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Myosin I and adaptation of mechanical transduction by the inner ear

Peter G. Gillespie

Oregon Hearing Research Center and Vollum Institute, Oregon Health & Science University, 3181 SW Sam Jackson Park Road, Portland, OR 97239, USA (*gillespp@ohsu.edu*)

Twenty years ago, the description of hair-cell stereocilia as actin-rich structures led to speculation that myosin molecules participated in mechanical transduction in the inner ear. In 1987, Howard and Hudspeth proposed specifically that a myosin I might mediate adaptation of the transduction current carried by hair cells, the sensory cells of the ear. We exploited the myosin literature to design tests of this hypothesis and to show that the responsible isoform is myosin 1c. The identification of this myosin as the adaptation motor would have been impossible without thorough experimentation on other myosins, particularly muscle myosins. The sliding-filament hypothesis for muscle contraction has thus led to a detailed understanding of the behaviour of hair cells.

Keywords: myosin; hair cells; mechanotransduction; adaptation; chemical genetics

1. INTRODUCTION

Residing within the inner ear, hair cells respond to mechanical stimuli, like those arising from sound or head movements (reviewed in Hudspeth 1989). At the apex of a hair cell is its hair bundle, a mechanically sensitive organelle: the bundle is made up of 30–300 actin-filled stereocilia and often a single microtubule-based kinocilium (figure 1*a*). Auditory or vestibular stimuli cause the bundle to move back and forth, leading to the opening and closing of transduction channels. When channels are open, they admit cations that depolarize the cell and trigger neurotransmitter release; when channels close, the cell hyperpolarizes and transmitter release ceases. Because of the unusual K^+-rich extracellular solution bathing hair bundles, the majority of this transduction current is carried by K^+; Ca^{2+} is also highly permeable and plays a key regulatory role in adaptation.

During bundle stimulation, adjacent stereocilia slide along each other, increasing tension in the extracellular tip link: this tension opens the channel. The tip link, an extracellular filament composed at least in part of cadherin 23 (Siemens *et al.* 2004), is probably not the elastic 'gating spring' inferred by biophysical measurements (Corey & Hudspeth 1983*a*) but instead is probably connected to it in series. The transduction channel responds to force in the gating spring and any attached components: when force is high, channels open, and when force is low, channels close (Corey & Hudspeth 1983*a*).

Hair cells are remarkably sensitive: at the limit of mammalian hearing, hair cells must respond to bundle movements as small as a few angstroms (Hudspeth 1989). Moreover, the hair cell electrical response can be fully saturated with bundle movements of only *ca*. 1°, or in the case of auditory hair cells, *ca*.100 nm. Assembly and maintenance of a transduction apparatus that responds with such sensitivity must require homeostatic mechanisms to continuously poise transduction channels near their most sensitive position. At least two independent adaptation mechanisms are responsible, at least one of which involves a myosin motor.

One contribution of 14 to a Discussion Meeting Issue 'Myosin, muscle and motility'.

2. ADAPTATION

In 1983, Corey and Hudspeth showed that hair cells adapt: a static excitatory displacement of the hair bundle initially leads to a large transduction current, which then declines over *ca*. 20 ms (Corey & Hudspeth 1983*b*). Although channels remain capable of being opened during adaptation, the size of the deflection required to open channels to the same extent increases: this behaviour suggests that the channels retain the same intrinsic sensitivity but have somehow been reset to a different operating range (Eatock *et al.* 1987). Adaptation is not exclusively a property of hair cells examined *in vitro*, as single neurons in the vestibular nerve contacting the frog's sacculus show very similar adaptation during acceleration of a live frog (Eatock *et al.* 1987).

Howard and Hudspeth (1987) showed that during an excitatory deflection with a flexible fibre, a bundle relaxed further in the positive direction with the same time-course as adaptation. This was a pivotal observation: the bundle undergoes a mechanical rearrangement during adaptation. In their model, during an excitatory stimulus, the upper attachment point of the tip link slides down the stereocilium, reducing tension felt by the channel and allowing it to close. When tension in the tip link decreases, such as during a negative stimulus, the insertion point climbed up the stereocilium and restored tension. Howard and Hudspeth argued that a myosin, and in particular one from the

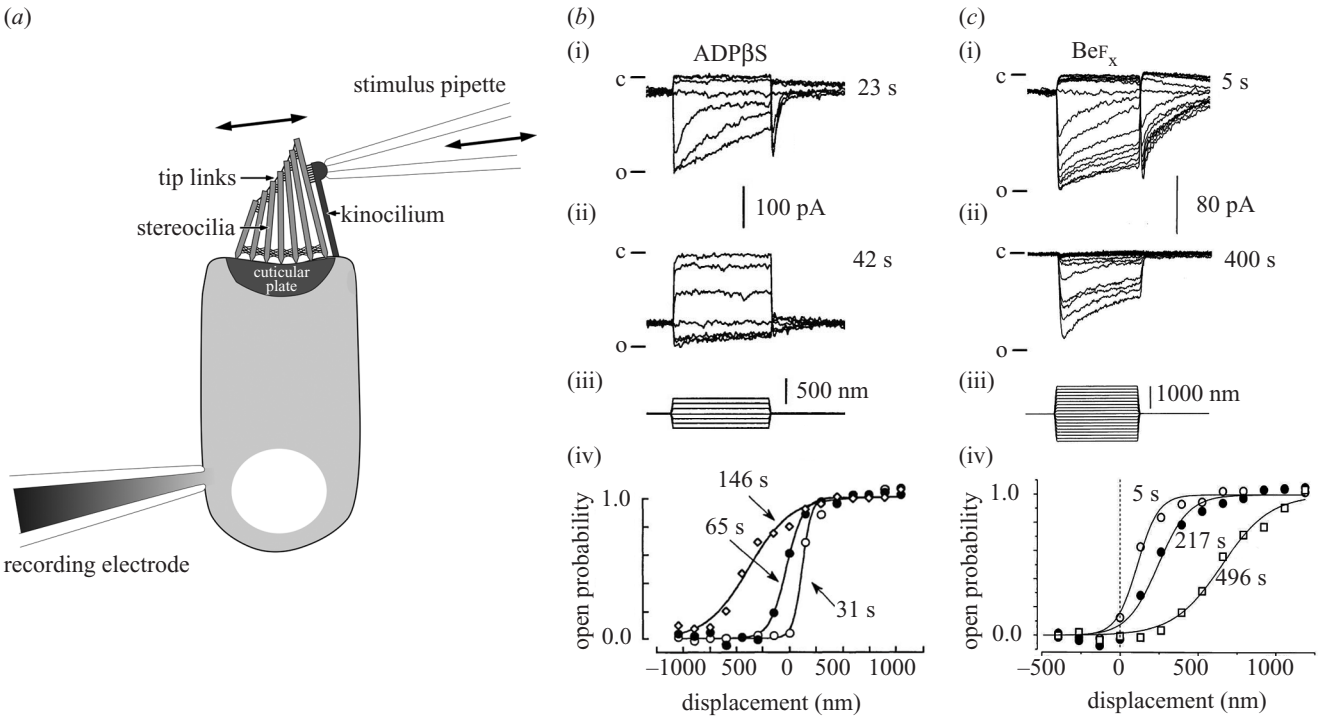

Figure 1. Inhibition of adaptation by ADP and phosphate analogues using whole-cell recording. (*a*) Mechanical stimulation and whole-cell recording of a transducing hair cell. A glass micropipette is adhered with suction to the top of the kinocilium of a hair cell: a piezoelectric device is used to move the micropipette and hence the hair bundle. Simultaneously, another glass pipette (the 'recording electrode') is used to make an electrically tight seal with the membrane: the whole-cell configuration is used, where the membrane within the pipette is sucked away. The contents of the recording electrode now have access to the cell: these contents could be a quasi-intracellular solution with a myosin inhibitor, for example. The recording electrode is connected via a Ag–AgCl wire to a patch-clamp amplifier, which allows the membrane potential of the cell to be fixed to a given level. As the transduction channels open during mechanical stimulation, cations flow into the cell; the clamp circuit counters this current flow with a current of opposite polarity, maintaining a constant potential. The clamp circuit records this feedback current back to the experimenter: it is equivalent to the transduction current, the cation flow during channel opening. (*b*)(i,ii) Transduction currents from a single hair cell using a whole-cell recording electrode filled with 10 mM ADPβS. Note block of adaptation and shift of the resting current level from near fully closed (c) to near fully open (o). (*b*)(iv) displacement–response relations from a different cell during dialysis with 10 mM ADPβS. Notice the leftward shift of the curve as dialysis proceeds. (Reprinted with modification from Gillespie & Hudspeth (1993) with permission.) (*c*) (i,ii) Dialysis of a hair cell with 1 mM beryllium fluoride (BeF_x). Currents shift from partly open to fully closed at rest. (*c*)(iv) Rightward shift in displacement–response relation as dialysis proceeds. (Reprinted, with modification, from Yamoah & Gillespie (1996), with permission from Elsevier.)

myosin I family, was well suited to control the tip-link attachment point.

Assad *et al.* (1989) showed that adaptation depended strongly on Ca^{2+} and that the adaptation motor must be very close to the transduction channel these results suggested a model in which the transduction channel is located at the top of a tip link and that the motor is connected in series in the immediate neighbourhood. Moreover, these authors also showed that the adaptation motor is an active force generator, capable of moving the hair bundle if it is activated (Assad & Corey 1992). These data strongly reinforced the concept that adaptation was mediated by a myosin.

3. FAST AND SLOW ADAPTATION

Although the properties of adaptation seemed to differ substantially in hair cell preparations studied by different laboratories, the past 5 years has brought a realization that there are two forms of adaptation, 'fast' and 'slow'. Although both forms of adaptation coexist in the same hair cell, they appear to be mediated by distinct mechanisms. The most commonly accepted model for fast adaptation, which is characterized by a time-constant for channel closing of a few milliseconds or less, suggests that Ca^{2+} entering through open transduction channels causes them to shut. By contrast, slow adaptation is characterized by a time-constant of *ca*. 20 ms and is caused by the myosin mechanism described above. Because slow adaptation can be blocked fully while fast adaptation remains (Holt *et al.* 2002), these two processes can be disentangled.

4. EVIDENCE FOR MYOSIN MOTORS IN ADAPTATION

Does adaptation have the right properties to be mediated by a myosin molecule? Inhibition of adaptation with reagents selective for specific classes of myosin, or even myosins in general, could provide evidence to test this assertion. We dialysed hair cells with two classes of ATPase inhibitors and looked for expected effects on transduction and adaptation (Gillespie & Hudspeth 1993; Yamoah & Gillespie 1996). ADP and ADP analogues should block myosins while bound to actin filaments and increase force production; by contrast, phosphate analogues should trap

myosin in a weakly bound state in which force production is inhibited. Although these agents should inhibit other ATPases, the specific predictions of their effects on myosin coupled with our detailed knowledge of the biophysical features of hair-cell transduction lead to strong predictions that assist in the interpretation of results.

We introduced ADP and ADP analogues into transducing hair cells and looked for their effects on the rate of adaptation and the force production of the adaptation motor (Gillespie & Hudspeth 1993). Although ADP alone had variable effects on adaptation, we suspected that metabolic enzymes such as adenylate kinase may have converted two molecules of ADP to one of AMP and one of ATP, the latter of which could power the adaptation motor. We obtained more reproducible results with ADPβS (figure 1*b*), which is not a substrate for adenylate kinase, and with a mixture of ADP and the adenylate kinase inhibitor Ap_5A. In both cases, adaptation was blocked but transduction remained intact. Moreover, the resting channel open probability, which depends on the force applied to the channel by the adaptation motor, increased in both cases (figure 1*b*). These data were in line with results seen in contracting muscle when ADP was introduced (Cooke & Pate 1985; Pate & Cooke 1989).

Reciprocal results were seen when we dialysed hair cells with the phosphate analogues vanadate, beryllium fluoride and sulphate (Yamoah & Gillespie 1996). In all cases, the force production of the adaptation motor was fully inhibited, reflected in the pronounced rightward shift of the displacement–response relation (figure 1*c*). Hair cells did not adapt at all to inhibitory displacements, which would have required the motor activity of the myosins making up the adaptation motor. Although there was some apparent adaptation to excitatory displacements, this behaviour could be explained by weakly bound myosin molecules transiently interacting with actin (Yamoah & Gillespie 1996). These results also reflected the behaviour of contracting muscle that has been exposed to phosphate analogues (Dantzig & Goldman 1985; Chase *et al.* 1993, 1994).

Taken together, the results of the ADP and phosphate analogue experiments suggested strongly that a myosin mediated adaptation. Most myosins share this spectrum of inhibitor sensitivity, however, and so the next step was to identify candidate myosin isoforms.

5. EVIDENCE FOR MYOSIN IN HAIR BUNDLES

Sustained efforts to identify the specific adaptation-motor myosin were initiated in the late 1980s. Given the lack of knowledge at the time of the number of myosin isozymes present in any genome, an antibody approach was unlikely to be successful, as antibodies were not available for most of the known (and for all of the unknown!) isoforms. Instead, a strategy to biochemically identify myosin in hair bundles needed to be promiscuous for all, or most, isozymes. To provide a preparation for such experiments, we developed a method for isolating hair bundles from the frog saccule (Gillespie & Hudspeth 1991): we could therefore carry out biochemical experiments selectively on bundles, avoiding any myosins found in cell bodies of the hair cells or supporting cells. Although the total amount of protein present in hair bundles is very small (*ca*. 40 ng from bundles of one saccule), sufficiently sensitive methods might reveal scarce adaptation-motor myosins.

Maruta & Korn (1981) showed that nucleotides could be used to photoaffinity-label myosins directly: subsequent work showed that the residue modified was Glu-185 (of smooth muscle myosin II), which occurs immediately after the conserved phosphate-binding sequence present in almost all myosins (Garabedian & Yount 1990, 1991). Most myosins do indeed have the appropriate sequence of GESGAGKTE, suggesting that this approach would be broadly applicable. Moreover, because the high specific activity of radioactive nucleotides offered tremendous detection sensitivity, a photoaffinity approach was appropriate for scarce hair bundles. To gain selectivity for myosins, which should be present in bundles along with many other nucleotide-binding proteins, we exploited vanadate trapping (Goodno 1982). After ATP hydrolysis, phosphate and ADP dissociate sequentially from myosin. Vanadate (or beryllium fluoride and aluminium fluoride) bind after phosphate dissociation and decrease the ADP dissociation rate by many orders of magnitude (Goodno 1982). Our photoaffinity-labelling strategy employed high specific activity UTP, which is more efficiently cross-linked to myosin than ATP, and vanadate, to increase specificity for myosins (Gillespie *et al.* 1993).

When hair bundles were purified, permeabilized, and labelled with this strategy, three prominent bands of 120, 160 and 230 kD were usually observed (figure 2*a*). Although all three of these proteins passed a series of tests diagnostic for myosins, the 120 kD hair-bundle myosin was always the most prominent. Because the photoaffinity-labelling strategy requires an active myosin, the strong signal obtained suggested that the 120 kD protein was the best candidate for the adaptation motor.

The size of the 120 kD hair-bundle myosin inspired us to look more closely with antibodies selective for myosin I isoforms. Albanesi and colleagues had purified an adrenal myosin I (Barylko *et al.* 1992) and raised monoclonal antibodies to it (Wagner *et al.* 1992). We found that these antibodies not only recognized a 120 kD band on protein immunoblot (Gillespie *et al.* 1993; figure 2*b*), but also labelled stereocilia tips (figure 3), the expected site of the adaptation motor (Gillespie *et al.* 1993). Taken together with the photoaffinity labelling, these results suggested that the hair-cell adaptation motor might be immunologically related to the myosin I of adrenal glands.

6. CIRCUMSTANTIAL EVIDENCE FOR MYOSIN 1C

Identification of a hair-bundle myosin I motivated several groups to identify myosin isoforms in hair cells using molecular biology methods. The Corey laboratory used a degenerate RT–PCR strategy and found 13 different myosin isozymes in frog sacculus (Solc *et al.* 1994): one of the most commonly identified ones was myosin 1c (Myo1c; then called myosin Iβ). The Hudspeth laboratory also identified myosin 1c in frog sacculus, and demonstrated that the inner-ear isozyme was identical to that found in brain (Metcalf *et al.* 1994). These results were not unexpected, as the Albanesi laboratory had shown that their monoclonal antibodies recognized the same myosin isozyme (Reizes *et al.* 1994).

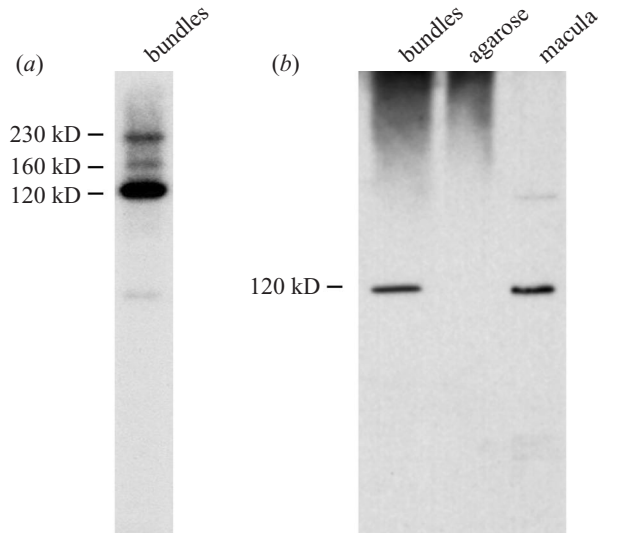

Figure 2. Identification of Myo1c in purified frog hair bundles. (*a*) Photoaffinity labelling; note prominent band at 120 kD, as well as 160 and 230 kD bands. (Reprinted, with modification, from Gillespie *et al.* (1993), with permission from Elsevier.) (*b*) Protein immunoblot with anti-Myo1c polyclonal antibody. A 120 kD protein was detected in the hair bundles and residual macula, the saccular tissue remaining after bundle isolation. (From Hasson *et al.* (1997).)

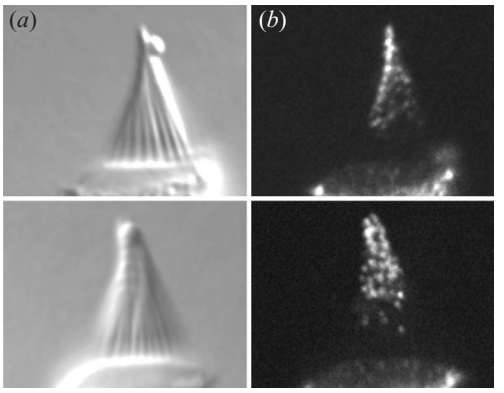

Figure 3. Immunolocalization of Myo1c at stereocilia tips. (*a*) DIC images of isolated hair cells. (*b*) Immunolocalization of Myo1c with mT3 monoclonal antibody. (Reprinted, with modification, from Gillespie *et al.* (1993), with permission from Elsevier.)

Polyclonal antibodies made against the tail of frog Myo1c recognized a band of the correct size on immunoblots (figure 2*b*) and labelled stereocilia tips (Hasson *et al.* 1997), just as the monoclonal antibodies had. Out of four myosins closely investigated (1c, 5, 6 and 7a), only Myo1c showed tip labelling, reinforcing its possible role as the adaptation motor. Myo1c was also found in additional bundle locations, most prominently at the base of stereocilia where the actin filaments of the bundle enter the cell (Hasson *et al.* 1997).

We also localized three other myosin isoforms in hair-cell epithelia (Hasson *et al.* 1997). Myosin 5 does not appear to be in hair cells, but is prominent in afferent nerve fibres contacting hair cells. Myosin 6 is essential for hair cells but is largely located at the base of the hair bundle: this isoform may be involved in maintaining membrane integrity. Finally, myosin 7a, also essential for hair cells, is found prominently in the stereocilia in a band *ca*. 1 μm above the base. Myosin 7a is co-localized with a class of links called the ankle links, which interconnect stereocilia: myosin 7a may anchor these links and assist in maintaining bundle integrity.

The most exhaustive immunolocalization of Myo1c was carried out using immunogold electron microscopy (Garcia *et al.* 1998). Myo1c was found to be concentrated in stereocilia at three locations: at the insertions of stereocilia into the apical surface of the cell, at the bases of the tip links, and near the tops of the tip links. Although Myo1c was not heavily concentrated within *ca*. 75 nm of the top of the tip link it was, instead, at the greatest concentration 100–300 nm higher, as well as at the very tip of each stereocilium. In sections with identifiable insertional plaques, structures visualized by electron microscopy that are thought to house the adaptation motors, Myo1c levels were elevated approximately fourfold. Moreover, the higher concentration found well above tip-link anchors could have been increased by Myo1c that moved there after tip links were broken or by poor antibody access to tightly packed myosin molecules in insertional plaques. The immunogold electron microscopy data therefore were consistent with identification of Myo1c as the adaptation motor, but certainly did not prove that assertion.

7. PROVING MYO1C IS THE ADAPTATION MOTOR

Immunolocalization of Myo1c strongly suggested that it could be the adaptation motor. Localization data can never substitute, however, for an experiment that provides a direct connection between a specific molecule and the physiological function being investigated. Although one such direct experiment would be to delete the gene for Myo1c, it is unlikely that hair cells from Myo1c knockout animals would retain transduction yet have no adaptation: because most models for transduction apparatus assembly invoke Myo1c to move channels and tip links into place, we expect that knockout animals would have no transduction. Our first attempt at knocking out Myo1c gave inconclusive results: although an interesting interpretation was that Myo1c shows developmental haploinsufficiency (lethality), our results could have been explained just as easily by a technical problem in the procedure (J. A. Mercer and P. G. Gillespie, unpublished data). Ultimately, although the knockout approach might determine whether Myo1c is essential for hair cells (assuming no compensation by another isozyme), it was very unlikely that this approach could prove whether Myo1c mediates adaptation itself.

Several investigators have devised more direct strategies for similar problems. For example, mutation of a specific Asp residue in G-proteins to an Asn changes the nucleotide specificity from guanosine nucleotides to xanthine nucleotides (Weijland & Parmeggiani 1993). If a cellular process that formerly depended on guanosine triphosphate converts its dependence to xanthine triphosphate when such a mutant G-protein is expressed, then the process must depend on that specific G-protein (Rybin *et al.* 1996). In another example, Shokat and colleagues devised a 'bump–hole' strategy for determining the proximal substrates of specific protein kinases: they made a mutation that opened up a cavity (the hole) in the nucleotide-binding site of a

kinase, then made an ATP analogue with a modification (the bump) that fitted into that cavity (Bishop *et al.* 2000). Only the mutant kinase could use the ATP analogue, and with a radiolabelled analogue, one could easily determine which proteins that kinase phosphorylated in a cellular context. Modification of the N^6 position of ATP and mutagenesis near where the adenine ring binds in a kinase gave an appropriate substrate–mutant pair (Shah *et al.* 1997; Liu *et al.* 1998).

These inspiring strategies suggested to us a general approach: make a mutation in a protein of interest that has no consequences for the activity of the protein *in vitro* or *in vivo*, then design a reagent that binds to the mutant protein but not the wild-type form. Depending on the protein studied and its activity in cells, the system could be designed so that the reagent either activates or inhibits the protein. By studying the cellular activity of interest and determining its sensitivity to, for example, a selective inhibitor, one could connect the specific molecule to that cellular activity if inhibition was seen only when the mutant protein was present.

Using this general approach, we devised a strategy that used selective inhibitors based on ADP. Because ADP keeps myosin bound tightly to actin, as with our ADPβS experiments, adaptation should be blocked without affecting transduction. We therefore exploited the N^6-modified nucleotide analogues developed by Shokat and colleagues: we supposed that the bulky side group on these analogues would prevent binding to wild-type Myo1c, but that a mutation could be designed that would accommodate the N^6 side group.

Close examination of structures of ADP-bound myosin (Fisher *et al.* 1995) indicated that Tyr-135 (of myosin II) would be an appropriate candidate for mutation. Tyr-135 forms a hydrogen bond with the N^6 amino group (figure 4*a*), demonstrating its close association with the adenine ring, yet is not absolutely conserved in myosins.

The equivalent residue in Myo1c is Tyr-61, so we mutated that residue to glycine and generated Y61G-Myo1c (Gillespie *et al.* 1999). Y61G-Myo1c hydrolysed ATP with a dependence on ATP or actin concentration that was nearly identical to that of wild-type Myo1c (wt-Myo1c): the mutant actually hydrolysed ATP at a slightly higher rate. We used N^6-modified ATP analogues to carry out an initial screen: ADP analogues corresponding to promising ATP analogues were then synthesized and retested. From this dual screen, we chose NMB-ADP (figure 4*b*), which inhibited Y61G-Myo1c and caused it to remain tightly bound to actin. Using an *in vitro* motility assay, we showed that the combination of a relatively small fraction of mutant myosin and a moderate concentration of NMB-ADP led to nearly complete immobilization of actin filament translocation by myosin (Gillespie *et al.* 1999).

The expected behaviour of the adaptation motor is shown in figure 4*c–e*. When a hair bundle containing only wild-type Myo1c molecules is deflected, the motors will slip down the cytoskeleton, even if NMB-ADP is present (figure 4*d*). By contrast, if Y61G-Myo1c and NMB-ADP are both present, then most of the mutant myosin molecules will be bound tightly to the actin cytoskeleton and the whole motor complex will be immobilized (figure 4*e*). Tension remains high so the channels will stay open: adaptation will be blocked.

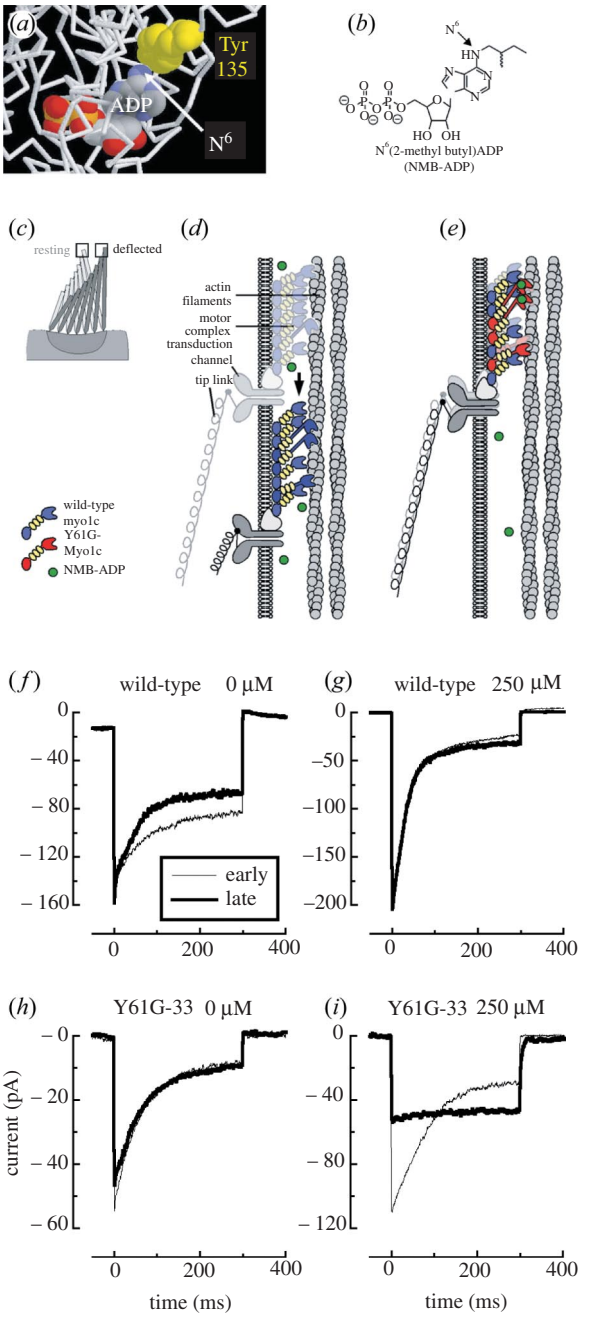

Figure 4. Inhibition of adaptation by NMB-ADP in mice expressing Y61G-Myo1c. (*a*) Structure of myosin 2 active site with ADP docked. Note the proximity of N^6 on ADP to Tyr-135 (Tyr-61 in Myo1c). (*b*) Structure of NMB-ADP. (*c*) Diagram indicating mechanical stimulation of a hair bundle. (*d*) Expected slipping of adaptation-motor complex in absence of NMB-ADP. (*e*) Immobilization of adaptation-motor complex by combination of Y61G-Myo1c and NMB-ADP. (*f*) Transduction in a wild-type hair cell early (grey line) and late (black line) during dialysis with control solution. (*g*) Stable adaptation in wild-type hair cell during dialysis with 250 µM NMB-ADP. (*h*) In the absence of NMB-ADP, adaptation is stable in mouse expressing Y61G-Myo1c. (*i*) As NMB-ADP enters a hair cell expressing Y61G-Myo1c, adaptation is blocked. (Figure modified from Holt *et al.* (2002).)

We made several transgenic mouse lines using a construct that incorporated the native mouse Myo1c promoter and a cDNA that incorporated the Y61G mutation (Holt

et al. 2002). mRNA for the mutant myosin was expressed at substantial levels, although mutant protein was difficult to detect directly. We found that purified mouse hair bundles from Y61G transgenic mice contained *ca*. 50% more Myo1c (combination of wild-type and mutant) than did the bundles from wild-type mice, suggesting that the mutant protein made up a third of the total (Holt *et al.* 2002).

In the crucial experiment testing the role of Myo1c in adaptation by mouse hair cells, we found that adaptation was blocked only if (i) recordings were done from mice expressing the mutant Myo1c and (ii) NMB-ADP was included in the patch-recording electrode (figure 4*i*). In all other situations, adaptation remained robust during extended dialysis (figure 4*f–h*). On average, the rate of adaptation to positive stimuli decreased by a factor of five: moreover, in 11/12 cells that lasted at least 8 min during dialysis, adaptation to negative stimuli was completely blocked. Although less visually striking, this latter result is extremely important: during adaptation to negative stimuli, myosins of the adaptation motor will move along stereocilia actin filaments towards the barbed ends, under no load. This situation is directly comparable to the behaviour of wild-type and mutant Myo1c in the *in vitro* motility experiment, and the decrease in negative adaptation is consistent with the nearly fully inhibited motility when Y61G-Myo1c makes up 33% of the total Myo1c mixture.

These results are similar to results obtained from genetic experiments in that although they establish a link between Myo1c and adaptation, they do not necessarily prove a particular molecular mechanism. Nevertheless, the data show conclusively that inhibition of Y61G-Myo1c inhibits adaptation, suggesting that wild-type Myo1c is the hair cell's adaptation motor.

8. LOOSE ENDS FOR MYOSIN 1C

Several important questions about Myo1c and adaptation remain unanswered. Because it remains formally possible that more than one myosin isozyme contributes to the adaptation motor, we have designed a reciprocal experiment in which all myosins in hair cells are inhibited with ADPβS and adaptation rescue is attempted with an N^6-modified analogue triphosphate. If Myo1c is the only adaptation motor, then adaptation should be fully restored with the appropriate nucleotide ratio in the Y61G mutant mice, but not in wild-type mice. In addition, it is unknown whether a slow form of adaptation is present in cochlear hair cells and whether Myo1c mediates this. For both of these experiments, we have knocked the Y61G mutation into the mouse genome and have begun to characterize the physiology of adaptation in these mice.

Moreover, much attention has been focused on identifying the adaptation motor because it must interact with other components of the transduction apparatus. Identifying the interacting partners of Myo1c may therefore lead to the identification of other critical elements needed for transduction, including, perhaps, the transduction channel itself. Nevertheless, despite a decade of attention on Myo1c, there are no reports of proteins with which this molecule interacts (aside from calmodulin and actin). We have recently found that slow adaptation is modulated by the concentration of phosphatidylinositol-4,5-bisphosphate (PIP_2) in the stereocilia membrane and that Myo1c interacts with this and other anionic lipids through two lipid-binding domains, one in the tail and one in the IQ domains (M. Hirono and P. G. Gillespie, unpublished data). We are investigating the possibility that interaction of Myo1c with the channel and other components of the transduction apparatus might be exclusively through lipids, which is a radical hypothesis but one that might explain the inability of several groups to identify proteins with which Myo1c interacts.

9. PERSPECTIVE

The 50 years of study of the molecular mechanism of muscle contraction that has followed the sliding-filament hypothesis has had a profound effect on the field of auditory and vestibular transduction. The rigour and attention to detail that characterizes the muscle-contraction field has meant that there is a rich literature from which those interested in the myosins may glean ideas. Now the auditory field can give something back to the myosin field: as the focus on myosins is now shifting towards understanding the mechanisms and cellular roles for the many forms of unconventional myosins, the detailed understanding of adaptation in hair cells might serve as a paradigm for those studying unconventional myosins in other contexts. There is no doubt that the hair cell has taken several myosin isozymes, including Myo1c, Myo6, Myo7a and Myo15a, and has adapted their general roles in other cell types towards specific requirements in the hair cell. Understanding the specialized roles of these myosins in hair cells will certainly lead to an elucidation of general principles that can be applied elsewhere.

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REFERENCES

Assad, J. A. & Corey, D. P. 1992 An active motor model for adaptation by vertebrate hair cells. *J. Neurosci.* **12**, 3291–3309.

Assad, J. A., Hacohen, N. & Corey, D. P. 1989 Voltage dependence of adaptation and active bundle movement in bullfrog saccular hair cells. *Proc. Natl Acad. Sci. USA* **86**, 2918–2922.

Barylko, B., Wagner, M. C., Reizes, O. & Albanesi, J. P. 1992 Purification and characterization of a mammalian myosin I. *Proc. Natl Acad. Sci. USA* **89**, 490–494.

Bishop, A.(and 11 others) 2000 Unnatural ligands for engineered proteins: new tools for chemical genetics. *A. Rev. Biophys. Biomol. Struct.* **29**, 577–606.

Chase, P. B., Martyn, D. A., Kushmerick, M. J. & Gordon, A. M. 1993 Effects of inorganic phosphate analogues on stiffness and unloaded shortening of skinned muscle fibers from rabbit. *J. Physiol.* **460**, 231–246.

Chase, P. B., Martyn, D. A. & Hannon, J. D. 1994 Activation dependence and kinetics of force and stiffness inhibition by aluminofluoride, a slowly dissociating analog of inorganic phosphate, in chemically skinned fibres from rabbit psoas muscle *J. Muscle Res. Cell Motil.* **15**, 119–129.

Cooke, R. & Pate, E. 1985 The effects of ADP and phosphate on the contraction of muscle fibers. *Biophys. J.* **48**, 789–798.

Corey, D. P. & Hudspeth, A. J. 1983*a* Kinetics of the receptor current in bullfrog saccular hair cells. *J. Neurosci.* **3**, 962–976.

Corey, D. P. & Hudspeth, A. J. 1983*b* Analysis of the microphonic potential of the bullfrog's sacculus. *J. Neurosci.* **3**, 942–961.

Dantzig, J. A. & Goldman, Y. E. 1985 Suppression of muscle contraction by vanadate. Mechanical and ligand binding studies on glycerol-extracted rabbit fibers. *J. Gen. Physiol.* **86**, 305–327.

Eatock, R. A., Corey, D. P. & Hudspeth, A. J. 1987 Adaptation of mechanoelectrical transduction in hair cells of the bullfrog's sacculus. *J. Neurosci.* **7**, 2821–2836.

Fisher, A. J., Smith, C. A., Thoden, J. B., Smith, R., Sutoh, K., Holden, H. M. & Rayment, I. 1995 X-ray structures of the myosin motor domain of *Dictyostelium discoideum* complexed with MgADP•BeF_x and MgADP•AlF_4^-. *Biochemistry* **34**, 8960–8972.

Garabedian, T. E. & Yount, R. G. 1990 Direct photoaffinity labeling of gizzard myosin with [^{3}H]uridine diphosphate places Glu185 of the heavy chain at the active site. *J. Biol. Chem.* **265**, 22 547–22 553.

Garabedian, T. E. & Yount, R. G. 1991 Direct photoaffinity labeling of gizzard myosin with vanadate-trapped adenosine diphosphate. *Biochemistry* **30**, 10126–10132.

Garcia, J. A., Yee, A. G., Gillespie, P. G. & Corey, D. P. 1998 Localization of myosin Ib near both ends of tip links in frog saccular hair cells. *J. Neurosci.* **18**, 8637–8647.

Gillespie, P. G. & Hudspeth, A. J. 1991 High-purity isolation of bullfrog hair bundles and subcellular and topological localization of constituent proteins. *J. Cell Biol.* **112**, 625–640.

Gillespie, P. G. & Hudspeth, A. J. 1993 Adenine nucleoside diphosphates block adaptation of mechanoelectrical transduction in hair cells. *Proc. Natl Acad. Sci. USA* **90**, 2710–2714.

Gillespie, P. G., Wagner, M. C. & Hudspeth, A. J. 1993 Identification of a 120 kd hair-bundle myosin located near stereociliary tips. *Neuron* **11**, 581–594.

Gillespie, P. G., Gillespie, S. K., Mercer, J. A., Shah, K. & Shokat, K. M. 1999 Engineering of the myosin Iβ nucleotide-binding pocket to create selective sensitivity to N(6)-modified ADP analogs. *J. Biol. Chem.* **274**, 31 373–31 381.

Goodno, C. C. 1982 Myosin active-site trapping with vanadate ion. *Meth. Enzymol.* **85**, 116–123.

Hasson, T., Gillespie, P. G., Garcia, J. A., MacDonald, R. B., Zhao, Y., Yee, A. G. & Corey, D. P. 1997 Unconventional myosins in inner-ear sensory epithelia. *J. Cell Biol.* **137**, 1287–1307.

Holt, J. R., Gillespie, S. K., Provance, D. W., Shah, K., Shokat, K. M., Corey, D. P., Mercer, J. A. & Gillespie, P. G. 2002 A chemical-genetic strategy implicates myosin 1c in adaptation by hair cells. *Cell* **108**, 371–381.

Howard, J. & Hudspeth, A. J. 1987 Mechanical relaxation of the hair bundle mediates adaptation in mechanoelectrical transduction by the bullfrog's saccular hair cell. *Proc. Natl Acad. Sci. USA* **84**, 3064–3068.

Hudspeth, A. J. 1989 How the ear's works work. *Nature* **341**, 397–404.

Liu, Y., Shah, K., Yang, F., Witucki, L. & Shokat, K. M. 1998 Engineering Src family protein kinases with unnatural nucleotide specificity. *Chem. Biol.* **5**, 91–101.

Maruta, H. & Korn, E. D. 1981 Direct photoaffinity labeling by nucleotides of the apparent catalytic site on the heavy chains of smooth muscle and *Acanthamoeba* myosins. *J. Biol. Chem.* **256**, 499–502.

Metcalf, A. B., Chelliah, Y. & Hudspeth, A. J. 1994 Molecular cloning of a myosin I beta isozyme that may mediate adaptation by hair cells of the bullfrog's internal ear. *Proc. Natl Acad. Sci. USA* **91**, 11 821–11 825.

Pate, E. & Cooke, R. 1989 A model of crossbridge action: the effects of ATP, ADP and P_i. *J. Muscle Res. Cell Motil.* **10**, 181–196.

Reizes, O., Barylko, B., Li, C., Südhof, T. C. & Albanesi, J. P. 1994 Domain structure of a mammalian myosin I. *Proc. Natl Acad. Sci. USA* **91**, 6349–6353.

Rybin, V., Ullrich, O., Rubino, M., Alexandrov, K., Simon, I., Seabra, C., Goody, R. & Zerial, M. 1996 GTPase activity of Rab5 acts as a timer for endocytic membrane fusion. *Nature* **383**, 266–269.

Shah, K., Liu, Y., Deirmengian, C. & Shokat, K. M. 1997 Engineering unnatural nucleotide specificity for Rous sarcoma virus tyrosine kinase to uniquely label its direct substrates. *Proc. Natl Acad. Sci. USA* **94**, 3565–3570.

Siemens, J., Lillo, C., Dumont, R. A., Reynolds, A., Williams, D. S., Gillespie, P. G. & Muller, U. 2004 Cadherin 23 is a component of the tip link in hair-cell stereocilia. *Nature* **428**, 950–955.

Solc, C. K., Derfler, B. H., Duyk, G. M. & Corey, D. P. 1994 Molecular cloning of myosins from bullfrog saccular macula: a candidate for the hair cell adaptation motor. *Audit. Neurosci.* **1**, 63–75.

Wagner, M. C., Barylko, B. & Albanesi, J. P. 1992 Tissue distribution and subcellular localization of mammalian myosin I *J. Cell Biol.* **119**, 163–170.

Weijland, A. & Parmeggiani, A. 1993 Toward a model for the interaction between elongation factor Tu and the ribosome. *Science* **259**, 1311–1314.

Yamoah, E. N. & Gillespie, P. G. 1996 Phosphate analogs block adaptation in hair cells by inhibiting adaptation-motor force production. *Neuron* **17**, 523–533.

GLOSSARY

ADP: adenosine diphosphate
AMP: adenosine monophosphate
ATP: adenosine triphosphate
DIC: differential interference contrast
NMB-ADP: N^6-(2-methylbutyl)ADP
RT–PCR: reverse transcriptase–polymerase chain reaction
UTP: uridine triphosphate