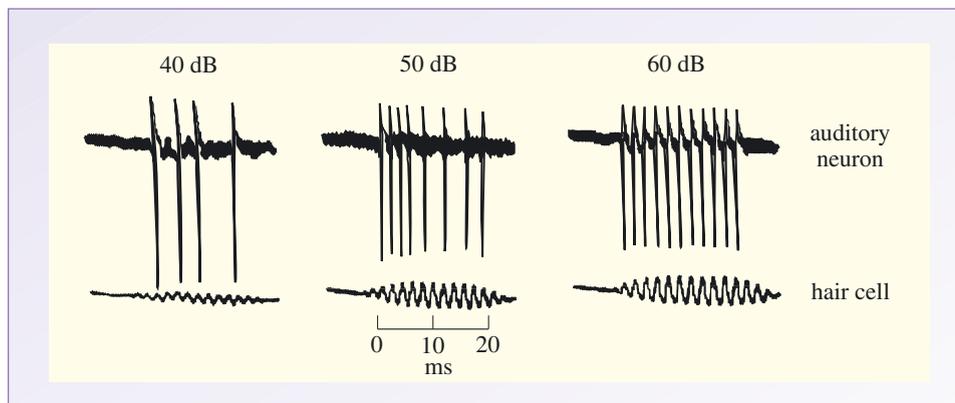
The receptor potential is a modulation in the resting potential of the hair cell that reflects the acoustic waveform. The receptor potential controls the release of chemical transmitter onto the afferent dendrite, where the signal is then converted into a train of action potentials. The synapse resembles other excitatory synapses in the brain by using the amino acid glutamate as the transmitter. However, unlike central synapses where each presynaptic action potential delivers a pulse of glutamate, the amount of glutamate released from the hair cell is graded with the membrane potential. Some transmitter leaks out in the absence of a stimulus and causes spontaneous firing of action potentials in the afferent dendrite. During a stimulus, depolarization of the hair cell releases more glutamate and increases the action potential firing rate. Conversely, hyperpolarization decreases the resting glutamate release and shuts off the spontaneous activity. Thus a pure tone of low frequency produces a sinusoidal receptor potential that modulates the firing of the afferent neuron in synchrony with the sound waveform. This is known as **phase locking** as the action potentials occur preferentially on one phase of the cycle.

In theory, the intervals between bursts of phase-locked action potentials can inform the brain about the frequency of the sound stimulus, but only if the frequency is less than 1000 Hz. At higher frequencies, phase locking is lost because the maximum rate of action potential firing in the auditory nerve fibres is exceeded. Furthermore, there are temporal limits imposed by the intrinsic properties of the hair cell and its transmitter release mechanism. At frequencies above 1000 Hz, the periodic receptor potential is transformed into a sustained depolarization of the hair cell known as the **summating potential** (Figure 7). The summating potential will augment transmitter release and cause a sustained increase in firing of action potentials throughout the stimulus.

The number of action potentials occurring during the sound stimulus depends on the intensity or loudness of that stimulus (Figure 8). For the lowest sound intensities, there is a small but reproducible increase in the mean firing rate over the spontaneous rate. The sound intensity at which this occurs is known as the neuron's **acoustic threshold**. As the sound intensity is raised above threshold, the action potential firing rate increases (Figure 8), but at the highest intensities it eventually saturates. The relationship between the action potential firing rate and sound level is thus S-shaped, similar to the hair cell's activation curve (Figure 4b and Figure 6b). However, different afferent neurons that synapse on the same inner hair cell have different thresholds. Some afferent fibres respond at the lowest sound pressure levels that are audible, but others are not recruited unless the sound pressure level is made 30-fold greater. The mechanism underlying this threshold variation is probably a difference in the sensitivity of the synapse connecting the hair cell to the afferent dendrite. The variation may enable the intensity of the sound to be represented over a wide dynamic range in terms of the firing rates in the population of afferent fibres.



**Figure 7** As the sound frequency is increased from 300 Hz to 4000 Hz, the hair cell receptor potential changes from a periodic to a sustained depolarization. The latter arises from the asymmetry of the activation curve of the mechanosensitive channel (Figure 4b).



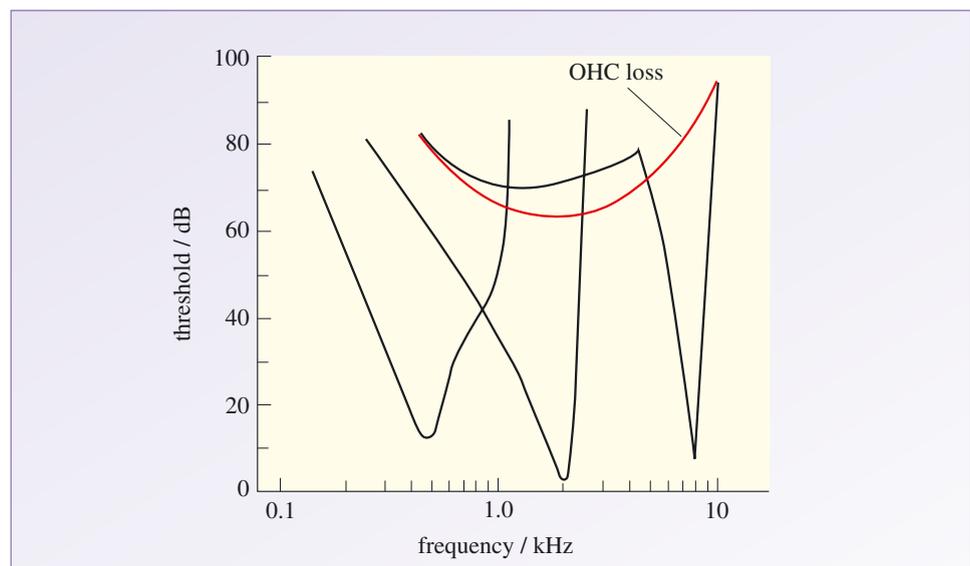
**Figure 8** The timing of action potentials in an auditory neuron is synchronized to the cycles of a 500 Hz sound stimulus. The sound level in decibels (dB) increases from left to right.

## 7 Hair cell frequency selectivity

Besides converting the acoustic waveform into an electrical signal, the cochlea also analyses the frequency composition of the sound stimulus. The relative proportions of the different frequency components can then be used by the brain to recognize the sound. For example different vowel sounds can be distinguished by the relative intensities of their constituent frequency bands or formants (see Chapter 5, *Hearing the world*). As already noted, low frequencies can be derived from the timing of action potentials phase-locked to the cycles of the sound. A more general method is the **place principle**, where each frequency is associated with a particular cochlear location. Such frequency analysis arises in part because the vibrations of the basilar membrane are tuned and, for a given frequency, are largest at one place. With progressively higher frequencies, the place of maximal vibration shifts systematically towards the base of the cochlea (see Chapter 1, *The mechanics of hearing*). The frequency components in a complex sound are thus segregated along the basilar membrane, and individual hair cells (and their associated auditory nerve fibres) are excited by stimuli containing only a small range of frequencies (Figure 9).

Although such frequency analysis appears at first sight to be caused by passive tuning of the basilar membrane (like the response of a guitar string), such passive tuning alone would be insufficient to generate the narrow frequency selectivity observed. It is believed that the outer hair cells play an active role in the process by supplying extra energy to reinforce the vibrations of the membrane. The effect of each outer hair cell is to amplify the vibrations of the basilar membrane locally, and as a consequence increase its frequency selectivity. The process is similar to augmenting the excursions of a child's swing by supplying appropriately timed pushes. Several lines of evidence support this idea. Electrical stimulation of the efferent nerve fibres damps the outer hair cell responses, which can diminish the frequency selectivity of the inner hair cells. Furthermore, preferential destruction of the outer hair cells by loud sounds or by aminoglycoside antibiotics degrades the frequency discrimination of the inner hair cells (Figure 9). These results are explained by proposing that outer hair cells can influence the relative motion of the basilar and tectorial membranes and thus govern the stimulus to the inner hair cells. This requires outer hair cells to possess an intrinsic force-generating mechanism.

**Figure 9** Frequency tuning curves in three auditory neurons, where the threshold sound level that just excites the neuron is plotted against sound frequency. Each neuron has a characteristic frequency where its threshold is a minimum. With destruction of outer hair cells (OHC loss), sound threshold is greatly elevated and frequency tuning deteriorates, as illustrated here for the neuron with the highest frequency.



Evidence supporting the outer hair cells' role in cochlear amplification comes from the observation that *in vitro* they can contract like muscle fibres when electrically stimulated. Depolarization shortens them and hyperpolarization elongates them. Transduction in outer hair cells is therefore bidirectional. Motion of the hair bundle is converted into a receptor potential, but a change in membrane potential can itself produce a mechanical response from the cell. The electromechanical performance of outer hair cells does not employ the same actin–myosin mechanism used to produce muscle contraction, but entails a special motor protein present at high density in the outer hair cell's lateral wall. It has been suggested that changes in membrane potential modify the shape of each motor protein, the net effect summed over all motors being to alter the surface area and hence length of the cell.

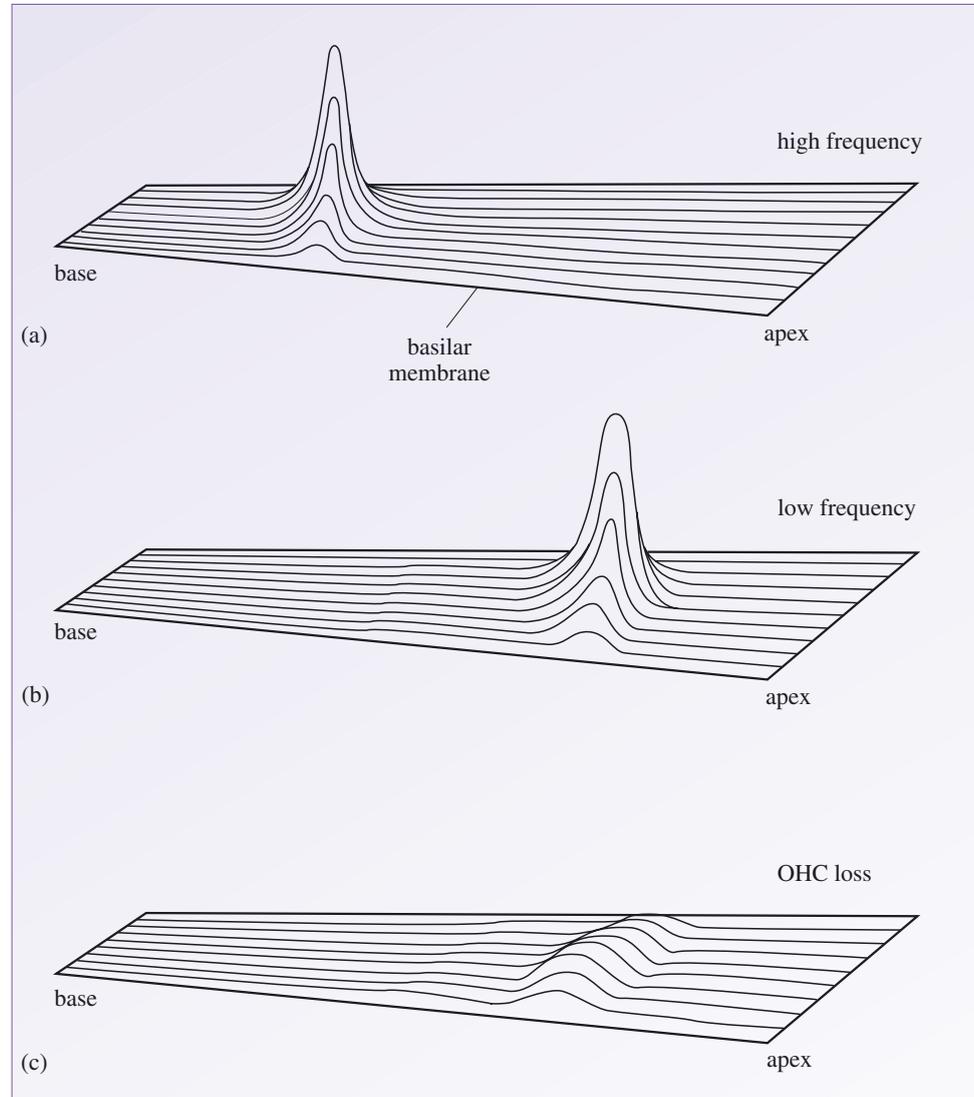
## 8 The tonotopic map

Each hair cell responds best to one particular sound frequency known as its **characteristic frequency** (Figure 9), which changes progressively with the distance of the cell along the basilar membrane. Cells at the base of the cochlea have the highest characteristic frequency, whereas cells at the apex have the lowest (Figure 10 overleaf). The projection of sound frequency on to cochlear location is known as the tonotopic map (see Chapter 1, *The mechanics of hearing*). The existence of a tonotopic map implies that the properties of the cochlea are not homogeneous along its length. A variation in the stiffness of the basilar membrane contributes to its tuning properties. The hair cells also exhibit diversity in both structure and biochemistry as a function of location. For example, in progressing from the low-frequency to high-frequency end of the cochlea, there is a three- to five-fold reduction in the length of the outer hair cell body and the height of the hair bundle. Such variations may be important for optimizing the outer hair cell's electromechanical performance. There are also gradients in the hair cell's complement of ion channels. Both the numbers and types of potassium channels in hair cells are quite different at the two ends of the cochlea. Hair cells responsive to high-frequency sounds may also have more mechanosensitive ion channels. These ion-channel gradients, though not fully understood, may be related to the differential sensitivity of the hair cells. High-frequency hair cells are more likely to be lost in old age or destroyed by loud sounds. Fathoming the origin of their vulnerability may provide a strategy for treating this significant medical problem.

## 9 Summary

The cochlea employs hair cells to convert sound-induced vibrations of the basilar membrane into electrical signals via deflection of their stereociliary bundles. Displacement of the bundle towards its tallest edge activates mechanosensitive ion channels located near the tips of the stereocilia, and allows potassium and calcium ions from the endolymph to enter the cell. The influx of these ions generates a receptor potential across the hair cell membrane, which in turn modulates the release of a chemical transmitter, most likely glutamate, onto the auditory afferent neurons. The sound intensity is represented by the size of the receptor potential in the hair cell, and the rate of firing of action potentials in the afferent neurons. Each hair cell responds preferentially, or is tuned, to a narrow band of sound frequencies, which change progressively with the cell's position along the cochlea. Such frequency selectivity is due to a mechanical tuning of the basilar membrane assisted by local amplification by a motor protein in the outer hair cells.

**Figure 10** (a) A high frequency sound causes the largest excursion of the basilar membrane near the base of the cochlea. (b) The vibration pattern shifts towards the apex for a low-frequency sound. (c) When the outer hair cells are destroyed (OHC loss), the vibration amplitude for the same stimulus as in (b) is reduced, indicating that the outer hair cells contribute to cochlear amplification.



## Further reading

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