Review

Cellular Physiology and Biochemistry

Cell Physiol Biochem 2011;28:1111-1122

Accepted: December 05, 2011

Cell Volume Regulation in Chondrocytes

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Key Words
RVI • RVD • Cell Volume Regulation • Membrane potential • Ion channel • Potassium ion channel • TRPV4 • TRPV5 • TRPV6

Abstract
Chondrocytes are the cells within cartilage which produce and maintain the extracellular matrix. Volume regulation in these cells is vital to their function and occurs in several different physiological and pathological contexts. Firstly, chondrocytes exist within an environment of changing osmolarity and compressive loads. Secondly, in osteoarthritic joint failure, cartilage water content changes and there is a notable increase in chondrocyte apoptosis. Thirdly, endochondral ossification requires chondrocyte swelling in association with hypertrophy. Regulatory volume decrease (RVD) and regulatory volume increase (RVI) have both been observed in articular chondrocytes and this review focuses on the mechanisms identified to account for these. There has been evidence so far to suggest TRPV4 is central to RVD; however other elements of the pathway have not yet been identified. Unlike RVD, RVI appears less robust in articular chondrocytes and there have been fewer mechanistic studies; the primary focus being on the Na^+-K^+-$2Cl^-$ co-transporter. The clinical significance of chondrocyte volume regulation remains unproven. Importantly however, transcript abundances of several ion channels implicated in volume control are changed in chondrocytes from osteoarthritic cartilage. A critical question is whether disturbances of volume regulation mechanisms lead to, result from or are simply coincidental to cartilage damage.

Introduction

Chondrocytes are the cells which synthesise and maintain the extracellular matrix of cartilage [1]. Since they exist within lacunae of load bearing articular cartilage itself, they are subject to an unusually harsh physiological environment [2]. Cartilage is also greatly hypertonic to most other tissues, typically in excess of 350mOsm [2]. This hydration state exists because whilst high con-

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centrations of polyanionic proteoglycan molecules (Fig. 1) draw water into the tissue by osmosis, water is simultaneously forced out by the tension of interwoven collagen "strands" [3]. In this review we will use the term "hypotonic" challenge to refer to decreases in osmotic pressure during an experiment, rather than reduced osmotic pressure compared to normal cartilage.

Cell volume regulation is important to chondrocyte physiology in a number of contexts: (i) Hypertrophic chondrocytes of growth plates become enlarged in the process of endochondral ossification [4-7]. Part of the enlargement is accounted for by cellular swelling [8]. (ii) Chondrocytes decrease in number, but increase in cellular volume with ageing and exercise and (iii) like other cells chondrocytes shrink as a part of apoptosis [9-12]. (iv) Compressive force applied to cartilage within load bearing joints will naturally flatten the cells within, necessitating a degree of membrane stretch (Fig. 2) [13-17]. Volume is then actively reduced which will return membrane stretch towards the pre-compression level [18, 19]. Such mechanical signals are key triggers for changes in biosynthetic activity [20, 21]. (v) Water content also increases in the early stages of osteoarthritis (OA) [22-24]. The role of chondrocytes in OA is unclear, but the loss of volume regulatory mechanisms could potentially contribute to the progression of such degenerative diseases. Finally, (vi) articular cartilage water content decreases with application of normal physiological loads and cell volume regulation mechanisms appear to be in place to allow chondrocytes to survive such changes [25-30]. When a volume regulating cell is exposed to a hypotonic challenge it swells due to the passive uptake of water via osmosis. As the cell takes up an increasing volume of water the cells will oppose this by efflux of ions, thus decreasing the intracellular osmolarity until it is once more in equilibrium with the extracellular environment. This recovery of volume is termed regulatory volume decrease (RVD) [31, 32]. In contrast, regulatory volume increase (RVI) is the process by which a cell recovers its volume following an initial shrinkage on exposure to hypertonic challenge [31, 32]. Such volume regulatory mechanisms in cartilage have been observed in several studies [33-35]. These authors were able to show clear evidence of RVD in isolated chondrocytes and in cartilage explants [28, 29]. RVI has also been demonstrated in chondrocytes [33-36], but has been studied rather less. This review will summarise the current state of articular chondrocyte cell volume regulation research, focussing on short term RVD and RVI. We will highlight those areas of consensus, but also several areas of controversy.

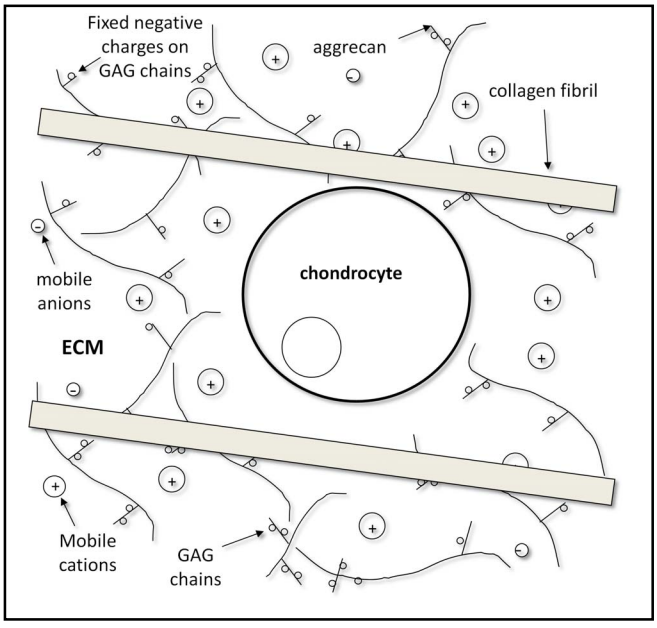

Fig. 1. Structure of articular cartilage. Articular cartilage is composed of a relatively low concentration of chondrocytes, surrounded by polyanionic glycoproteins and long thin strands of collagen fibres. The collagen fibres provide structure to the matrix and the fixed negative charges on the GAG chains attract positive cations. Water is drawn in to the tissue because of the negatively charged GAG chains, whilst simultaneously being forced out by the rigid collagen structure. ECM; extracellular matrix, GAG; glycosaminoglycan.

Articular Chondrocyte Regulatory Volume Decrease (RVD)

Whilst it has been known for many years that the osmolarity of cartilage changes, and that isolated chondrocytes exhibit RVD, arguably the key study of chondrocyte volume control was that of Bush and Hall [28]. Confocal microscopy was used to demonstrate that on exposure to hypotonic solutions chondrocytes swell passively *in situ*, and then exhibit an active volume recovery process [28]. Passive movement of water through the articular chondrocyte membrane is readily accounted for by the presence of several different aquaporin channels [37-40]. The next fundamental problem has been to unravel the active mechanisms underlying chondrocyte RVD. There have been several recent advances in this area, but understanding of the full mechanism is still far from complete. Generalized models of RVD in animal cells suggest that following exposure to hypotonic solution, cells initially swell passively, and then solutes are released from the cell, facilitating osmotic efflux of water and hence volume recovery [31, 32]. Early experi-

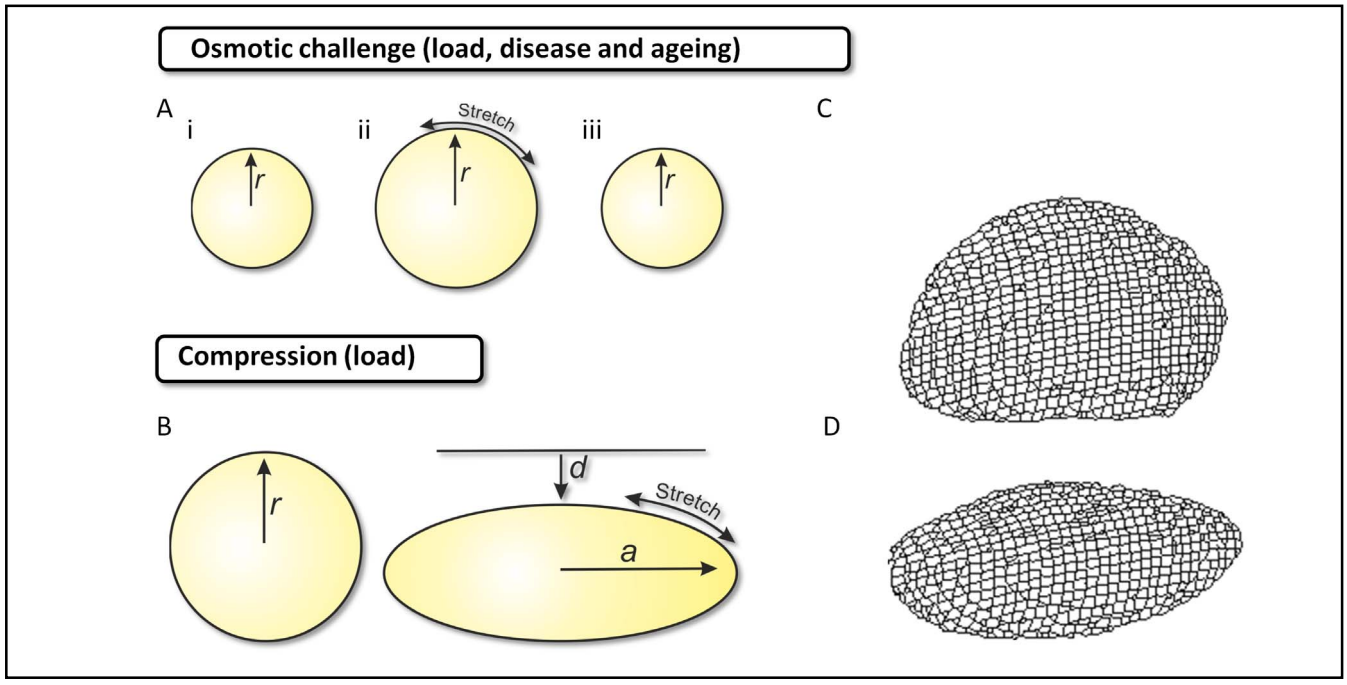

Fig. 2. Deformation of chondrocyte during hypo-osmotic shock and joint compression. (A) Prior to active volume regulation (i), decreased osmolarity would be expected to lead to cellular swelling (increasing cell radius, *r* μm) (ii). This is indeed the case with chondrocytes, either isolated or *in situ* [28, 29]. Following this regulatory volume decrease occurs, returning the cell to its starting volume (iii). (B) Static compression (by *d* μm) of joints also leads to membrane stretch. This is because the sides of the joints are relatively compliant, and so chondrocytes deform from relatively spherical to progressively flatter ellipsoids [13-17] ($a > r$). It can be proved algebraically, that for any given volume, an ellipsoid has a greater surface area than the parent sphere. Therefore, assuming this deformation takes place faster than water can leave the cell this leaves, vertical compression leads to membrane stretch (figure not draw to scale). (C) and (D) 3D reconstructions of chondrocyte surfaces from *in situ* confocal microscopy. Figure adapted from [14] (Permission Granted).

ments therefore attempted to determine the identity of potential solutes. One of the first species shown to be released by chondrocytes on exposure to hypotonic solutions was taurine [41]. This efflux paralleled the process of RVD [35]. However, Hall points out, that since chondrocytes can recover volume from a 20% increase, and taurine content of cells rarely exceeds 2mM, there is "a negligible role for taurine as an osmolyte during recovery from cell swelling" [41]. Investigations have therefore focused both on identifying which solutes (or "osmolytes") do directly mediate volume recovery and the membrane transporters which conduct these osmolytes. The term "osmolyte channel" is used, which is a useful term, since it does not exclude any charged or uncharged species from consideration. Ion channels are also obvious candidates for the mediation of solutes during membrane stretch since many are activated by mechanical deformation of the plasma membrane [42]. Little is known about the macromolecular complexes in which stretch activated ion channels are likely to function, but some channels may be linked to the cytoskeleton via β1-integrins [43, 44]. This may be responsible for their gating by transmitting extracellular physical forces of stretch or pressure to the channels, causing them to undergo a conformational change. Activation of these ion channels may lead to changes in cell activity via alteration of the resting membrane potential (*RMP*) and thus the global functions of the cell. This hypothesis is supported by studies using ion channel blockers that disrupt the process of mechanotransduction and alter chondrocyte biosynthetic activity [21, 45]. Although the identity of a key "osmolyte channel" itself remains unknown, the transient receptor potential vanilloid type 4 channel (TRPV4) is becoming widely established as a molecular sensor in many different cells and is clearly present in chondrocytes and involved with RVD [46-48]. TRPV4$^{-/-}$ mice fail to exhibit the classical RVD response [49]. Since TRPV4 is predominantly a non-specific or, functionally, a Ca^{2+} channel one would expect its osmotic shock or compression to result in increased intracellular Ca^{2+} [50]. This has now been shown to occur in a number of studies [51-53]. A proportion of the mechanically in-

duced increase in cytosolic Ca^{2+} also appears to derive from intracellular stores because it is also reduced by thapsigargin [52-54]. How the increase in intracellular Ca^{2+} ions leads to RVD is yet to be established. One possibility is that it activates Ca^{2+} activated channels, which in turn allow the exit of sufficient ions and/or other osmolytes.

Potassium ions have frequently been implicated in RVD, but the evidence is contradictory. The work of Hall and colleagues in the 1990s showed large increases in K^+ efflux upon hypotonic shock [55], but the pharmacological profile of this conductance did not neatly fit with any known ion channel of the time. In particular it should be noted that the large Ca^{2+} activated potassium (BK) channel inhibitors TEA (5mM) and charybdotoxin (8% inhibition with 10μM), and the intermediate Ca^{2+} activated potassium (IK) channel inhibitor nitrendipine [56], had little effect on hypotonically activated K^+ efflux [55]. Furthermore, the authors failed to detect any ionomycin induced (i.e., Ca^{2+} activated) K^+ efflux [55]. Interestingly, the potent inwardly rectifying potassium channel inhibitor pimozide [57] was shown to be a powerful inhibitor of hypotonic K^+ efflux [55], but this has not been followed up on (to the authors' knowledge). Electrophysiological studies show that chondrocytes express many different K^+ channels [58-68], including Ca^{2+} activated K^+ channels [60, 65, 66, 69]. Chondrocyte K^+ channels have also been shown to be activated by membrane stretch and hypo-osmotic challenge [62, 65, 69]. Two of these studies suggested that BK channels were activated by direct membrane stretch [62, 65] and RVD is blocked by REV5901 [29, 30, 35] which can inhibit the BK channel [70]. At least some of the mechanically activated K^+ conductance of chondrocytes is mediated by channels other than BK however, since it is sensitive to the low conductance calcium-activated potassium (SK) channel blocker, apamin [69].

Another possible candidate osmolyte driving RVD in other cells is anion efflux [32]. If the reversal potential for permeant anions lies positive to the *RMP* then activation of Cl^- ion channels would allow efflux of Cl^- ions and could contribute to RVD. However, the permeability of many Cl^- channels is quite promiscuous allowing other species such as HCO_3^- [71] or taurine [72] to permeate. Therefore the reversal potential for the "chloride" channel is not known for sure, and even the natural value of the chondrocyte *RMP* is highly controversial (see below) so the involvement of Cl^- channels will need to be directly shown, by use of selective Cl^- channel blockers. Disappointingly, there are no highly selective inhibitors of

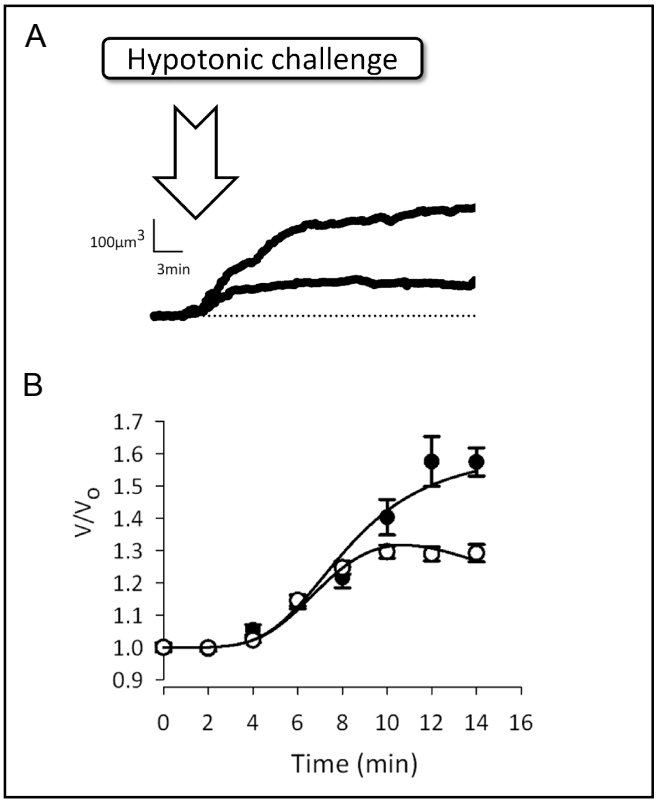

Fig. 3. Effect of membrane potential on Volume Regulation. The level of the membrane potential is a key player in determining the efficiency with which a chondrocyte can maintain its volume in the face of hypotonic challenge. RVD is more effective at 10mV than at -80mV. (A) Raw data from a chondrocyte exposed to hypo-osmotic challenge at either 10mV or -80mV. It is clear that the cell swells considerably more when held at -80mV (using single sharp electrode switch-clamp electrophysiology) than when it is held at 10mV. (B) Mean data from a number of experiments such as that shown in (A), all data fitted with the equations $OSMin(t) = Osmin(t)/vol(t)$, where $Osmin(t)$ is the number of moles of intracellular solute and is related to potassium ion efflux $dOsmin/dt = G_{KCa(t)} (RMP-E_{K(t)}/e.NA)$ with *RMP* being the ONLY variable changed between the two fits. Modified from [77], please see original source for full justification.

Cl^- channels and chondrocytes probably express several types of these channels, including Ca^{2+} -activated Cl^- channels (CaCC, which may be synonymous with anoctamin 1, TMEM16A) [72-76].

Our own recent experiments certainly support the notion that RVD involves ion transport, rather than transport of neutral species, since the cell membrane potential has a direct influence on the efficiency of chondrocyte volume control [77]. The native level of the chondrocyte *RMP* is also quite controversial. The first recorded val-

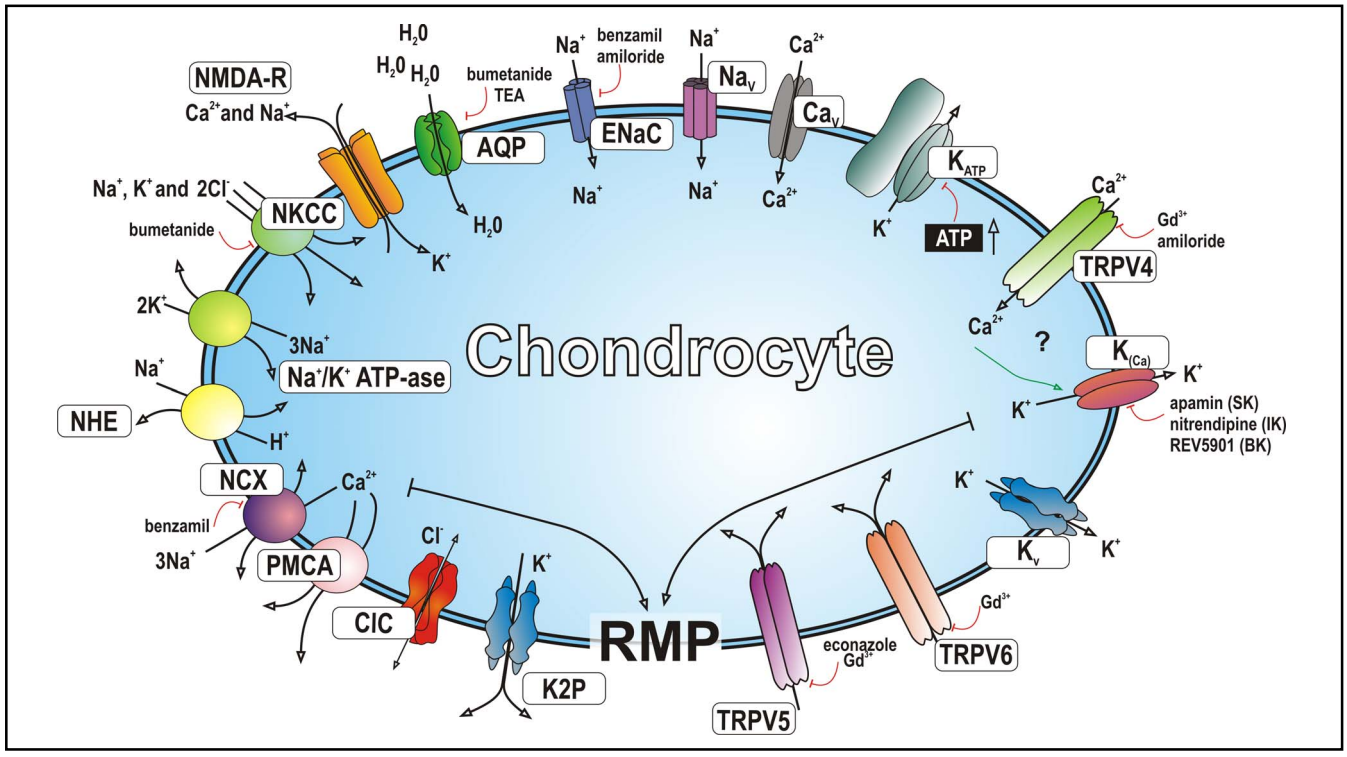

Fig. 4. Ion channels and transporters in articular chondrocytes. Ion channels and transporters identified in articular chondrocytes (for citations, see text or [109]). All of these proteins will indirectly influence the *RMP* and thus volume control, however, those proposed as key players have been highlighted. The *RMP* will be equivalent to the equilibrium (steady state) value of the membrane potential (*Vm*) calculated from $Cm \cdot dV_m/dt = -\sum_i^n I_i$ for all *n* permeant ionic species. *Ii* is the ionic current of ion *i* and *Cm* is the membrane capacitance (adapted from [146]). The membrane potential itself is a key player in determining the efficiency of volume regulation [77]. We have included some of the less well known channel blocking effects of several of the drugs mentioned in the text. References to these are given in the text, with the exception of AQP block by TEA [147], and benzamil inhibition of ENaC [148]. We have not attempted to include every ion channel blocker in this figure; for example charybdotoxin and TEA are well known blockers of BK channels and TEA has subunit dependent effects on Kv channels [149]. The figure is modified from [109].

ues of *RMP* in chondrocytes were approximately -11mV [78], but a number of authors have found potentials as low as -46mV [58, 74, 79]. Clearly, absolute measurement of *RMP* is difficult since each method has a number of potential caveats [for example: 80], however, in our own experiments we used microelectrodes, patch clamp and optical measurements and consistently arrived at values nearer to the original chondrocyte values of Wright et al. [78]. Few other cell types have been shown to have *RMP* as depolarised as chondrocytes, although mast cells are one such example [81]. Our mathematical simulations showed that this could well be a cellular adaptation to facilitate powerful RVD [77] and may also be applicable to other such depolarised cell types. RVD is a dynamic process which most likely takes place from the first instant of volume increase and membrane stretch. Therefore rather than use the usual protocol of allowing chondrocytes to swell and then measuring the time taken for them to return to the starting level, we followed the whole dynamic cycle starting with passive swelling as hypotonic solutions reached the cell (Fig. 3). Other studies have shown dynamic changes in membrane potential resulting from changes in volume [82], but in our study, we did the opposite. We used voltage-clamp and showed that swelling was much greater (i.e., RVD was significantly weakened) when membrane potential is held at very negative levels [77]. The principle ion channel (or channels) setting the chondrocyte *RMP* is also highly controversial. Fig. 4 illustrates those ion channels which have been shown in chondrocytes and highlights those which have been proposed to set the *RMP*, and thus exert an indirect effect on RVD. Our basic published model [77] is clearly a huge simplification and does not include the full range of ion channels and transporters known to exist in chondrocytes (Fig. 4) or more complex mechanisms, such as the concurrent reduction in Na^+ perme-

Transporters implicated in Chondrocyte volume regulation				
Signal	Transporter	Species	Process Implicated	Reference
Membrane stretch	BK	K^+	RVD	[65]
Hypotonicity	osmolyte	K^+, taurine	RVD	[35,41]
Pressure	SK	K^+	Response to pressure	[69]
	IClswell, VRAC?	Cl^-, taurine	RVD	[72]
	Aquaporins 1 & 3	H_2O	Passive volume change, RVI and RVD.	[37-40]
Hypertonic	NKCC	Na^+, K^+, Cl^-	RVI	[33,36,84-88]
	NCX (reverse mode)	Na^+, Ca^{2+}	Response to hypertonic shock	[89]
	Ca^{2+}-ATPase (PMCA)	Ca^{2+}	Response to hypertonic shock	[90]
Increased extracellular Na^+	NHE		Up-regulated in response	[91,92]
Mechanical	TRPV4	Cations	Volume regulation	[48,49]
None*	TRPV5	Cations	RVD via *RMP*	[77]

Table 1. Ion channels and membrane transporters implicated in chondrocyte volume regulation or response to osmotic/mechanical stimulation. "Signal" is the stimulus which was applied to the chondrocyte in order to change activity of the transporter, "species" is the identity of the proposed transported or permeant osmolyte. *TRPV5 was shown to be constitutively active [77].

ability central to the Vereninov model of U937 cell apoptotic shrinkage [83].

Articular Chondrocyte Regulatory Volume Increase (RVI)

The capacity of chondrocytes to undergo RVI has been known since the 1990s [93], but there have been fewer studies than those on RVD and in chondrocytes the response appears to be more fickle than RVD. So, for example, there have been reports of no RVI [94], slow RVI [85], or RVI in only a small proportion of cells [36]. Other studies have shown very clear robust RVI in chondrocytes [26, 93]. There are probably several reasons for this, excluding species differences. Firstly, chondrocytes are themselves a heterogeneous population. Even if one considers just articular cartilage, there are phenotypic differences between superficial and deep layer chondrocytes [1]. Secondly, chondrocytes only retain a "realistic" phenotype for the first few passages in culture [95] and so cells used after this time are likely to have altered volume regulatory properties. *In situ* chondrocytes appear less prone to RVI than isolated chondrocytes [36]. Thirdly, the exact protocol used appears to make a considerable difference. Some authors report that changing osmolarity with NaCl evokes RVI, whereas changing it with sucrose does not [33]. Further reports show, as for other cell types [31] that RVI following a previous cycle of RVD is greater than that on a naïve chondrocyte [93]. In cells other than chondrocytes, a variety of transporters have been proposed to mediate RVI including the Na^+/H^+ (NHE/SLC9Ax) transporters [92], the Na^+ - K^+ - $2Cl^-$ (NKCC/SLC12A1/2) co-transporter [87, 88], an organic osmolyte transporter protein [32] and hypertonically activated ion channels [96]. Early experiments on chondrocytes showed an influx of K^+ concomitant with RVI [55]. Since one would expect the opening of a cation channel to produce K^+ *efflux* rather than *influx* it was proposed that RVI may involve the activity of an appropriate transporter protein [55]. The proposed protein was NKCC and this has remained the main focus of chondrocyte RVI research to date.

$^{86}Rb^+$ flux experiments provided very strong evidence for Na^+/K^+ -ATPase (quabain sensitive) and NKCC cotransporter (bumetanide sensitive, Na^+/Cl^- dependent) influx in the chondrocyte resting state [55]. These $^{86}Rb^+$ flux experiments were to strongly support the theory that NKCC contributes to, but that Na^+/K^+ -ATPase activity results from RVI [55]. It should be remembered that any change in intracellular ionic composition, either resulting from changes in extracellular ionic changes, or mechanosensitive ion channel activity will lead to secondary activity of such pumps. The NKCC hypothesis received support from a number of

further studies. For example, RVI responses enhanced by either the cytoskeletal disrupter latrunculin B or the ion channel blocker Gd^{3+} were found to be significantly inhibited by the NKCC inhibitor bumetanide [36, 85]. Whilst any pharmacological approach, including use of bumetanide, suffers from a danger of possible contamination with off target effects such as aquaporin inhibition [97], it is worth noting that NKCC is also present at high levels in growth-plate chondrocytes. This is a location where chondrocyte volume regulation is thought to be particularly important [98]. The importance of NKCC to chondrocyte RVI was supported by a very recent study on the cultured chondrocyte cell line C-20/A4 [86]. NKCC was knocked down with an siRNA duplex and RVI was reduced by approximately 50% [86]. Several studies have reported increases in intracellular Ca^{2+} associated with hypertonic shock [26, 89, 99, 100]. The generation of this Ca^{2+} spike is condition dependent. Studies have frequently shown that it requires the presence of extracellular Ca^{2+} [26, 89]. However, the spike is in some cases sensitive to the non-selective ion channel blocker Gd^{3+} and intracellular store depletion with thapsigargin [26], but insensitive to these treatments in others [89]. Interestingly, the latter study did show that the Ca^{2+} spike was sensitive to KBR7942, the TRPC channel [101] and Na^{+}-Ca^{2+} exchange-reverse mode (NCX) [102] inhibitor [89]. Whether or not the Ca^{2+} influx is a necessary step for RVI in chondrocytes has also been investigated. Whilst removal of extracellular Ca^{2+} leads to greater initial hypertonic shrinkage [26], which could correlate to a loss of RVI, the presence or absence of intracellular Ca^{2+} spikes did not correlate with the presence or absence of RVI [36] itself. Furthermore, RVI was more rapid in avian chondrocytes when the ion channel inhibitor Gd^{3+} was applied, or extracellular Ca^{2+} was removed [85]. This suggests hypertonic Ca^{2+} spikes may form part of a parallel pathway, perhaps linking volume to metabolic activity rather than volume recovery itself. Much the same may be true for the NHE transporters too. There is abundant data to show that chondrocytes express both bicarbonate [103] and NHE transporters [104-107]. Experiments also show that there is clearly an increase in chondrocyte intracellular pH [108] on exposure to hypertonic shock, but there is no direct evidence that these changes contribute to the process of RVI. No shrink activated ion channels have yet been reported in chondrocytes, although ENaC has been shown to be present in chondrocytes and ENaC is activated by membrane shrinkage and contributes to RVI in rat hepatocytes [107, 109, 110].

Clinical Relevance of Chondrocyte Volume Control?

OA is the most common form of arthritis in both humans and domestic animals. In humans the incidence is 60% in men and 70% in women over the age of 65 [111]. Cartilage loss is usually only detected once the patient has experienced significant joint pain, by which time damage to the tissue may be irreversible. The pathogenesis of this heterogeneous disease is complex, with contributory factors including genetic predisposition and occurrence of joint trauma [112, 113]. Modern definitions of OA focus on "joint failure" rather than disease of cartilage itself, since it is yet to be proven whether the initial abnormality occurs in the articular cartilage or in the subchondral bone [113-116]. Nevertheless, a number of clear and characteristic changes are commonly associated with OA; including degeneration of cartilage [111]. Ageing also results in structural and compositional changes within cartilage, causing a loss of strength and volume of the tissue [116-118]. Since chondrocytes are responsible for secretion of the extracellular matrix proteins an interesting question is whether chondrocyte physiology changes during onset of OA. In healthy cartilage, water content is finely balanced by compressive force driving water out and swelling pressure drawing water in [119]. Collagen fibres exert the compressive force, whereas the Gibbs-Donnan effect and cartilage proteoglycans create osmotic pressure which tends to draw water in [119]. It is well established that in the later stages of joint degeneration, proteoglycan production decreases and as a result less water is bound within the network [118]. Paradoxically, the early stages of OA are marked by increases of cartilage water content [22, 24, 120-122]. This occurs because whilst there is an overall loss of proteoglycans [23, 121], it is outweighed by a loss of collagen [23, 119]. Following the increase in water content there is an increase in the volume of individual chondrocytes in osteoarthritic cartilage [19, 123, 124] although this can largely be accounted for by the changes in extracellular osmolarity [125]. Chondrocytes that are distended by hypotonic challenge are distinctly more vulnerable to physical damage [126] and in other cell types (HeLa cells) dysfunction of volume regulation has been shown to increase loss of cells by apoptosis [127]. Apoptosis is clearly increased in OA [128, 129], but abnormal chondrocyte morphology could be an even earlier marker for cartilage degeneration [130]. This has been suggested because abnormal chondrocyte

morphology in macroscopically non-degenerated tissue appears to correlate with the appearance of the inflammatory mediator IL-β and a loss of collagen type VI [130]. Experiments also show that osmotic challenge [26, 52, 53, 89], hydrostatic pressure [131] and compression [132, 133] lead to mobilisation of chondrocyte intracellular Ca^{2+} and to changes of cartilage synthesis [15, 134-140]. Furthermore, Gd^{3+} tends to increase cellular volume [85] and decreases protein and proteoglycan synthesis [45]. Therefore, even if loss of chondrocyte volume regulation is initially a consequence of cartilage degeneration, it seems plausible that it will contribute to further cartilage degeneration. There have been few studies targeting chondrocyte ion channels in animal models of OA, but a recent study investigated the "genome wide" transcription of mRNA in chondrocyte from the cartilage of control and OA patients [141]. Whilst this study was not concerned with cell volume regulation, examination of the data tables reveals that significant changes (criterion ≥2 fold change) of only 7 channel transcripts were detected (Table 2). Each of these has a putative role in volume regulation or apoptosis. KCNK5 (Task-2) was recently shown to influence the chondrocyte membrane potential and has been shown elsewhere to be involved in volume regulation and apoptosis [59, 142, 143]. The next three channels in Table 2 are all Ca^{2+}-activated potassium channels, some of which may be central to RVD (see above). ENaC transcript abundance is reduced over three-fold in chondrocytes from osteoarthritic patients, and ENaC has a role in RVI in hepatocytes [144]. TMEM16A, an anoctamin [76], is believed to be the Ca^{2+}-activated Cl^- channel frequently discussed in connection with RVD (see above). The last element of the table is AQP1, first reported in chondrocytes by Trujillo et al [107] and this shows a remarkable 39 fold increase in chondrocytes from OA patients, this parallels the situation with rheumatoid arthritis where AQP1 is also up-regulated [145]. Assuming that expressed protein levels to some degree correlate to transcript abundance, it is hard to imagine that all these channels can be altered in OA cartilage without significant impact on the cell volume regulatory properties of chondrocytes.

Gene Symbol	Encoded Ion channel	Abundance Ratio	*p*-value
KCNK5	K2P5.1 (Task-2)	-4.7	4.8E-16
KCNMA1	K_{Ca} 1.1 (BK)	3.1	5.0E-10
KCNN4	K_{Ca} 3.1 (SK or IK)	10.2	2.0E-17
KCNT2	K_{Ca} 4.2 (BK)	-2.2	2.0E-07
SCNN1A	ENaC	-3.6	1.2E-08
TMEM16A	Ca^{2+} activated Cl^-	3.2	1.4E-20
AQP1	Aquaporin 1	39.8	7.3E-22

Table 2. Plasma membrane ion channels and porin transcripts changed in chondrocytes from cartilage of patients with OA [141]. Clearly there may be other ion channel changes which were not detected in this microarray study, but this list includes ALL detected ion channel transcripts significantly changed by 2-fold or more, but excludes ion channel modifiers and accessory proteins.

Conclusion

Cell volume regulation by chondrocytes appears to be important to cartilage physiology in a number of situations and this review has focussed on experimental models of regulatory volume control in articular chondrocytes. One of the great strengths of this small field is that several groups are approaching key mechanistic questions with a range of complimentary techniques including; mechanical, confocol microscopic, mathematical modelling, flux studies and patch clamp studies together with transcriptomic and quantitative protein expression analysis. All these techniques are equally valuable, since cellular volume regulation appears to involve a complex network of inter-related processes where the activity of one transporter is likely to directly or indirectly affect another. In the past, pharmacological studies have been hampered by a lack of selective ligands, but now a whole range of new and highly selective ligands have become available which are allowing even more precise exploration of the mechanisms of chondrocyte cell volume regulation.

Acknowledgements

CHF is a BBSRC funded student, RL is funded by the PetPlan Charitable Trust. Thanks to Dr. Madu Batuwangala for kindly providing the original artwork which we have now modified.

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