Chapter 3
Biophysical Properties of Mechanotransduction

Wei Xiong

Abstract In early time, the coarse signals, such as cochlear microphonics and summating potentials, were recorded by electrocochleography by placing a metal electrode on the round window, which reflected the sound-induced electrical responses mostly mediated by hair cells. However, these signals are mainly a summated response from a group of hair cells. The direct evidence came from the intracellular recording of hair cells in the tail lateral line of mudpuppy *Necturus maculosus* (Harris et al., Science 167(3914):76–79, 1970). Similarly, auditory response from the cochlear hair cells was probed by intracellular recording in guinea pig (Russell and Sellick, Nature 267(5614):858–860, 1977; J Physiol 284:261–290, 1978). In isolated bullfrog saccule tissue, the mechanotransduction (MET) current was recorded in hair cells, which provided the first evidence that the deflection of hair bundle induced receptor potential change of hair cells (Hudspeth and Corey, Proc Natl Acad Sci USA 74(6):2407–2411, 1977). Nevertheless, whole-cell patch clamp was applied to hair cells to achieve a detailed information of the MET channel including some single-channel behaviour (Ohmori, J Physiol 359:189–217, 1985). From then on, researchers have studied most of the biophysical properties of the channel systematically by electrophysiology, pharmacology, and optical imaging without knowing the molecular identity of the MET channel. Now several important questions have been tackled in this chapter, including the following: Where does the channels localize in the hair cells? What kind of ions do the channels pass through? How are the channels activated and then adapted? How many channels are there opened per tip link? What are the single-channel properties?

Keywords Channel · Permeability · Activation · Adaptation · Reverse polarity

W. Xiong (✉)
School of Life Sciences, IDG/McGovern Institute for Brain Research, Tsinghua University, Beijing, China
e-mail: wei_xiong@mail.tsinghua.edu.cn

W. Xiong, Z. Xu (eds.), *Mechanotransduction of the Hair Cell*,
SpringerBriefs in Biochemistry and Molecular Biology,
https://doi.org/10.1007/978-981-10-8557-4_3

3.1 Channel Localization

It is not surprising to imagine that the cilia-based hair bundle is developed in the hair cells for the vibration sensation. In addition, the filamentous tip-link structure was observed between a stereocilium and its taller neighbouring stereocilium, which was proposed to play roles in transduction. An intriguing hypothesis was that the hair bundle hosts the MET channels and allocates the channels specifically to be around the tip links. However, the problem was how to prove it experimentally. Three decades ago, several pioneer research teams have used very delicate methods to locate the MET channels on hair bundle in a subcellular scale. In 1982, Hudspeth applied extracellular recording to draw a heat map for the channel distribution in the hair bundle of bullfrog saccular hair cells. Operationally, a fine-tip electrode was placed at an array of sites on the hair bundle to probe the flow of transduction current. By this way, the maximal transducer response took place at or near the top of the hair bundle, i.e. the distal ends of the stereocilia [6]. Late on, Jaramillo and Hudspeth used another strategy to further confirm the localization of the transducer channels at the hair bundles. This time, they locally applied a channel blocker gentamicin to scan the possible hot spot that can inhibit the transduction currents. Similarly, the most sensitive site for channel blocker was at the top of the hair bundle [7]. These studies suggested that the top surface of hair bundles was the most sensitive part to mechanical stimulation and aminoglycoside inhibition. However, it still remained puzzled whether the MET channels were exactly here for the functionality. To really point out where the ion fluxes in, cellular calcium imaging was recruited to visualize the channel activity; however, the epifluorescence microscopy just gave an ambiguous result due to low resolution [8]. Late on the confocal microscopy, a state-of-the-art technology back at that time was used to address this question. The higher temporal and spatial resolution endowed the confocal microscopy with the power to solve the question. The focal scan and line scan both showed that the calcium enters from the very top of the hair bundle and then diffuses along the stereocilia [9, 10], which is exactly the location of tip links.

Then the emerging question is how does the channels distribute around the tip link, symmetrically at both ends of the tip link or asymmetrically at one end of the tip link? If it is one end, then which end should host the channel, i.e. upper tip-link density (UTLD) or lower tip-link density (LTLD)? In previous studies, chicken and frog hair cells were used as model. The structure of lower vertebrate hair cells was similar to mammalian vestibular hair cells that had less manifestation of polarity. The pattern of calcium influx was not clear enough to draw a crystal conclusion that the MET channels localized on either or both end of the tip links [9]. It was until 2009 that the accurate location was firmly determined by Fettiplace and Ricci groups. They used rats as a model since the mammalian cochlear hair cells possessed three rows of stereocilia that showed an obvious staircase structure. Utilizing an ultrafast swept-field confocal microscope and calcium indicator with fast kinetics, they showed that the calcium influx only happened from the top of the second and third rows of stereocilia while leaving the tallest one intact during mechanical

deflection. Then it was assumed that the channel is at the lower tip-link side both in IHCs and OHCs [11]. Therefore, the asymmetry applied to not only the bundle structure but also the molecular distribution including the MET channels.

3.2 Channel Selectivity and Permeability

A follow-up question was which ions passed through the transducer channels in hair cells? Early in 1979, Corey and Hudspeth used adult bullfrog preparation to probe this question. Back at that time, dual sharp electrodes were applied with a simple voltage clamp configuration. The hair bundle was deflected with a triangle pattern at a magnitude of 1–2 μm and frequency of 10 Hz. By exchanging ionic solution on the apical side but keeping the basolateral side in perilymph bath, the selectivity of the transducer channel was examined as a nonselective cation channel. Comparing to K^+, the relative permeability reflected by microphonic current was 0.9 by Li^+, 0.9 by Na^+, 1.0 by Rb^+, and 1.0 by Cs^+. And more interestingly, ammonium ion (NH_4^+) was 1.3 [12]. Calcium seemed to be an important cofactor of MET current with Sr^{2+} as a replacement to Ca^{2+}, but Mg^{2+} and Ba^{2+} do not [5, 12].

Aminoglycoside has been a well-known toxic to hair cells. A major effect pathway was that aminoglycoside entered the hair cell through the MET channels and blocked the channels [5]. Despite of the ototoxicity of aminoglycoside, these molecules also provided an insight how big the channel was. It means that the channel pore is big enough to let a molecule such as streptomycin in. Interestingly, FM1-43 that has been intensively used in monitoring vesicle trafficking was shown to block the channels and compete with aminoglycoside [13]. As a summary, Farris et al. made a series of pharmacological examination of MET channel with multiple antagonists known targeting those common channels. The pharmacological profile indicated that the MET channel was closer to cyclic nucleotide-gated (CNG) and transient receptor potential (TRP) channels [14].

Further, many components were verified to change the channel transduction. Depletion of PIP2 in hair cells reduced the channel amplitude [15, 16]. Also it was reported that loss of TMC proteins caused an altered calcium permeability in hair cells [17]. Corey and Hudspeth checked the permeability of an organic cation TMA that had a size of 0.54 nm in diameter [12]. This evidence indicated that the transducer channels had an internal diameter of at least 0.65 nm. Farris et al. systematically discussed the appropriate pore size by testing a series of small organic molecules, with an idea that the narrowest diameter of the pore was 1.25 nm. The channel was around 3.1 nm in length and less than 1.7 nm in width [14].

3.3 Activation and Adaptation

Adaptation is broadly used as a general concept for sensory cue processing that the organisms gain a distinguished capability to collect useful information out of noise. In terms of each hair cell, it strongly adapts to sustained mechanical stimuli in millisecond time constant. The first piece of work on hair-cell adaptation has been studied systematically in bullfrog sacculus hair cells [18]. By directly exposing hair cells from frog, the nerve activity and MET current were examined by in vivo recording. The discharge rate of saccular nerve was increased during onset and termination of acceleration stimulation. The displacement-response curve of MET was not changed obviously in shape but only shifted when superimposing a step-like stimulation. This evidence strongly showed that an adaptation existed in hair cells together with many studies from others [19–24]. And this millisecond-level adaptation was defined as slow adaptation since the channel was later recognized to have amazingly fast kinetics.

It was possible to be studied more deeply when introducing a novel type of piezoelectric actuator with microsecond responsivity [24–27]. Surrendered to a step-like deflection of hair bundle, there were two components of adaptation after the activation phase [27]. The manifestation of currents showed dramatic kinetics difference with previously observed current property. Immediately following a rapid activation (usually less than 100 μs), there is a fast adaptation that is quite similar to fast inactivation of voltage-gated sodium channel but with a time constant of sub-millisecond in mammalian hair cells. Then there is a slow adaptation [25, 28]. It is not sure whether this fast activation time was still underestimated due to physical limitation of stimulation apparatus. The channel opens and recloses so rapidly that it is consistent with the coding requirement of the sound information, especially for middle-to-high frequencies. In physiological status, sinusoid sound wave seems not challenging the MET channels too much. However, it is still an interesting question how high the frequency hair cell can detect by itself, such as whether a 10 kHz hair cell responses accurately to the 10 kHz wave. It means that the rising/falling phase of stimulation is around 25 μs theoretically, though the basilar membrane has done most of the job to analyse the frequencies.

Adaptation can be affected by many factors. Early study has found the adaptation was sensitive to voltage and calcium [19, 20, 29]. It was mainly resulted from calcium inhibition to the channel [30]. Recently, many MET components were characterized as essential regulator to the channel kinetics. It has been reported that Myo7a, harmonin, and LHFPL5 (also known as TMHS) ablation reduced the fast adaptation [31–33]. Actually membrane lipid around is also the important player for normal function of MET channels. A representative case is PIP2 that deeply contributed to current kinetics, such as conductance and adaptation [15, 16].

3.4 Single-Channel Properties

The channels are highly clustered at LTLD, so the number of tip links determines the number of active channels. By manipulating the number of the tip links to a few, it provided an opportunity to study the MET channel at single-channel level. A step-like MET current has been observed with a triangular stimulation in whole-cell patch-clamped chicken hair cells [34]. It was considered as single-channel recording of transducer channels that was later studied intensively by several groups though the conductance was reported ranging from 10 pS to 110 pS [5, 34–39]. Especially, Crawford et al. proposed that the conductance is around 110 pS in turtle hair cells, and late Geleoc et al. had a similar observation on mice [36, 37]. In 2003, Ricci et al. systematically analysed the single-channel behaviour of MET complex in turtle. The single-channel events were perfectly matching the macroscopic current kinetics after average assembly. Extracellular calcium deprivation from 2.8 mm to 0.05 mm increased the channel conductance from 118 pS to 215 pS by average [38]. There was a tonotopic distribution of channel conductances both in turtle [38] and in rat [39]. However, IHCs show no tonotopic variation on MET channel conductances that is equal to maximal conductance of OHCs, known as high-frequency hair cells.

With a relatively accurate single-channel conductance measurement, it is easy to calculate number of the channels per tip link. In 2006, Beurg et al. measured number of stereocilia and amplitude of transducer current. By average, there was 91 stereocilia that represented 60 tip links per OHC in middle coil. By measuring the saturated MET current as 1.2 nA and the single channel current as 12.1 pA, it was counted 1.65 channel per tip link. It was also confirmed that there are two channels per tip link by Ca^{2+} imaging calculation. By validating number of active stereocilia in IHCs with calcium imaging, the linearized fit showed 35.4 pA MET current per stereocilium. Consider the single-channel conductance as 15 pA at −80 mV, there were estimated two channels per tip link [11, 39].

3.5 Reverse-Polarity Mechanotransduction

In mature hair cells, deflection of the hair bundles towards the tallest stereocilia increases the open probability of the sensory MET channels, while deflection in the opposite direction decreases the open probability [40]. However, in some conditions, there is a type of MET current other than the classic properties of transducer channels [41–47]. Controlled by a sinusoid wave, a fluid jet generated a MET current at the negative phase in addition to the positive phase [42, 46]. In TMC1 and TMC2 double knockout mice, the hair cells showed this type of MET current even

there was no classic MET current once the hair bundle was negatively deflected intensively (hence short as reverse-polarity MET current) [41]. This reverse-polarity current came out also when the hair bundle was treated by BAPTA, a calcium chelator breaking up tip links [41, 47]. It seems that the reverse-polarity current showed up once the MET complex was dissembled or immature [48]. This reminds us a series of observation that MET current was recorded in the inhibitory direction in null mice with MET component deficit [41–47] and a developing hair cell showed a lack of directional sensitivity [47–49]. The ion selectivity and responsiveness to pharmacological blockers of the reverse-polarity current are similar but not identical to that of the regular MET current [41, 47, 50]. High-speed Ca^{2+} imaging suggested that the reverse-polarity channels are not localized to the hair bundle but distributed at the apical surface of hair cells [48].

Then a debate arose that whether this reverse-polarity channel is identical to the classic MET channel. A new type of MET channel might be responsible for this reverse-polarity current, or the two channels are the same one but just locating at different position to give different biophysical behaviour. The TMC1 and TMC2 double knockout hair cells present the reverse-polarity current, which suggests either the two types of MET currents were from two types of the channels or the TMCs are not the channels for reverse polarity. In 2016, Mueller lab excluded the possibility of one-channel hypothesis. They found Piezo2 is specifically expressed in OHCs, while Piezo1 is not detected in hair cells. It has been well known that Piezos are MET channels that play pivotal roles in proprioception, sheath stress, lung airway, and so on. The molecular mechanism of Piezo2 in hair-cell MET is discussed in Chap. 4. Since Piezo2 null mice were embryonic lethal, they made conditional knockout by introducing Pax2-specific deletion of Piezo2 in the inner ear cells but keep the animal alive. The Piezo2 conditional knockout mice showed classic MET currents and relatively normal auditory function but only lost the reverse-polarity current. It is an amazing phenotype though we still have no idea about what is the physiological function of reverse-polarity current. Ironically, we have known so many properties about auditory MET, but the molecular identity is still elusive. Of course, it have placed TMCs again the first candidate for the MET channel.

3.6 Discussion

In this chapter, we summarized the biophysical properties for the mechanotransducer channels of hair cells. As a nonselective cation channel, it is highly restricted to the membrane patch proximal to the lower end of the tip link in mature hair cells. It generally allows most of the regular cations to flux in, but calcium inhibits the channel from intracellular side once it comes in. With the modulation of calcium and scaffold proteins, the channels are endowed with kinetics of fast and slow adaptation. Surprisingly, a type of “reverse-polarity” MET current exists in addition to

the classic MET current in hair cells. Clearly, they are resulted from different channel proteins, especially known as Piezo2 contributing on reverse-polarity MET. However, our biophysical understanding of the classic MET channel is still limited by the fact that the channel was not cloned yet, or at least the channel cannot be reconstructed in an exogenously expressing system. TMCs are the top candidates, but their role in MET complex is still in debate. To know everything about the MET of the hair cells, we need significant input from molecular genetics and biochemistry. Of course, it is very inspiring to get a whole picture by embedding abundant morphological and biophysical knowledge with molecular basis of MET machinery that will be discussed in next chapter.

References

1. Harris, G.G., L.S. Frishkopf, and A. Flock. 1970. Receptor potentials from hair cells of the lateral line. *Science* 167 (3914): 76–79.
2. Russell, I.J., and P.M. Sellick. 1977. Tuning properties of cochlear hair cells. *Nature* 267 (5614): 858–860.
3. ———. 1978. Intracellular studies of hair cells in the mammalian cochlea. *The Journal of Physiology* 284: 261–290.
4. Hudspeth, A.J., and D.P. Corey. 1977. Sensitivity, polarity, and conductance change in the response of vertebrate hair cells to controlled mechanical stimuli. *Proceedings of the National Academy of Sciences of the United States of America* 74 (6): 2407–2411.
5. Ohmori, H. 1985. Mechano-electrical transduction currents in isolated vestibular hair cells of the chick. *The Journal of Physiology* 359: 189–217.
6. Hudspeth, A.J. 1982. Extracellular current flow and the site of transduction by vertebrate hair cells. *The Journal of Neuroscience* 2 (1): 1–10.
7. Jaramillo, F., and A.J. Hudspeth. 1991. Localization of the hair-cells transduction channels at the hair bundles top by iontophoretic application of a channel blocker. *Neuron* 7 (3): 409–420.
8. Ohmori, H. 1988. Mechanical stimulation and Fura-2 fluorescence in the hair bundle of dissociated hair cells of the chick. *The Journal of Physiology* 399: 115–137.
9. Denk, W., et al. 1995. Calcium imaging of single stereocilia in hair cells: localization of transduction channels at both ends of tip links. *Neuron* 15 (6): 1311–1321.
10. Lumpkin, E.A., and A.J. Hudspeth. 1995. Detection of Ca2+ entry through mechanosensitive channels localizes the site of mechanoelectrical transduction in hair cells. *Proceedings of the National Academy of Sciences of the United States of America* 92 (22): 10297–10301.
11. Beurg, M., et al. 2009. Localization of inner hair cell mechanotransducer channels using high-speed calcium imaging. *Nature Neuroscience* 12 (5): 553–558.
12. Corey, D.P., and A.J. Hudspeth. 1979. Ionic basis of the receptor potential in a vertebrate hair cell. *Nature* 281 (5733): 675–677.
13. Gale, J.E., et al. 2001. FM1-43 dye behaves as a permeant blocker of the hair-cell mechanotransducer channel. *Journal of Neuroscience* 21 (18): 7013–7025.
14. Farris, H.E., et al. 2004. Probing the pore of the auditory hair cell mechanotransducer channel in turtle. *The Journal of Physiology* 558 (Pt 3): 769–792.
15. Hirono, M., et al. 2004. Hair cells require phosphatidylinositol 4,5-bisphosphate for mechanical transduction and adaptation. *Neuron* 44 (2): 309–320.
16. Effertz, T., et al. 2017. Phosphoinositol-4,5-bisphosphate regulates auditory hair-cell mechanotransduction-channel pore properties and fast adaptation. *The Journal of Neuroscience* 37 (48): 11632–11646.

17. Kim, K.X., and R. Fettiplace. 2013. Developmental changes in the cochlear hair cell mechanotransducer channel and their regulation by transmembrane channel-like proteins. *The Journal of General Physiology* 141 (1): 141–148.
18. Eatock, R.A., D.P. Corey, and A.J. Hudspeth. 1987. Adaptation of mechanoelectrical transduction in hair-cells of the Bullfrogs Sacculus. *Journal of Neuroscience* 7 (9): 2821–2836.
19. Assad, J.A., N. Hacohen, and D.P. Corey. 1989. Voltage dependence of adaptation and active bundle movement in bullfrog saccular hair cells. *Proceedings of the National Academy of Sciences of the United States of America* 86 (8): 2918–2922.
20. Crawford, A.C., M.G. Evans, and R. Fettiplace. 1989. Activation and adaptation of transducer currents in turtle hair cells. *The Journal of Physiology* 419: 405–434.
21. Assad, J.A., and D.P. Corey. 1992. An active motor model for adaptation by vertebrate hair cells. *The Journal of Neuroscience* 12 (9): 3291–3309.
22. Shepherd, G.M., and D.P. Corey. 1994. The extent of adaptation in bullfrog saccular hair cells. *The Journal of Neuroscience* 14 (10): 6217–6229.
23. Holt, J.R., D.P. Corey, and R.A. Eatock. 1997. Mechanoelectrical transduction and adaptation in hair cells of the mouse utricle, a low-frequency vestibular organ. *The Journal of Neuroscience* 17 (22): 8739–8748.
24. Ricci, A.J., Y.C. Wu, and R. Fettiplace. 1998. The endogenous calcium buffer and the time course of transducer adaptation in auditory hair cells. *The Journal of Neuroscience* 18 (20): 8261–8277.
25. Kennedy, H.J., et al. 2003. Fast adaptation of mechanoelectrical transducer channels in mammalian cochlear hair cells. *Nature Neuroscience* 6 (8): 832–836.
26. Ricci, A.J., and R. Fettiplace. 1998. Calcium permeation of the turtle hair cell mechanotransducer channel and its relation to the composition of endolymph. *The Journal of Physiology* 506 (Pt 1): 159–173.
27. Wu, Y.C., A.J. Ricci, and R. Fettiplace. 1999. Two components of transducer adaptation in auditory hair cells. *Journal of Neurophysiology* 82 (5): 2171–2181.
28. Ricci, A.J., et al. 2005. The transduction channel filter in auditory hair cells. *The Journal of Neuroscience* 25 (34): 7831–7839.
29. Hacohen, N., et al. 1989. Regulation of tension on hair-cell transduction channels: displacement and calcium dependence. *The Journal of Neuroscience* 9 (11): 3988–3997.
30. Kimitsuki, T., and H. Ohmori. 1992. The effect of caged calcium release on the adaptation of the transduction current in chick hair cells. *The Journal of Physiology* 458: 27–40.
31. Xiong, W., et al. 2012. TMHS is an integral component of the mechanotransduction machinery of cochlear hair cells. *Cell* 151 (6): 1283–1295.
32. Grillet, N., et al. 2009. Harmonin mutations cause mechanotransduction defects in cochlear hair cells. *Neuron* 62 (3): 375–387.
33. Kros, C.J., et al. 2002. Reduced climbing and increased slipping adaptation in cochlear hair cells of mice with Myo7a mutations. *Nature Neuroscience* 5 (1): 41–47.
34. Ohmori, H. 1984. Mechanoelectrical transducer has discrete conductances in the chick vestibular hair cell. *Proceedings of the National Academy of Sciences of the United States of America* 81 (6): 1888–1891.
35. Holton, T., and A.J. Hudspeth. 1986. The transduction channel of hair cells from the bull-frog characterized by noise analysis. *The Journal of Physiology* 375: 195–227.
36. Crawford, A.C., M.G. Evans, and R. Fettiplace. 1991. The actions of calcium on the mechanoelectrical transducer current of turtle hair cells. *The Journal of Physiology* 434: 369–398.
37. Geleoc, G.S., et al. 1997. A quantitative comparison of mechanoelectrical transduction in vestibular and auditory hair cells of neonatal mice. *Proceedings of the Biological Sciences* 264 (1381): 611–621.
38. Ricci, A.J., A.C. Crawford, and R. Fettiplace. 2003. Tonotopic variation in the conductance of the hair cell mechanotransducer channel. *Neuron* 40 (5): 983–990.
39. Beurg, M., et al. 2006. A large-conductance calcium-selective mechanotransducer channel in mammalian cochlear hair cells. *The Journal of Neuroscience* 26 (43): 10992–11000.

40. Shotwell, S.L., R. Jacobs, and A.J. Hudspeth. 1981. Directional sensitivity of individual vertebrate hair cells to controlled deflection of their hair bundles. *Annals of the New York Academy of Sciences* 374: 1–10.
41. Kim, K.X., et al. 2013. The role of transmembrane channel-like proteins in the operation of hair cell mechanotransducer channels. *The Journal of General Physiology* 142 (5): 493–505.
42. Alagramam, K.N., et al. 2011. Mutations in protocadherin 15 and cadherin 23 affect tip links and mechanotransduction in mammalian sensory hair cells. *PLoS One* 6 (4): e19183.
43. Zhao, B., et al. 2014. TMIE is an essential component of the mechanotransduction machinery of cochlear hair cells. *Neuron* 84 (5): 954–967.
44. Beurg, M., et al. 2015. Subunit determination of the conductance of hair-cell mechanotransducer channels. *Proceedings of the National Academy of Sciences of the United States of America* 112 (5): 1589–1594.
45. Michalski, N., et al. 2007. Molecular characterization of the ankle-link complex in cochlear hair cells and its role in the hair bundle functioning. *The Journal of Neuroscience* 27 (24): 6478–6488.
46. Stepanyan, R., and G.I. Frolenkov. 2009. Fast adaptation and Ca2+ sensitivity of the mechanotransducer require myosin-XVa in inner but not outer cochlear hair cells. *The Journal of Neuroscience* 29 (13): 4023–4034.
47. Marcotti, W., et al. 2014. Transduction without tip links in cochlear hair cells is mediated by ion channels with permeation properties distinct from those of the mechano-electrical transducer channel. *The Journal of Neuroscience* 34 (16): 5505–5514.
48. Beurg, M., et al. 2016. Development and localization of reverse-polarity mechanotransducer channels in cochlear hair cells. *Proceedings of the National Academy of Sciences of the United States of America* 113 (24): 6767–6772.
49. Waguespack, J., et al. 2007. Stepwise morphological and functional maturation of mechanotransduction in rat outer hair cells. *The Journal of Neuroscience* 27 (50): 13890–13902.
50. Beurg, M., K.X. Kim, and R. Fettiplace. 2014. Conductance and block of hair-cell mechanotransducer channels in transmembrane channel-like protein mutants. *The Journal of General Physiology* 144 (1): 55–69.