Chapter 15
The Role of the Primary Cilium in Chondrocyte Response to Mechanical Loading

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15.1 Introduction

All cells experience external mechanical forces whether it is compression, tension, fluid shear, hydrostatic pressure or a combination of these. Mechanical signals are converted to biochemical and structural changes within the cell through a process of mechanotransduction which ultimately regulates cell function. Indeed this process of mechanotransduction and response to mechanical environment is fundamental to many aspects of cell behaviour including stem cell differentiation, cell polarity and developmental tissue patterning, tissue homeostasis and response to injury. However the fundamental mechanotransduction pathways and how they are specialised for different cell types and associated biomechanical environments, is as yet unclear. Emerging into this context is the primary cilium, a hitherto under-rated and ignored cellular structure whose function and importance are only just being recognised. In the 70s and 80s, pioneering studies by Jensen, Poole and others, described the structure of the primary cilium in a variety of tissues (Albrecht-Buehler and Bushnell 1980; Jensen et al. 1979; Poole et al. 1985, 1997). However it took another 30 years before the primary cilium began to be recognised as an organelle of fundamental importance for cell and tissue function and pathology. It is now acknowledged that the primary cilium plays a central role in processes that include cell fate and development, cell cycle regulation, chemosensation and cell migration (Gerdes et al. 2009). Of particular relevance to this book is the finding that primary cilia function as mechanoreceptors in an increasing range of cell types. In this chapter we explore the role of the chondrocyte primary cilium in responding to the complex and demanding mechanical environment which is so critical to articular cartilage homeostasis and function.

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15.2 The Structure of the Primary Cilium

The primary cilium is a specialized membranous projection or compartment with a unique framework of microtubules made of acetylated α-tubulin. Unlike motile cilia such as those found on the airway epithelium, there is only one primary cilium per cell. The primary cilium consists of an axoneme that extends out from the basal body which is a modified form of the more mature of the two centrioles. The axoneme exhibits what is described as a "9 + 0" microtubule structure possessing nine outer doublet microtubules arranged around a central core as shown in Fig. 15.1. This structure differs from that of motile cilia which exhibit a "9 + 2" structure comprising the same 9 doublet microtubules as the primary cilium but with an additional central pair which helps to confer motility on these cilia. The ciliary axoneme is ensheathed in a lipid bilayer, the ciliary membrane, which is contiguous with the plasma membrane but has a distinct composition of membrane proteins (Ostrowski et al. 2002; Teilmann et al. 2005; Teilmann and Christensen 2005). A region at the base of the cilium called the ciliary necklace separates these two membrane compartments (Gilula and Satir 1972).

Ciliogenesis, the formation of a cilium, is intrinsically linked with the cell cycle. In proliferating cells cilia assembly typically occurs during G_1 whilst cilia resorption and disassembly occurs upon entry into the cell cycle prior to mitosis (Christensen et al. 2008; Kim et al. 2011; Li et al. 2011; Pan and Snell 2007; Robert et al. 2007). Upon exiting mitosis the mother-centriole dissociates from the core of the mitotic spindle to become the basal body. The basal body moves to the cell surface, associating with golgi-derived vesicles en-route, and docks at an actin-rich assembly site where it nucleates outgrowth of ciliary microtubules (Dawe et al. 2007). Electron microscopy has identified several cilia structures that support the basal body in its role not only as an anchor for the cilium, but also as a gatekeeper of protein import and export. These include the striated rootlet (Hagiwara et al. 1997), distal and sub-distal appendages (Ringo 1967) and transition zone fibres (Anderson 1972). The latter, in particular, forms a region at the base of the cilium called the transition zone which contains several protein complexes that regulate selective import and transport of ciliary proteins (Garcia-Gonzalo et al. 2011; Williams et al. 2011). This occurs in a cell type-specific manner, thus controlling the protein composition of the cilium and potentially enabling cilia structure and function to be specialised in different cell types. Thus in virtually every tissue, a set of specific receptors becomes Localised or localized to the ciliary membrane which are adapted to detect particular environmental signals.

The process of intraflagellar transport (IFT) is responsible for building and maintaining the structure of the primary cilium, as shown schematically in Fig. 15.2 (Berbari et al. 2009; Haycraft et al. 2007; Huangfu and Anderson 2005; Pazour and Witman 2003). IFT is the bidirectional transport of raft-like transport modules, IFT particles, along the length of the cilium (Davenport and Yoder 2005; Haycraft and Serra 2008). Assembly of IFT particles occurs at the base of the cilium such that proteins, including the structural protein tubulin, are transported in the antereograde

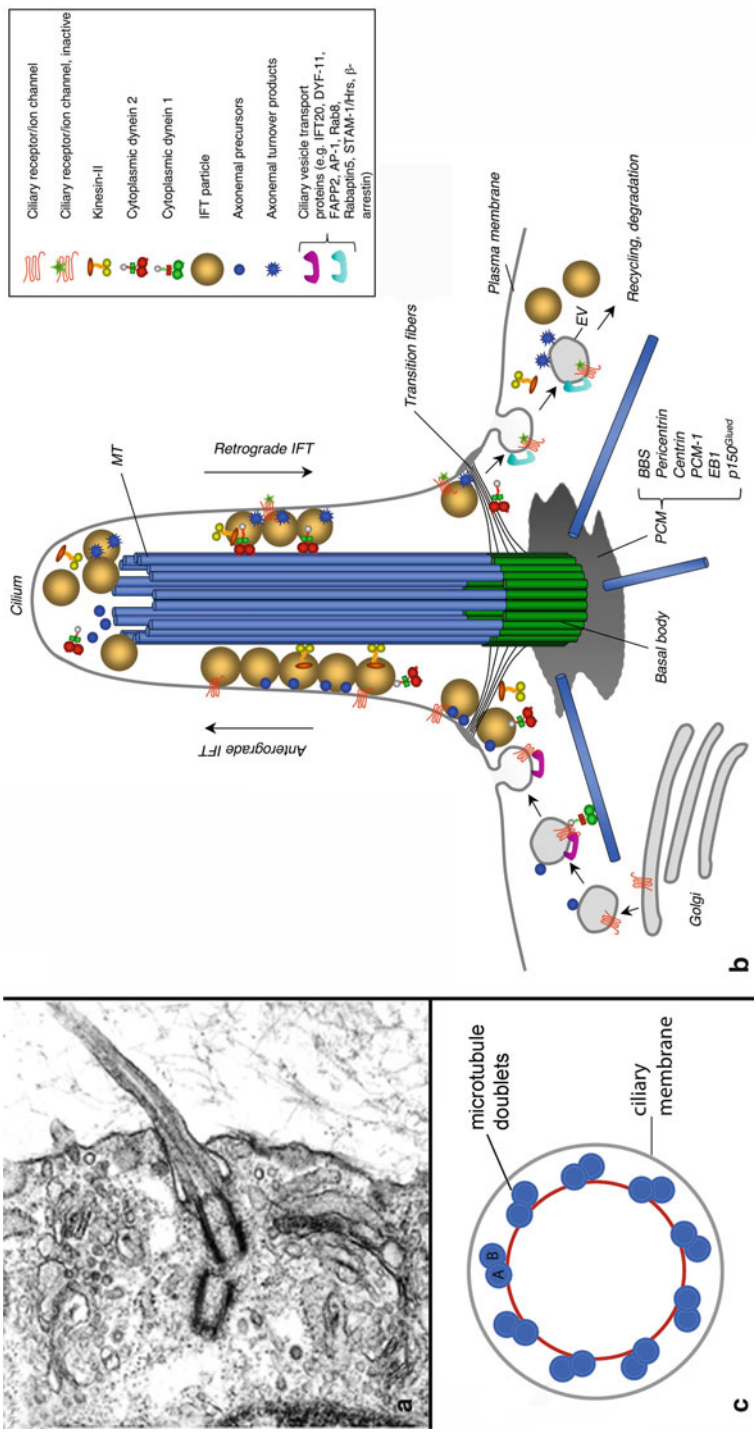

Fig. 15.1 The primary cilium. **a** TEM image showing the primary cilium for a chondrocyte within articular cartilage (Reproduced with permission from CA Poole). **b** Schematic diagram showing the structure of the primary cilium and the mechanism of assembly and disassembly via intraflagellar transport. (Based on figure from Pedersen and Rosenbaum, and reproduced with permission (Pedersen and Rosenbaum, 2008)). **c** Schematic cross section of the primary cilium showing the characteristic '9 + 0' formation of microtubule doublets

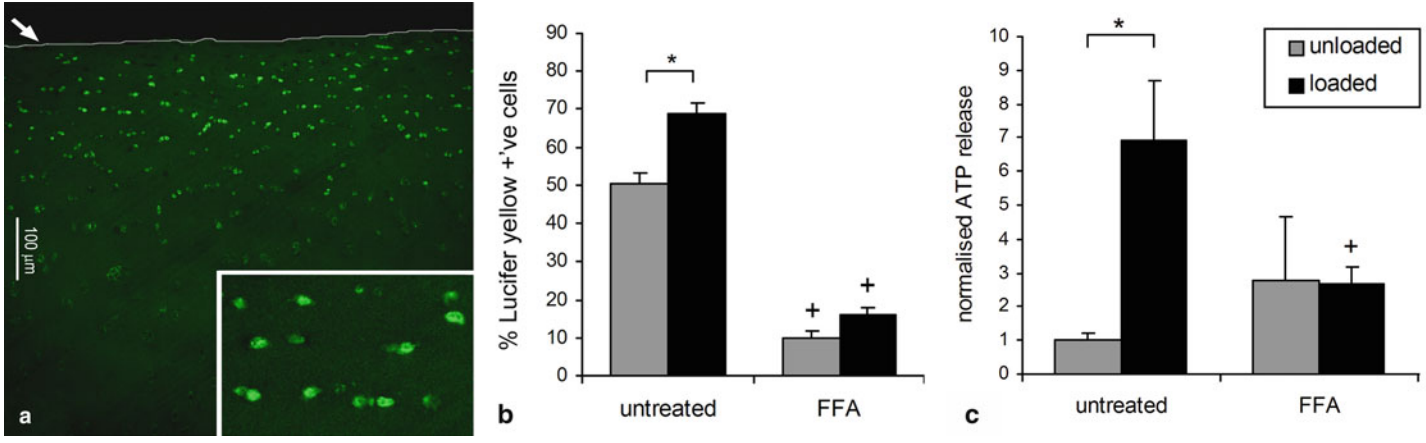

Fig. 15.2 Chondrocytes express hemichannels which open to release ATP upon mechanical stimulation. **a** Confocal immunofluorescence showing the expression of the hemichannel protein, connexin 43, in human articular cartilage. Inset shows higher magnification. Adapted from (Knight et al. 2009). **b** Hemichannel opening in response to mechanical loading as shown by the percentage of Lucifer yellow cells in unloaded and loaded chondrocyte-agarose constructs. Lucifer yellow uptake was blocked by the hemichannel inhibitor, flufenamic acid (*FFA*) confirming the specificity of the Lucifer yellow assay. **c** Mechanical loading activates ATP release measured in the culture media following a 1hour period of cyclic loading. The release of ATP was blocked by the hemichannel inhibitor, flufenamic acid (*FFA*). (Adapted from (Garcia and Knight 2010))

direction to the tip of the cilium, where they become fully assembled and correctly localized (Qin et al. 2004). Antereograde transport is driven by the Kinesin II motor complex which is composed of three subunits; KIF3A, KIF3B and KAP (Davenport and Yoder 2005). Once at the tip Kinesin II is inactivated, this facilitates both cargo release and return of the raft to the base of the cilium- a process driven by cytoplasmic dyenin 1b (Krock et al. 2009; Perrone et al. 2003; Schafer et al. 2003).

Ciliary tubulin undergoes several highly conserved post-translational modifications which include; detyrosination, glutamylation, glycylation and acetylation (Verhey and Gaertig 2007; Westermann and Weber 2003). Such modifications function to stabilize the axonemal microtubules and can be used to visualize the cilium with immunocytochemistry as they are more abundant than elsewhere in the cell (Jensen et al. 2004). Despite these modifications the cilium remains a highly dynamic structure. Assembly continually occurs at the axonemal tip, but once a set length is reached, the cilium does not extend further as microtubule assembly is balanced by simultaneous disassembly. Microtubule disassembly is an active process and several mechanisms for how this occurs have been identified (Cao et al. 2009; Pugacheva et al. 2007; Prodromou et al. 2012).

15.3 The Primary Cilium as a Mechanosensor

Mechanotransduction is the process by which mechanical force or associated deformation or strain is translated into a cellular response (for review see Farge 2011; Kolahi and Mofrad 2010; Schwartz 2010; Schwartz and DeSimone 2008; Shivashankar 2011). The mechanisms and signalling pathways involved appear to depend on the cell type and the precise nature of the mechanical environment. Even within a single cell type different loading modalities, durations, magnitudes and rates elicit a variety of cellular responses. Consequently a plethora of mechanotransduction and mechanosensitive processes have been identified with associated interplay and redundancy within these pathways. However, the primary cilium has emerged as a putative mechanotransducer involved in mechanotransduction in a variety of cell types. In particular the primary cilium has been identified as a flow sensor in osteocytes, vasculature endothelium and kidney tubular epithelia (Lu et al. 2008; Malone et al. 2007; Nauli et al. 2011; Praetorius and Spring 2003). Studies suggest that flow rate-dependent deflection of the cilium initiates a signalling cascade involving the polycystin ion channel complex on the axoneme and related intracellular calcium signalling (Lu et al. 2008; Nauli et al. 2003; Praetorius and Spring 2001). In bone, it is suggested that loading initiates fluid flow through the canaliculae which is detected by deflection of the primary cilia present on the osteocytes (Malone et al. 2007). This mechanotransduction process regulates bone resorption and formation which underpins bone mechanoregulation as defined by Wolff's Law and the Mechanostat principal (Frost 1987).

15.4 Cartilage Mechanotransduction

Articular cartilage is the specialised soft tissue that covers the articulating surfaces within synovial joints where it functions to reduce stress to the underlying bone and to provide a low friction, low wear weight-bearing surface. As such, articular cartilage is subjected to a demanding and complex mechanical environment, consisting of compressive and shear strain, hydrostatic pressure and fluid flow. This mechanical loading environment is critical to the health and homeostasis of the tissue maintaining the balance between synthesis and catabolism of the extracellular matrix which provides the tissue with its mechanical functionality. It is well established that mechanical loading regulates matrix synthesis and composition based on *in vivo* studies and those using cartilage explants or isolated cells *in vitro*. Furthermore removal of this physiological loading or exposure to excessive loading is linked to cartilage degradation and association pathologies such as osteoarthritis. In addition, cartilage development and patterning is also dependent on transduction of appropriate mechanical forces.

The chondrocyte is the only cell type within articular cartilage and is responsible for detecting the mechanical environment and regulating the composition, structure and function of the extracellular matrix. In particular this is achieved by mechanoregulation of the synthesis of extracellular matrix proteins, such as collagen II and the proteoglycan aggrecan, as well as proteases, such as ADAMTS5 and MMP13, which breakdown the matrix. Although chondrocyte mechanotransduction is clearly of immense importance in cartilage physiology, the mechanisms involved are unclear.

Extensive studies suggest that chondrocytes respond to a wide range of physiological mechanical stimuli including cell deformation, fluid shear, hydrostatic pressure, and associated physicochemical changes such as electrical streaming potentials, pH and osmolarity (for review see Urban, 1994). However the mechanotransduction pathways involved are less well defined. Studies indicate that mechanical loading may initiate downstream changes in cell function through the activation of intracellular calcium signalling pathways (D'Andrea et al. 2000; Edlich et al. 2004; Edlich et al. 2001; Erickson et al. 2001; Guilak et al. 1999; Kono et al. 2006; Mizuno, 2005; Ohashi et al. 2006; Pingguan-Murphy et al. 2005; Roberts et al. 2001; Wilkins et al. 2003). More recently, our group, and others, have reported that chondrocytes subjected to mechanical loading release ATP (Garcia and Knight 2010; Millward-Sadler and Salter 2004) which activates P2 purine receptors leading to global calcium transients (Pingguan-Murphy et al. 2006, 2005). Until recently the mechanosensitive mechanism of ATP release in chondrocytes was unknown. In all cell types there is still debate about the physiological transport mechanisms that facilitate ATP release with three putative mechanisms namely; anion channels, connexin hemichannels and exocytosis of ATP-filled vesicles. Studies from our group have now established that chondrocytes express connexin 43 hemichannels (Fig. 15.2a) and that cyclic compression opens these hemichannels as shown by the uptake of Lucifer Yellow which is blocked by the inhibitor flufenamic acid (Fig. 15.2b). Furthermore the mechanically induced opening of the hemichannels facilitates the release of ATP as

confirmed by inhibition with flufenamic acid (Fig. 15.2c). Chondrocytes express the apparatus for the reception of the extracellular ATP in the form of a selection of P2X and P2Y receptors (Knight et al. 2009) such that blocking of these receptors prevents mechanically activated calcium signalling (Pingguan-Murphy et al. 2006). Furthermore we have also shown that this purinergic mechanotransduction ATP-calcium pathway is responsible for the characteristic compression induced up-regulation of proteoglycan synthesis in articular chondrocytes (Chowdhury and Knight 2006).

15.5 The Role of the Chondrocyte Primary Cilium in Mechanotransduction

The chondrocyte primary cilium has been postulated to play a role in cartilage tissue homeostasis and development (Kaushik et al. 2009; McGlashan et al. 2007). However it is only very recently that studies have shown for the first time that the primary cilium is involved in chondrocyte mechanotransduction (Wann et al. 2012). These studies from Knight's group at Queen Mary University of London in collaboration with Poole and McGlashan in New Zealand used immortalized chondrocytes from Wild-type (WT) and *Tg737* Oak Ridge Polycystic Kidney (ORPK) mice provided by collaboration with Haycraft at Medical University of South Carolina. Hypomorphic allele mutation of the *Tg737* gene (IFT88) disrupts polaris expression, interrupting ciliogenesis and resulting in severely stunted or absent primary cilia (Fig. 15.3a). ORPK chondrocytes did not exhibit the classic compression-induced calcium transients induced in WT cells subjected to compressive loading (Fig. 15.3c, d, e). However, interestingly ORPK cells did respond to load with increased release of ATP (Fig. 15.3e). Thus the absence of mechanically induced calcium signalling in ORPK cells without a primary cilium is not caused by the loss of mechanically activated ATP release. Instead the primary cilium appears to regulate mechanotransduction downstream of the initial connexin mediated release of ATP.

Studies also examined whether the disruption of the mechanotransduction pathway in ORPK cells influenced extracellular matrix synthesis (Wann et al. 2012). Indeed, whilst chondrocytes derived from wild type mice showed a characteristic mechanically-induced up-regulation of proteoglycan synthesis, no such mechanosensitive regulation of matrix synthesis at gene or protein level was seen in ORPK cells (Fig. 15.4). Thus these studies reveal that the primary cilium is essential for chondrocyte mechanotransduction via ATP induced calcium signalling (Wann et al. 2012). However, in contrast to separate cilia-mediated mechanotransduction pathways in other cell types (Malone et al. 2007; Masyuk et al. 2006; Praetorius and Spring 2001), it is the connexin hemichannels, independent of the chondrocyte primary cilium, that function as the initial mechanoreceptors. Interestingly, the fact that the primary cilium is essential for both purinergic signalling and transduction of extracellular ATP into intracellular calcium transients, suggests that the cilium may be involved in cell physiology beyond mechanotransduction.

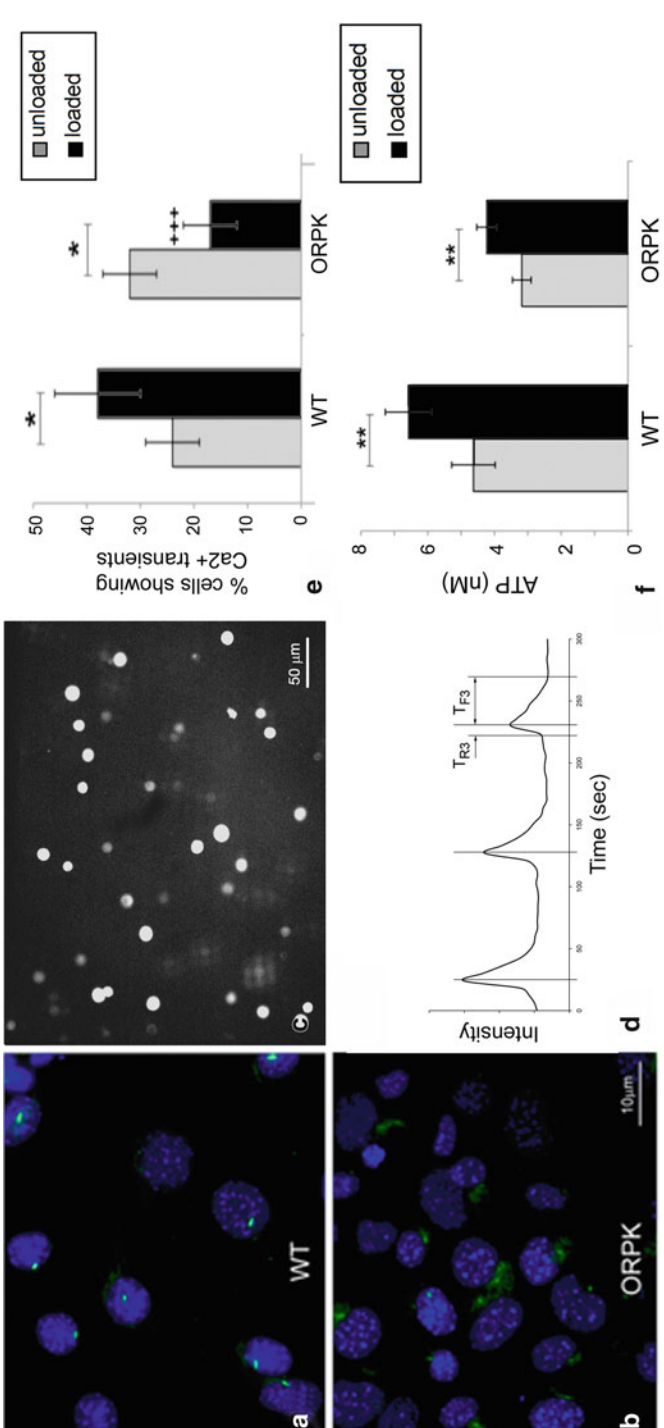

Fig. 15.3 Loss of primary cilia disrupts mechanically induced calcium signalling but does not influence mechanically induced ATP release. **a** Confocal immunofluorescence showing the presence of primary cilia labelled with acetylated α-tubulin (*green*) in wild type (*WT*) chondrocytes cultured in monolayer. Nuclei labelled with DAPI (*blue*). **b** Primary cilia are absent from ORPK chondrocytes which lack IFT88. **c** A single image from a confocal time series showing isolated chondrocytes in agarose labelled with the intracellular calcium indicator Fluo4-AM. **d** Typical intracellular calcium transients within a single chondrocyte quantified by measuring temporal changes in Fluo4 intensity. **e** Cyclic compressive loading of chondrocytes in agarose constructs significantly increases the percentage of cells showing calcium transients in WT chondrocytes ($p < 0.05$). By contrast, in ORPK cells loading produces a significant reduction in the percentage of cells showing calcium transients ($p < 0.05$). **f** Compressive loading also up-regulates the release of ATP in both WT and ORPK chondrocytes ($p < 0.01$). Cumulative ATP release was measured in the culture media surrounding an individual construct after a 1 h loading period or equivalent unloaded control. (Adapted from (Wann et al. 2012))

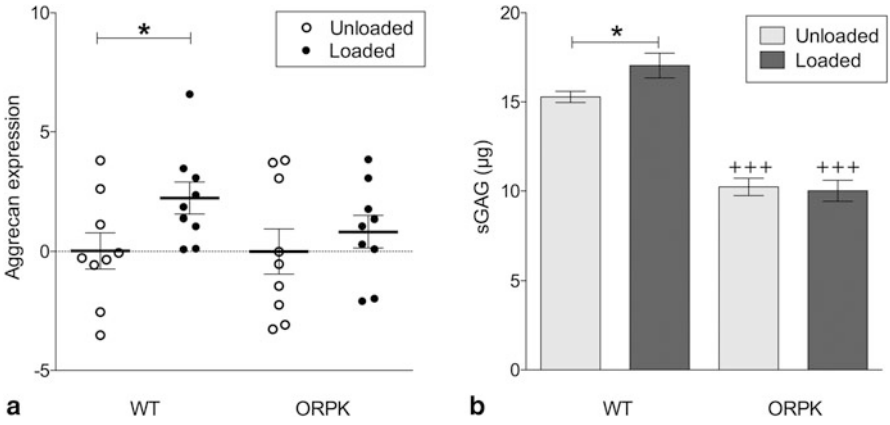

Fig. 15.4 Primary cilia are required for mechanosensitive up-regulation of extracellular matrix synthesis in chondrocytes. **a** Compressive loading of chondrocytes in agarose significantly up-regulates aggrecan gene expression for WT chondrocytes ($p < 0.05$) but not ORPK chondrocytes which lack a primary cilium. Gene expression was measured by qPCR following 1 h cyclic compression. **b** Compressive loading of WT chondrocytes in agarose stimulates synthesis of sulphated glycosaminoglycan (*sGAG*) measured after a 24 period of cyclic compression ($p < 0.05$). By contrast ORPK cells in agarose exhibit significantly reduced levels of sGAG synthesis compared to WT cells ($p < 0.001$) and a complete absence of any mechanosensitive changes in sGAG synthesis. (Adapted from (Wann et al. 2012))

15.6 Hedgehog Signalling and Other Cilia-Mediated Pathways

In addition to mechanotransduction, primary cilia are also involved in a variety of other signalling pathways. Loss of cilia, or cilia dysfunction has been linked to a series of related genetic disorders such as Bardet Biedel Syndrome and Polycystic Kidney Disease, which are collectively termed ciliopathies (for review see (Waters and Beales 2011)). In addition to defects in cell cycle regulation and mechanotransduction, these ciliopathies have helped to identify a number of other fundamental signalling pathways which are dependent upon a fully functioning primary cilium. Interestingly, emerging evidence suggests that many of these pathways are themselves mechanosensitive.

Common characteristics of ciliopathies include skeletal patterning defects such as polydactyly, and abnormalities of the central nervous system, both of which are indicative of defects in Hedgehog (Hh) signalling. Hh signalling is crucial for embryonic development and regulates the morphogenesis of a variety of tissues and organs (Athar et al. 2006; Ehlen et al. 2006; King et al. 2008; Nagase et al. 2008). The Hh receptor, Patched localizes to the primary cilium and maintains the pathway in an 'OFF state' through the inhibition of a second transmembrane protein, Smoothened (Rohatgi et al. 2007). In vertebrates, Patched not only inhibits Smoothened activation, but also its localization in the cilium (Rohatgi et al. 2007). Smoothened regulates the processing of a family of bi-functional transcription factors called Gli proteins. In the absence of Hh ligands full-length Gli activators are processed to their truncated repressor forms, this is suggested to take place within the cilium and is dependent

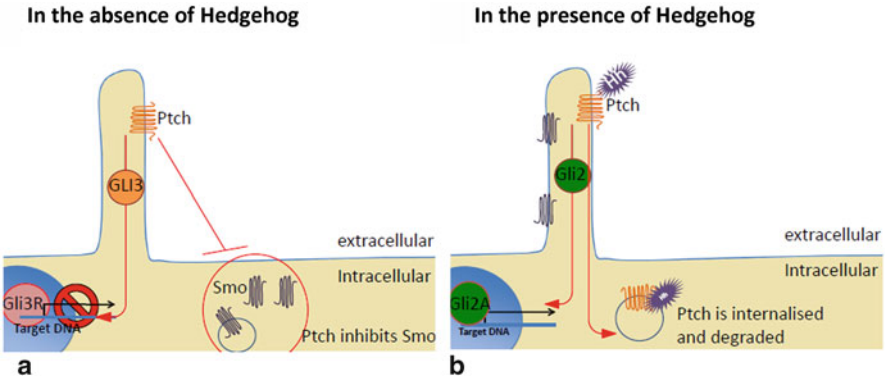

Fig. 15.5 Schematic diagram showing a simplified overview of the Hedgehog signalling pathway. **a** In the absence of Hedgehog (*Hh*) ligands the hedgehog receptor Patched (*Ptch*) localises to the primary cilium where it inhibits the function of Smoothened (*Smo*). Smo is held in an inactive conformation and prevented from entering the cilium, the mechanism by which this is achieved is unclear. Smo regulates the processing of a family of bifunctional transcription factors called Gli proteins. As a consequence of Smo inhibition, Gli transcription factors are either degraded or processed to their repressor forms (Gli3R) within the cilium resulting in the repression of Hh target genes. **b** When Hedgehog (*Hh*) ligands bind to Ptch, the receptor is internalised and targeted for proteasomal degradation releasing the inhibition on Smo. Smo undergoes an activating conformational change and enters the cilium where it inhibits the degradation and processing of Gli transcription factors, and promotes formation of Gli activators (Gli2A) and thus gene transcription. Several additional components are required for this signalling pathway such as Supressor of fused (*SuFu*), KIF7 and Rab23 (not shown) for review see. (Cohen 2010)

upon components of the IFT machinery (Haycraft et al. 2005; May et al. 2005). When Hh ligands bind to Patched, Smoothened inhibition is released. Smoothened then translocates to the primary cilium where it inhibits Gli processing, allowing full length Gli activators to move to the nucleus where they activate the expression of Hh-regulated genes (Day and Yang 2008; Huangfu and Anderson 2005; Huangfu et al. 2003; Milenkovic et al. 2009; Rohatgi et al. 2007; Varjosalo and Taipale 2008; Veland et al. 2009). The Hh signalling pathway described above is shown schematically in Fig. 15.5 and reviewed by Wong et al (Wong and Reiter 2008).

The cilium also houses other signalling pathway components important to both development and homeostasis. These include receptor tyrosine kinases (Christensen et al. 2012) and the PDGF receptor (PDGFR), which is trafficked into the cilium in growth arrested cells (Schneider et al. 2005). Ligand-dependent activation of PDGFR is followed by Akt activation and activation of the Mek1/2-Erk1/2 pathways, with Mek1/2 being phosphorylated within the cilium and at the basal body (Schneider et al. 2005) which ultimately regulates cell cycle progression and cellular migration via NHE-1 (Christensen et al. 2008; Jones et al. 2012; Kim et al. 2011; Schneider et al. 2010).

Non-canonical wnt signalling also takes places on the cilium resulting in the breakdown of β-catenin and the inhibition of wnt target genes (Corbit et al. 2008). For review of the interaction between the primary cilium and the wnt signalling pathway

see Gerdes et al (Gerdes and Katsanis 2008). The signalling of polycystin 1 and 2 is another cilia-dependent pathway thought to converge on many downstream effectors including STAT1, P100, beta catenin and intracellular calcium stores (Dalagiorgou et al. 2010; Kim et al. 1999; Lal et al. 2008; Low et al. 2006; Nauli et al. 2003; Pazour et al. 2002; Praetorius and Spring 2001). Furthermore, in some cell types certain signalling proteins are specifically localised to the ciliary axoneme suggesting that the primary cilium is critical in these signalling pathways. Examples of these ciliary signalling proteins include somatostatin receptors in neurons (Handel et al. 1999) and adenylate cylclase isoforms in neurons, osteocytes and synovial fibroblasts (Bishop et al. 2007; Malone et al. 2007; Ou et al. 2009).

15.7 Mechanoregulation of Hedgehog Signalling in Chondrocytes

In cartilage, Indian hedgehog (Ihh) is the major hedgehog protein regulating chondrocyte proliferation and differentiation during skeletal development. Ihh is essential for endochondral ossification which is the predominant mechanism of bone formation (for review see (Ehlen et al. 2006)).

In 2001, Wu et al demonstrated a novel function for Ihh in cartilage, as a mechanotransduction mediator (Wu et al. 2001). Using cyclic compression of isolated embryonic sternal chondrocytes in 3D-culture, they determined Ihh gene expression was induced by mechanical stress. Ihh induction was sensitive to the stretch-activated ion channel blocker gadolinium and stimulated chondrocyte proliferation via the induction of BMP2/4 (Wu et al. 2001). The mechanoregulation of Ihh expression appears to be under the control of specific mechanosensitive microRNAs (Guan et al. 2011) and is influenced by the presence of the oligomeric extracellular matrix proteins, matrillins (Kanbe et al. 2007; Le et al. 2001). Consequent elimination of functional matrillins in the chondrocyte pericellular matrix abrogates mechanical activation of Hh signalling (Kanbe et al. 2007). The classification of Ihh as a 'mechanosensitive' gene has been further strengthened by studies in the avian embryonic limb (Nowlan et al. 2008). Nowlan et al. compared the in vivo gene expression pattern of Ihh with patterns of biophysical stimuli induced by embryonic muscle contraction (Nowlan et al. 2008a, b). These studies revealed the expression pattern of Ihh colocalises with regions of high strain and fluid velocity and that this colocalisation is disrupted in limbs immobilised with the neuromuscular blocking agent decamethonium bromide (Nowlan et al. 2008). The rat tempromandibular joint (*TMJ*) has been used to investigate the function of mechanosensitive of Ihh expression in vivo during post-natal development (hjTang et al. 2004; Rabie and Al-Kalaly 2008). Mechanical stress to the TMJ induces Ihh gene expression within the proliferative layer of the condylar cartilage growth plate (hjTang et al. 2004), expression increases with greater loading (Ng et al. 2006; Rabie and Al-Kalaly 2008). Ihh expression is associated with increased proliferation of chondroprogenitor cells resulting in increased cartilage growth (hjTang et al. 2004; Ng et al. 2006).

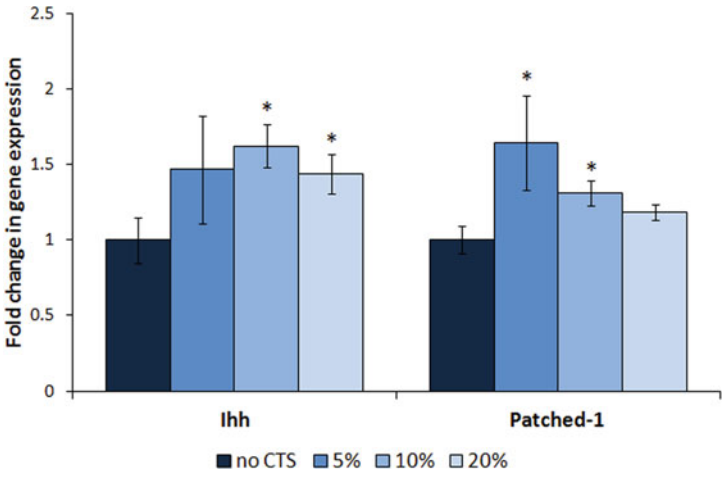

Fig. 15.6 Cyclic tensile strain upregulates Ihh gene expression and activates hedgehog signalling in adult articular chondrocytes. The expression of Ihh is significantly increased in chondrocytes subjected to cyclic tensile strain (*CTS*) at 5, 10 and 20 % strain compared to no CTS controls ($p < 0.05$). Gene expression was measured by qPCR following 1 h CTS. Changes in Patched1 gene expression were monitored as a measure of hedgehog pathway activation. Patched1 gene expression was significantly increased by 5 and 10 % CTS compared to no CTS controls ($p < 0.05$), however no significant changes were observed at 20 % strain indicating the pathway is not activated by this regime

The function of Ihh in adult cartilage is poorly understood. Current studies from the authors based at Queen Mary, London, have shown Ihh expression is also mechanosensitive in bovine articular chondrocytes isolated from adult tissue and subjected to cyclic tensile strain, which leads to strain-dependent Hh pathway activation (Fig. 15.6). The magnitude of Ihh gene expression is much lower than previously reported (Shao et al. 2011; Wu et al. 2001). This difference potentially arises due to the use of adult articular chondrocytes rather than chondrocytes isolated from embryonic chick sterna (Wu et al. 2001) or rat cartilage growth plates (Shao et al. 2011), as used in previous studies. It is also unlikely that chondrocytes in these different animals and different locations will be exposed to the same mechanical environment and may have adapted their responses accordingly.

Recent studies explore the role of the primary cilium in loading-induced Ihh signalling. Chondrocyte-specific ablation of *kif3a*, a component of the kinesin II IFT motor complex, using Col2a-Cre-mediated recombination, results in a loss of primary cilia in the post-natal murine TMJ (Kinumatsu et al. 2011). Loss of primary cilia in condylar cartilage results in abnormal hedgehog signalling producing defects in chondrocyte maturation, intramembranous bone formation, and chondrogenic condylar growth (Kinumatsu et al. 2011). Similarly, *in vitro* studies using rat growth plate chondrocytes demonstrate hedgehog signal transduction in response to hydrostatic pressure requires a fully functioning primary cilium (Shao et al. 2011). A role for the primary cilium in the maintenance of articular cartilage has also recently been demonstrated using the *Tg737*^{orpk} mouse (Ift88-deficient, see above) (Chang

et al. 2012). Mutant articular cartilage was thicker with a reduced overall stiffness and was consequently more prone to the development of osteoarthritis. Hh signalling was increased in the cartilage of ORPK mice, a phenomenon previously reported in osteoarthritis (Lin et al. 2009). This increase in Hh signalling was proposed to occur due to reduced cilia-mediated repression of the Hh signal (Chang et al. 2012).

Cartilage is not the only tissue in which the expression of Hh proteins is mechanically regulated. In vascular smooth muscle, strain produced a reduction in the expression of sonic hedgehog (Shh), another member of the Hedgehog protein family (Varjosalo and Taipale 2008). This resulted in decreased expression of several components of the hedgehog signalling pathway leading to increased apoptosis and reductions in cell number which could be rescued by addition of recombinant Shh (Morrow et al. 2007). This study implies there may be tissue-specific mechanisms regulating Hh signalling in response to mechanical cues.

15.8 Mechanoregulation of Primary Cilia Structure

As cilia-mediated signalling pathways, such as Hh signalling and mechanotransduction, are starting to be characterised, other studies have focused on primary cilia structure with a view to understanding the complex structure-function relationship. Indeed, an increasing number of studies are showing that length and associated anterograde and retrograde IFT, are correlated to functionality (Besschetnova et al. 2010; Tran et al. 2008) and in some case to disease as in certain ciliopathies (Mokrzan et al. 2007). In the case of motile, cilia-like flagella this is considerably easier, the molecular characterization of the mechanisms that regulate cilia length is much further ahead (Berman et al. 2003; Nguyen et al. 2005; Rosenbaum 2003; Tam et al. 2003, 2007; Wang et al. 2004). However, regulation of primary cilia length appears to involve a wide range of possible mechanisms including the cAMP-PKA system, the PKC- Mitogen-activated (MAP) Protein Kinases, a large range of actin and tubulin related proteins, many cell-cycle related proteins, Gelectins, FGF signaling, and Hypoxia-inducible factors (HIFs) (Abdul-Majeed et al. 2011; Besschetnova et al. 2010; Cruz et al. 2010; Kim et al. 2010; Kinzel et al. 2010; Li et al. 2011; Lopes et al. 2010; Massinen et al. 2011; May-Simera et al. 2010; Miyoshi et al. 2009; Neugebauer et al. 2009; Ou et al. 2009; Palmer et al. 2011; Pugacheva et al. 2007; Rondanino et al. 2011; Sharma et al. 2011; Thiel et al. 2011; Verghese et al. 2009, 2011). The primary cilia disassembly pathways are perhaps better defined and include prominent roles for tubulin de-acetylases and cell cycle related kinases such as Aurora A (Hubbert et al. 2002; Pugacheva et al. 2007; Prodromou et al. 2012).

At its most extreme cilia length regulation, in the form of rapid disassembly, often takes place in polarized cell types where the cilium is facing into a lumen. Here fluid-flow induced shear forces exert influence over structure producing a feedback loop which regulates epithelial cilia-mediated mechanotransduction (Besschetnova et al. 2010; Iomini et al. 2004). Thus fluid shear-mediated deflection of the primary cilium activates calcium signalling thereby reducing intracellular cAMP concentrations leading to cilium shortening and decreased mechanotransductive signalling

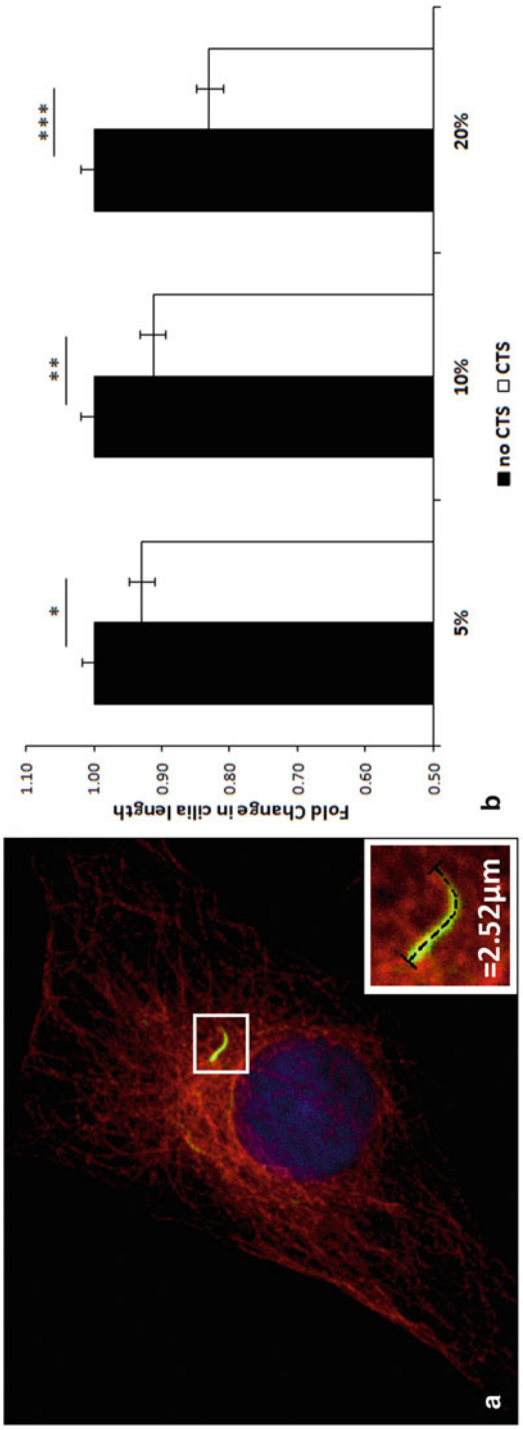

Fig. 15.7 Cyclic tensile strain induces primary cilia disassembly and reduces cilia length in a strain-dependent manner. **a** Immunofluorescent labelling of the chondrocyte primary cilium. Primary cilia in bovine articular chondrocytes were labelled with acetylated α-tubulin (*green*) and β-tubulin (*red*), the cilia appears yellow as these two labels colocalise. Nuclei labelled with DAPI (*blue*). The length of the primary cilium was determined by measuring the distance from base to tip (*dashed line*) using Leica Lite confocal software. **b** Chondrocytes were subjected to cyclic tensile strain (*CTS*) at 5, 10 and 20 % strain for 1 h, 0.33 Hz. Mean primary cilia length was significantly reduced by CTS ($p < 0.05$) in a strain dependent manner compared to no CTS controls

(Besschetnova et al. 2010). Similarly for articular chondrocytes, mechanical loading influences primary cilia structure and length with studies showing strain-dependent reductions in cilia length following cyclic compression (McGlashan et al. 2010) or cyclic tension (Fig. 15.6). These data suggests the reported variation in primary cilia length observed between different zones of the articular cartilage (Farnum and Wilsman 2011; McGlashan et al. 2008) may be the result of established differences in the mechanical environment within each zone (Guilak et al. 1995). This is supported by the fact that zonal differences in cilia length and prevalence are more pronounced in load bearing regions of the joint and that cilia are shorter and more oriented in regions experiencing high levels of strain compared with low (Farnum and Wilsman 2011). Interesting recent studies by the authors suggest that the reduction in primary cilia length observed at high strain magnitudes (20 % cyclic tensile strain), prevents the mechanosensitive up-regulation of Hh signalling (Fig. 15.6 and 15.7). It remains to be seen whether this is part of a physiological feedback mechanism as in epithelial cells subjected to fluid flow or whether this is a pathological injury response.

15.9 Conclusion and Perspectives

The first pioneering studies by Poole and Jensen and others, characterising the presence of primary cilia in cartilage and other musculoskeletal tissues were largely over looked for many years. It is only recently that the importance of the chondrocyte primary cilium in cartilage physiology has begun to be recognised. In particular chondrocyte primary cilia are now know to be essential for cartilage development. More specifically cilia are required for chondrocyte mechanotransduction and the maintenance of a functional extracellular matrix in response to a dynamic mechanical environment. The primary cilium also functions as a centre for hedgehog signalling which is required for development and which has recently been found to be involved in the pathogenesis of osteoarthritis. Interestingly, hedgehog signalling is stimulated by mechanical loading which also regulates primary cilia structure. Furthermore, recent studies demonstrate the inflammatory cytokines, present in the osteoarthritis, regulate primary cilia structure as part of the mechanism controlling downstream catabolic response (Wann and Knight 2012). All these studies support an emerging link between mechanical forces, primary cilia structure and cilia function. This is likely to be of fundamental importance for articular cartilage in health and disease. Furthermore the understanding of these mechanosensitive relationships may lead to the development of novel therapeutic strategies.

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