

Invited Review

Ion transport in chondrocytes: membrane transporters involved in intracellular ion homeostasis and the regulation of cell volume, free $[Ca^{2+}]$ and pH

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Summary. Chondrocytes exist in an unusual and variable ionic and osmotic environment in the extracellular matrix of cartilage and are responsible for maintaining the delicate equilibrium between extracellular matrix synthesis and degradation. The mechanical performance of cartilage relies on the biochemical properties of the matrix. Alterations to the ionic and osmotic extracellular environment of chondrocytes have been shown to influence the volume, intracellular pH and ionic content of the cells, which in turn modify the synthesis and degradation of extracellular matrix macromolecules. Physiological ion homeostasis is fundamental to the routine functioning of cartilage and the factors that control the integrity of this highly evolved and specialized tissue. Ion transport in cartilage is relatively unexplored and the biochemical properties and molecular identity of membrane transport mechanisms employed by chondrocytes in the control of intracellular ion concentrations and pH is not fully defined and this review focuses on these processes. Chondrocytes have been shown to express voltage and stretch activated ion channels, passive exchangers and ATP dependent ion pumps. In addition, recent studies of transport systems in chondrocytes have demonstrated the presence of isozyme diversity that includes Na^+/H^+ exchange (NHE1, NHE3), Na^+ , K^+ -ATPase (several isoforms) and others each of which possess considerably different kinetic properties and modes of regulation. This multitude of isozyme diversity indicates the highly specialized handling of ions and protons in order to accomplish a fine regulation of their transmembrane fluxes. The complexities of these transport systems and

their patterns of isoform expression underscore the subtlety of ion homeostasis and pH regulation in normal cartilage. Perturbations in these mechanisms may affect the physiological turnover of cartilage and thus increase the susceptibility to degenerative joint disease.

Key words: Chondrocyte, Cartilage, Ion transport, pH regulation, Na^+ , K^+ -ATPase, Na^+/H^+ exchange, Ca^{2+} -ATPase

Introduction

Articular cartilage is a mechanically unique connective tissue designed to withstand load and act as an elastic shock absorber and wear resistant surface to the articulating joints. Within articular cartilage, only one cell type is found: the chondrocyte. This is a highly specialized cell solely responsible for the synthesis and maintenance of cartilage matrix, a meshwork of collagen II, non-collagenous proteins and aggregating proteoglycans (see Fig. 1) (Muir, 1995; Buckwalter and Mankin, 1997a). Cell density in cartilage is low compared to other tissues. In adult human cartilage, chondrocytes occupy less than 1% of the total tissue volume (Stockwell, 1971, 1991) (Fig. 2A). In foetal human cartilage and cartilage from small animals the cell density is significantly higher. Although chondrocytes vary in size, shape, morphology and metabolic activity, they all possess the ability and intracellular organelles for the biosynthesis and degradation of the extracellular matrix. They surround themselves with extracellular matrix but do not form cell-to-cell contacts (Fig. 2C). The tissue is organized into three principal layers or zones, each of which has distinct biochemical, biomechanical and physiological characteristics. The chondrocytes in each zone also have characteristic shapes, sizes and orientations with respect to the

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articular surface. For example, in the surface zone, chondrocytes are flattened and ellipsoid-shaped arranged in parallel to the articular surface (Fig. 2A). In the middle and deep zones the cells are more spheroidal in shape and align themselves in columns perpendicular to the articular surface and the zone of calcification. The composition of the extracellular matrix also varies in each zone; in the surface layer the matrix is rich in collagen but the concentration of proteoglycans is relatively low whereas in the middle and deep layers the concentration of proteoglycans is significantly higher (Stockwell and Scott, 1967). The concentration of proteoglycans is highest in the deep zone and the chondrocytes express biochemical markers normally associated with matrix mineralization and calcification (e.g. alkaline phosphatase; Fig 2B).

The unique load bearing and physico-chemical properties of articular cartilage depend on their high concentrations of complex, negatively charged proteoglycans. The glycosaminoglycan side chains of proteoglycans contain up to two anionic groups (carboxyl and sulphate) per disaccharide subunit and thus provide a net negative charge to the matrix of cartilage (Fig. 1). Since these ionic groups are covalently attached to the solid matrix, they are often referred to as "fixed negative charges" and thus result in a fixed charge density (FCD) (Lesperance et al., 1992). Electrostatic interactions between the fixed charged groups provides up to 50% of the tissue compressive stiffness and the ability to withstand load. The ionic composition of the matrix is strictly dictated by the FCD; the negative charges on the proteoglycans draw cations (principally Na^+ , K^+ , and Ca^{2+}) into the matrix to balance the negative charge distribution (Maroudas, 1980). Consequently, the

concentrations of these cations are higher within cartilage relative to serum and synovial fluid (Table 1). In addition, the high fixed negative charge density results in a high concentration of protons, leading to a lowering of cartilage pH (Gray et al., 1988). Anions (Cl^- and HCO_3^-) are repelled by the negative charges and their concentration is relatively low in cartilage (Urban and Hall, 1992). In general, the movement of monovalent cations in the matrix is not restricted and is similar to that in free solution whereas divalent cations such as Ca^{2+} are less mobile (Maroudas, 1980). The ionic environment of chondrocytes is thus unusual compared with most other cell types (Table 1). The movement of cations into the matrix is followed by the movement of osmotically obliged water which may contribute as much as 80% of the wet weight of articular cartilage. The interaction between water and the ions in the matrix substantially influences the load bearing performance of cartilage. The increase in the total ionic strength results in an increase in tissue osmolarity, creating a Donnan effect; the collagen network resists the osmotic pressure exerted by the tissue water and the inflated proteoglycans. Mechanical loading itself results in changes to the ionic and osmotic environment of chondrocytes; loading expresses interstitial fluid from the matrix of cartilage increasing the local proteoglycan concentrations (Maroudas, 1979; Urban, 1994) and hence the local ionic strength and osmotic pressure (Fig 3). The loading induced cell shrinkage will then activate volume regulatory ion and osmolyte transport mechanisms that allow the chondrocytes to return to their original volume. This regulatory volume increase (RVI) effectively raises intracellular $[\text{K}^+]$, resulting in cell swelling towards normal volume (Hoffmann and Simonsen, 1989; Hall et al., 1996).

In normal cartilage, proteoglycans help to maintain the osmotic pressure within the cartilage matrix and concentrations of electrolytes in the interstitial fluid. The influence of tissue FCD on the ion and water content of the matrix may have important biochemical, cellular and patho-physiological consequences. For example, the synthesis of cartilage matrix by chondrocytes is sensitive to extracellular Na^+ concentrations (Urban and Bayliss, 1989). Furthermore, an acidic tissue pH has deleterious effects on matrix synthesis in many types of cartilage (Schwartz et al., 1976; Wilkins and Hall, 1995). More importantly, in the early stages of osteoarthritis, proteoglycans are lost from the matrix (Stockwell, 1991;

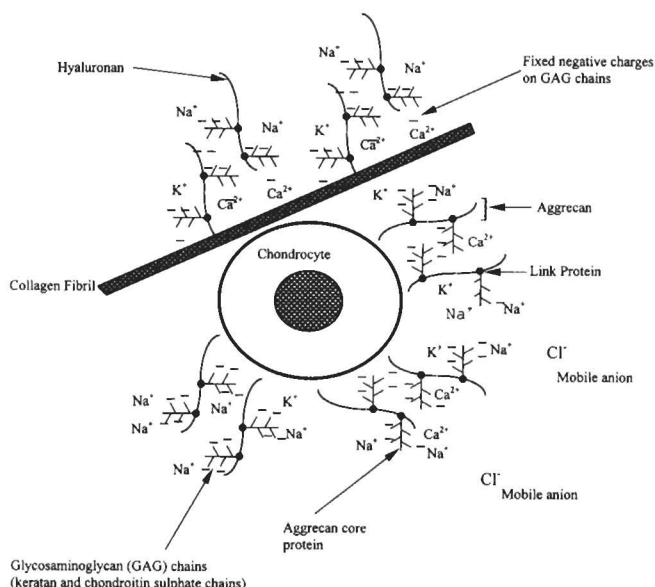


Fig. 1. Schematic illustration of the major constituents of cartilage matrix and the ionic environment of the chondrocyte.

Table 1. The ionic composition of articular cartilage.

	Na^+ (mM)	K^+ (mM)	Ca^{2+} (mM)	Cl^- (mM)	pH
Cartilage	240-350	7-12	6-20	60-100	6.9-7.1
Serum/Synovial fluid	140	5	1.5	145	7.4

The concentrations of the principal cations and anions in cartilage matrix depends on the local concentration of proteoglycans and their fixed charge density (Maroudas, 1980). The range of values given above are from human femoral head cartilage.

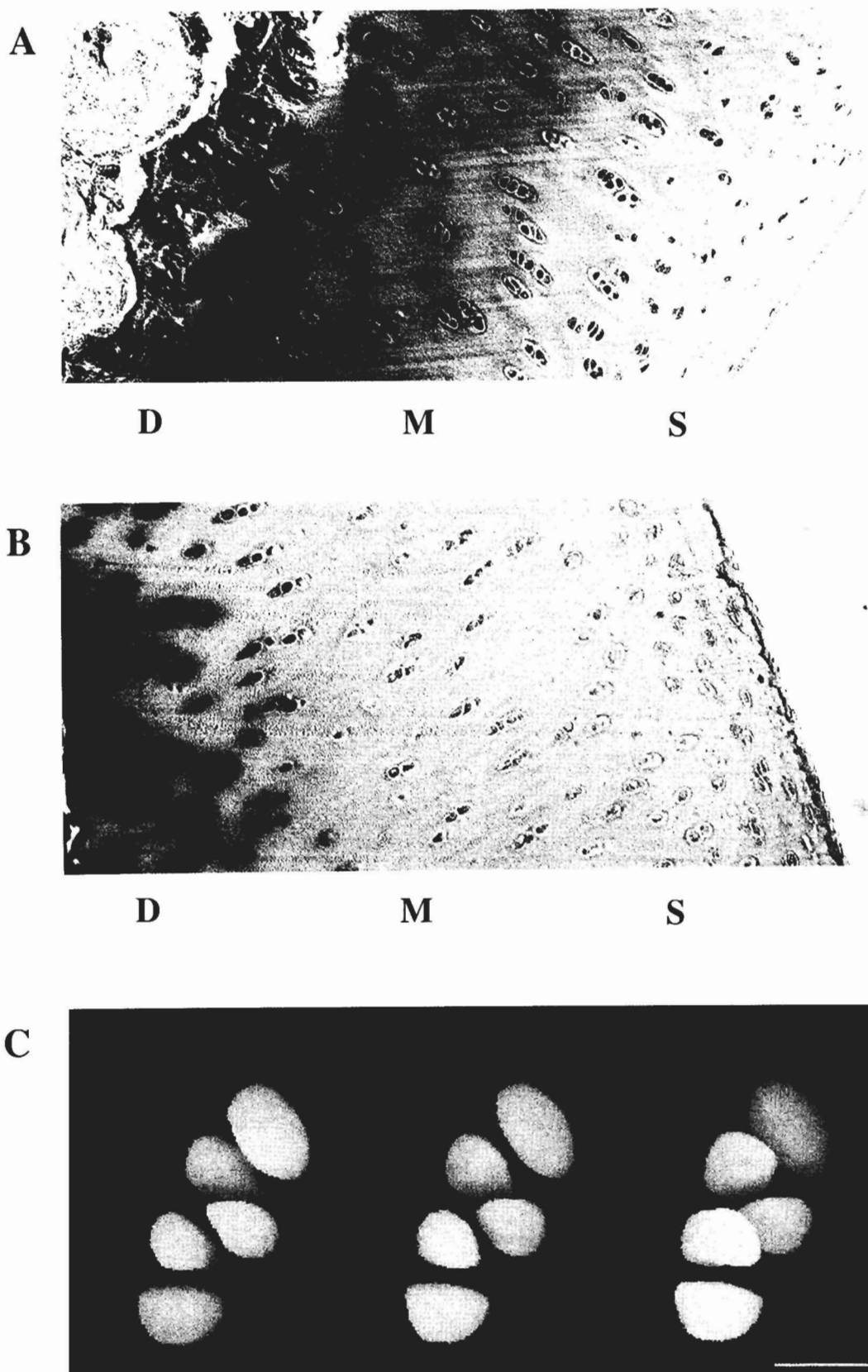


Fig. 2. **A.** Micrograph of thionin stained bovine articular cartilage from the metacarpophalangeal joint of a 2 year old steer. The tissue is organized into three principal layers which consist of the surface (S), middle (M) and deep (D) zones. The calcified cartilage which is adjacent to the underlying bone may be seen immediately below the deep layer. The morphology of chondrocytes depends on the layer in which they are found. **B.** This micrograph illustrates the deep zone specific expression of alkaline phosphatase, a marker of matrix mineralization and calcification. **C.** Confocal micrograph of live chondrocytes loaded with the fluorescent indicator CMFDA in a characteristic cluster or column of five cells that is typical of the middle zone. Bar: 10 μ m.

Buckwalter and Mankin, 1997b) and these early pathological changes result in a reduced FCD in the matrix creating physico-chemical alterations that modify the ionic environment of the chondrocyte and, ultimately, its metabolic behaviour. Chondrocytes sense and respond adaptively to changes in their ionic and osmotic environment. In the short-term they regulate the intracellular concentration of ions in an attempt to return the ion concentrations to their physiological levels. In the long-term such changes may affect the equilibrium between matrix synthesis and degradation. This may compromise the mechanical properties of the tissue and result in greater variation in the ionic and osmotic environment, thus playing a significant role in early cartilage pathology.

The transport of ions across the plasma membrane is a fundamental property of all living cells including chondrocytes. Major advances have been made over the past few years in the molecular identification and characterization of the major ion transport proteins expressed abundantly in neuronal and epithelial cells and in unravelling their pivotal roles in cellular ion homeostasis. The information gained has advanced our understanding of many essential cell functions particularly volume regulation, control of intracellular pH and free calcium homeostasis. However, our current knowledge of ion transport in articular chondrocytes is rudimentary compared to these other cell types. Further understanding of the ion transport processes involved in

chondrocyte ion, volume and pH homeostasis in normal cartilage is essential and a pre-requisite to understanding the processes that may occur in abnormal cartilage. In this review, the principal ion transport pathways in chondrocytes are discussed and compared in an attempt to understand how ion transport proteins allow chondrocytes to cope with their challenging ionic environment and how the altered expression of ion transporters may be important in pathologies of cartilage. It is hoped that this review will enhance our current understanding of ion transport in cartilage and stimulate further work in this potentially important but neglected area.

The importance of Na^+ transport in cartilage

Large transmembrane ion gradients exist in chondrocytes and Na^+ is by far the most abundant ion in cartilage. Its extracellular concentration in the matrix is estimated at around 250-350 mM (depending on the species and the zone of cartilage studied) whereas its intracellular concentration is around 40 mM which is still relatively high compared with other cell types (15-16 mM) (Maroudas, 1980; Urban and Hall, 1992). The difference between the intracellular and extracellular concentration of Na^+ results in a steep inward gradient for this important cation (Stein, 1990). This inward Na^+ gradient is exploited to transport a variety of ions, essential metabolites (amino acids and glucose) and organic osmolytes into the chondrocyte (Byers et al., 1983; Hall, 1995; Hall et al., 1996a,b). However, increased accumulation of intracellular Na^+ is not tolerated by chondrocytes since it affects the intracellular $\text{Na}^+:\text{K}^+$ ratio which needs to be maintained at a low level for optimal cell metabolism. The Na^+, K^+ -ATPase is the plasma membrane enzyme that performs this vital physiological function in all cells including chondrocytes. Its activity which depends upon the intracellular Na^+ and extracellular K^+ concentrations is regulated by the transmembrane fluxes of Na^+ and K^+ and thus indirectly regulated by the function of other ion transport mechanisms whose designated functions may also alter the intracellular $\text{Na}^+:\text{K}^+$ ratio. The Na^+, K^+ -ATPase also plays a vital role in maintaining cell volume; active extrusion of Na^+ counterbalances the colloidal osmotic pressure contributed by charged intracellular macromolecules (Basavappa et al., 1998).

Na^+, K^+ -ATPase expression in chondrocytes

The Na^+, K^+ -ATPase functions in almost all animal cells as the principal regulator of intracellular Na^+ and K^+ concentrations using ATP as an energy source. During each active cycle 3 Na^+ ions are pumped out of the cell in exchange for 2 incoming K^+ ions for every ATP molecule consumed (Sweadner, 1989, 1995). Thus, Na^+, K^+ -ATPase function leads to intracellular accumulation of K^+ which is essential for the activity of many intracellular enzymes (Kernan, 1980). The

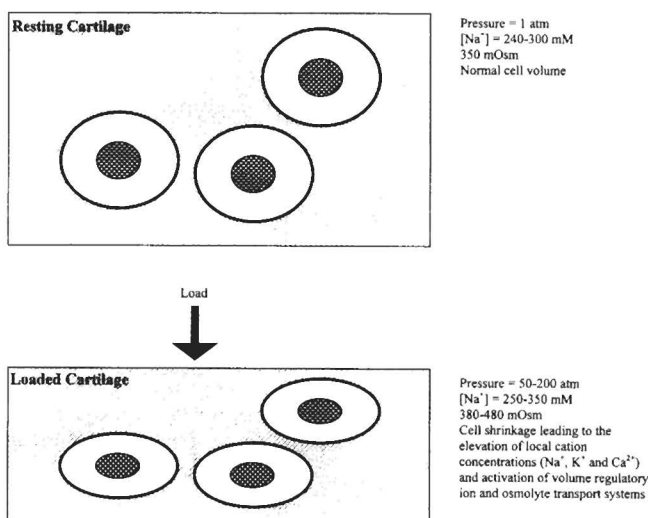


Fig. 3. The effect of load on the ionic and osmotic environment of chondrocytes compared in resting and loaded cartilage. Mechanical load deforms articular cartilage, directly elevating hydrostatic pressure, which results in the expression of tissue fluid, with high local fluid flows (streaming potentials), and increases tissue fixed charge density. This results in significant increases in local cation concentrations and tissue osmolarity (Urban, 1994) and compression induced changes in chondrocyte shape that decrease cell volume (Guilak and Bachrach, 1989). Changes in cell volume and ion content activate volume regulatory ion and osmolyte transport pathways in an attempt to restore the original cell volume (Hall, 1995; Hall et al., 1996a,b).

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intracellular concentration of K^+ must be maintained at around 120-140 mM by active pumping from the extracellular environment where the K^+ concentration is around 5 mM, against a steep concentration gradient. Thus, the Na^+ , K^+ -ATPase maintains the low intracellular $Na^+ : K^+$ ratio in face of an inward concentration gradient for Na^+ and an outward gradient for K^+ . The Na^+ , K^+ -ATPase is most abundantly expressed in excitable tissues such as the brain, skeletal muscle, cardiac muscle and epithelia and moderately expressed in other tissue and cell types. The enzyme is composed of an α and a β subunit; the 112 kDa catalytic α subunit contains the binding sites for Na^+ , K^+ , ATP and cardiac glycosides, a class of steroid compounds which serve as specific inhibitors of the Na^+ , K^+ -ATPase. The 45 kDa β subunit is often referred to as the regulatory subunit and is required for biogenesis and activity of the enzyme complex which is believed to be a heterodimeric protomer (α - β)₂ (Brotherus et al., 1983; Fambrough et al., 1994). Thus far, 4 α and 3 β isoforms have been identified in mammals (Martín-Vasallo et al., 1989; Shamraj and Lingrel, 1994; Malik et al., 1996). The α 1 isoform serves as the "housekeeping" isoform, as judged by its abundance and ubiquitous cellular distribution. The remaining isoforms exhibit a more restricted tissue specific and developmental pattern of expression; the α 2 isoform is expressed most abundantly in cardiac muscle, skeletal muscle and adipose tissue (Sweadner et al., 1994) and its expression is insulin sensitive (Russo and Sweadner, 1993). The α 3 isoform is found in high concentrations in neurons of the central nervous system (Sweadner, 1995). The α 4 isoform appears to be specific to the testis (Shamraj and Lingrel, 1994). The β 1 isoform is ubiquitously expressed (except in reticulocytes, Stengelin and Hoffman, 1997) whereas β 2 appears to be concentrated in the nervous system (Martín-Vasallo et al., 1989; Lecuona et al., 1996; Peng et al., 1997); β 2 is an adhesion molecule on glial cells (AMOG) specifically involved in mediating interactions between neurons and glia (Gloor et al., 1990). The β 3 isoform is the most recently described member of the β isoform gene family and is expressed predominantly in the testis but also in the brain, kidney, lung, spleen, liver and intestines (Malik et al., 1996; Peng et al., 1997; Arystarkhova and Sweadner, 1997).

Studies on bovine articular cartilage and freshly

isolated bovine chondrocytes have demonstrated the presence of 3H -ouabain binding sites and hence the presence of Na^+ , K^+ -ATPase pump units in chondrocytes (Mobasher et al., 1994). Quantitative studies on the isolated chondrocytes have demonstrated that the density of the Na^+ , K^+ -ATPase is relatively high for these small cells ($1.5-2.0 \times 10^5$ Na^+ , K^+ -ATPase sites per 8-12 μ m cell; Mobasher et al., 1994, 1996b). Autoradiographic studies using 3H -ouabain suggest that Na^+ , K^+ -ATPase density is sensitive to the ionic and osmotic environment of the chondrocyte and long-term increases in extracellular Na^+ result in the upregulation of the Na^+ , K^+ -ATPase in cartilage explants (Fig. 4) (Mobasher et al., 1995b, 1997a) and isolated cells (Mobasher et al., 1997b). Evidence indicates that freshly isolated chondrocytes are also sensitive to their ionic and osmotic environment and are capable of adaptive responses to ionic environmental perturbations, particularly changes to extracellular $[Na^+]$. Studies using isoform-specific monoclonal antibodies (Fig. 5) (Mobasher et al., 1995a, 1996b, 1997a,b) and cDNA probes (Mobasher et al., 1996a) have indicated that bovine chondrocytes express at least two catalytic α and two regulatory β isoforms of the Na^+ , K^+ -ATPase (results summarized in Table 2). There is preliminary evidence that human articular chondrocytes express three α (α 1, α 2, α 3) and three β subunit isoforms (β 1, β 2, β 3) the expression of which is developmentally regulated and is altered in cartilage pathologies (Trujillo, Alvarez de la Rosa and Martín-Vasallo, unpublished observations).

These above findings and physiological studies performed by Hall and co-workers (1996) suggest a central role for the Na^+ , K^+ -ATPase in intracellular ion homeostasis and cell volume regulation in chondrocytes. The presence of multiple Na^+ , K^+ -ATPase isoforms in the plasma membrane of chondrocytes and the unique kinetic properties of each isoform variant may reflect a cartilage specific specialization in order to maintain a low $Na^+ : K^+$ ratio in face of steep inward Na^+ gradients. The catalytic α isoforms have been shown to exhibit significantly different kinetic properties; the affinity of the α 3 isoform for intracellular Na^+ is orders of magnitude lower than that of the α 1 isoform (Jewell and Lingrel, 1991). Thus, the expression of the α 3 isoform may be related to the high extracellular concentration of

Table 2. ATPase pumps present in articular chondrocytes.

	PROPERTIES	REFERENCE
Na^+ , K^+ -ATPase (NKA)	High density ($1.5-2.0 \times 10^5$ /chondrocyte); multiple catalytic α and regulatory β subunits identified in bovine cartilage (α 1, α 3, β 1 and β 2); more isoforms identified in human cartilage	Mobasher et al., 1996a,b; Mobasher et al., 1997a,b; Trujillo and Martín-Vasallo, unpublished observations
Ca^{2+} -ATPase (PMCA)	Abundant plasma membrane expression (one isoform PMCA1 identified)	
H^+ , K^+ -ATPase/ H^+ -ATPase (HKA/HA)	Expressed in cultured avian growth plate chondrocytes	Dascalu et al., 1993

Na⁺ in cartilage and the substantial transmembrane concentration gradient for Na⁺ that exists in chondrocytes. The $\alpha 3$ isoform with its low affinity for Na⁺ working in parallel with the $\alpha 1$ isoform may allow chondrocytes to respond more effectively to the physiological changes in intracellular Na⁺ that occur frequently *in vivo*. In addition the affinity of the $\alpha 3$ isoform for the cardiac glycoside ouabain is higher than $\alpha 1$ raising the possibility of independent regulation of Na⁺, K⁺-ATPase units containing these different isoforms at the tissue and transcriptional levels by endogenous inhibitors or endo-ouabain-like compounds (Schreiber, 1991; Mobasher, 1998).

The possible roles of the β isoforms (particularly $\beta 2$) in cell cytoskeleton-extracellular matrix interactions and cell adhesion is intriguing. The β isoforms are glycoproteins containing three N-linked oligosaccharides on their extracellular domain. Since branched extra-cellular carbohydrates have been implicated in cell recognition events, it may be possible that the β isoforms are involved in transmitting extracellular information by permitting a physical interaction between the pump and components of the cellular cytoskeleton (i.e. ankyrin, actin filaments and actin associated proteins; Mandreperla et al., 1989), cell surface mechano-sensory complexes and possibly also extracellular matrix macromolecules (Mobasher et al., 1996b). There is no current concrete evidence for such interactions and clearly these possibilities require further investigation. However, it is clear that the chondrocyte Na⁺, K⁺-ATPase exists in multiple molecular forms and plays a vital role in the physiology of chondrocytes in normal cartilage.

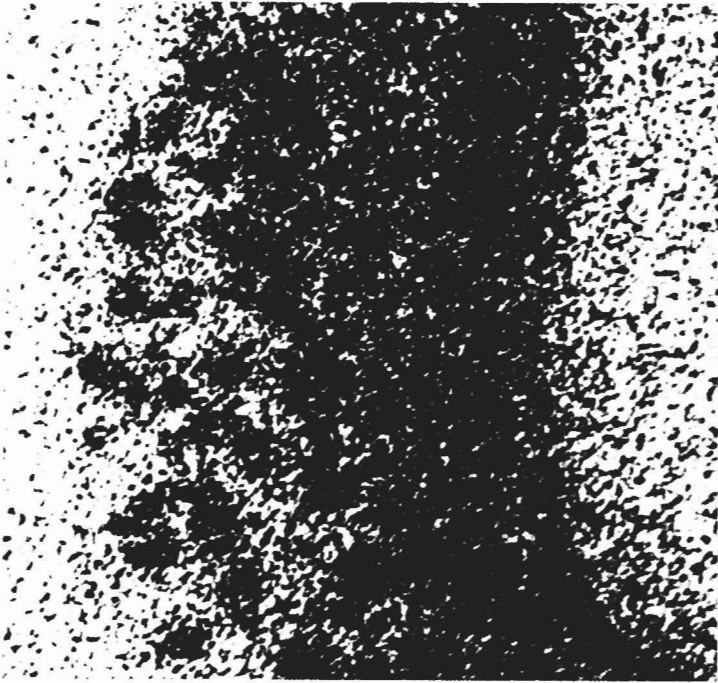
The observation that Na⁺, K⁺-ATPase density depends on the ionic and osmotic environment suggests that chondrocytes are sensitive to their extracellular environment. The upregulation normally observed only takes place following long-term changes to extracellular [Na⁺] (Mobasher et al., 1997a) or osmolarity (A. Mobasher, unpublished observations). Across the tissue the density of the Na⁺, K⁺-ATPase also varies. In the middle zone where proteoglycan concentrations are higher than the surface zone, Na⁺, K⁺-ATPase density is also higher (Mobasher et al., 1997a). The middle zone experiences the greatest compressive loading compared to the other zones and thus the changes in local extracellular [Na⁺] resulting from fluid expression are likely to be high. This may explain the necessity for higher Na⁺, K⁺-ATPase densities in this zone (Mobasher, 1998).

The precise role of the Na⁺, K⁺-ATPase in pathologies of cartilage is unknown at the present time. Since the Na⁺, K⁺-ATPase is a principal regulator of cellular ion homeostasis, it may well be involved in situations where the ionic environment of chondrocytes is drastically altered. Changes in the ionic and osmotic environment of chondrocytes occur during the initial stages of human osteoarthritis where tissue hydration and proteoglycan loss occur leading to a fall in tissue [Na⁺] and [K⁺] content (Maroudas, 1979, 1980). If the chondrocyte Na⁺, K⁺-ATPase does not maintain the optimal Na⁺:K⁺ ratio within prerequisite physiological limits, there may be deleterious alterations to extracellular matrix synthesis rates in the short-term (Urban et al., 1993) and possibly also in the long-term. The intracellular concentration of K⁺ is particularly significant because of the requirements of intracellular enzymes; its presence at high intracellular concentrations is essential for the proper functioning of a wide range of enzymes (Kernan, 1980). The presence of intracellular K⁺ is also vital for binding to the extracellular K⁺ binding site on the α subunit of the Na⁺, K⁺-ATPase, ATP hydrolysis and subsequent conformational changes involved in ion transport (Jørgensen, 1986). Other factors, for example, an adequate supply of glucose for the provision of metabolic energy in the form of ATP provided by glycolysis in these strongly glycolytic cells may also be important. Since the Na⁺, K⁺-ATPase is an ATP dependent pump, the concentration of ATP may become limiting to ion homeostasis and normal cell physiology if the diffusion dependent supply of glucose is compromised in pathological states. Blockade of the Na⁺, K⁺-ATPase by endogenous inhibitors or depleted ATP stores will compromise pump activity leading to subsequent cytotoxic edema or cell swelling and eventually to pathological hypertrophy. The failure of the Na⁺, K⁺-ATPase and other ion pumps resulting from persistent ATP depletion may also result in ischemic cell death. Shortage of ATP will inhibit the activity of the Na⁺, K⁺-ATPase which in turn will have a deleterious effect on the optimal function of the plasma membrane Ca²⁺-ATPase and intracellular [Ca²⁺]. This may then trigger cell death since intracellular and intranuclear Ca²⁺ fluctuations can affect chromatin organization, induce gene expression and also activate cleavage of nuclear DNA by nucleases during programmed cell death or apoptosis (Nicotera and Rossi, 1994; Nicotera et al., 1994).

During the later stages of osteoarthritis large quantities of proteoglycans are lost from the matrix of

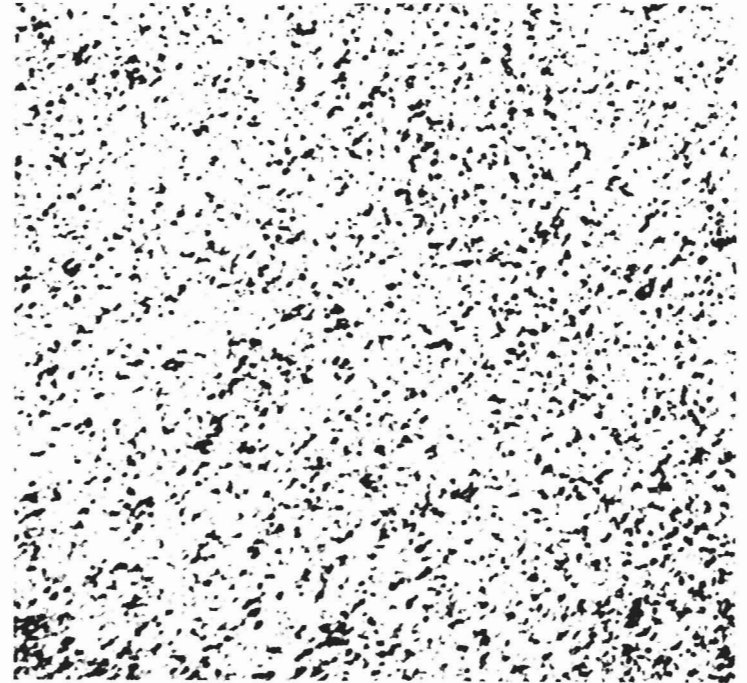
Fig. 4. Evidence for the abundant expression of the Na⁺, K⁺-ATPase from autoradiographic studies using ³H-ouabain, a specific inhibitor of the Na⁺, K⁺-ATPase that binds to the enzyme (molar 1:1 ratio) allowing accurate quantitative analysis. **A.** An autoradiograph of ³H-ouabain binding to a cryostat section of bovine articular cartilage. The silver grains are concentrated around clusters of chondrocytes known as chondrons in the middle (M) and deep zones of cartilage. On the articular surface (S) the silver grains are more diffuse due to the smaller size, different morphology, higher density and close proximity of chondrocytes. **B.** Non-specific binding obtained in the presence of excess non-radioactive ouabain (10⁻⁴M) (Erdmann, 1982). **C.** Upregulation of chondrocyte Na⁺, K⁺-ATPase density in response to long-term (18 hr) exposure to elevated [Na⁺]. Specific ³H-ouabain binding measurements were made on cartilage explants and binding normalized to DNA content. **D.** Binding of ³H-ouabain to isolated chondrocytes compared in the presence and absence of external K⁺ which competes for the ouabain binding site on the α subunit of the Na⁺, K⁺-ATPase (Sweadner, 1995).

A

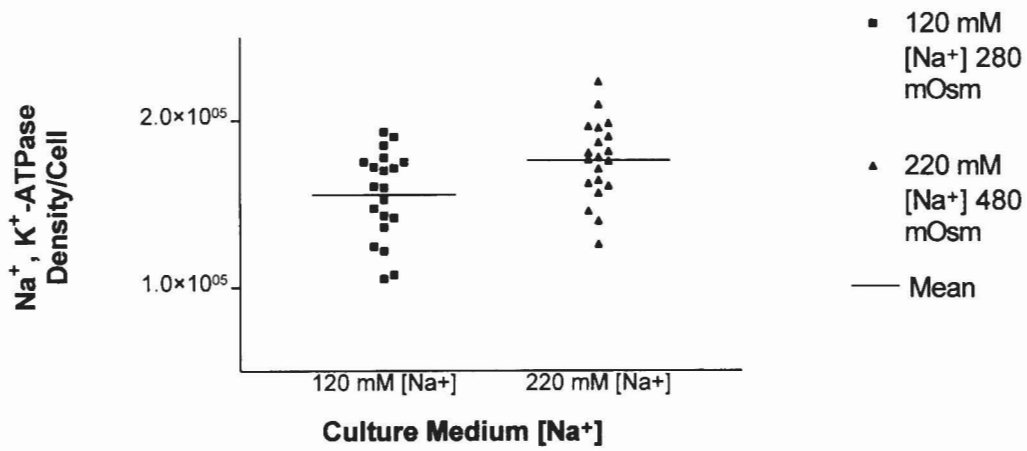


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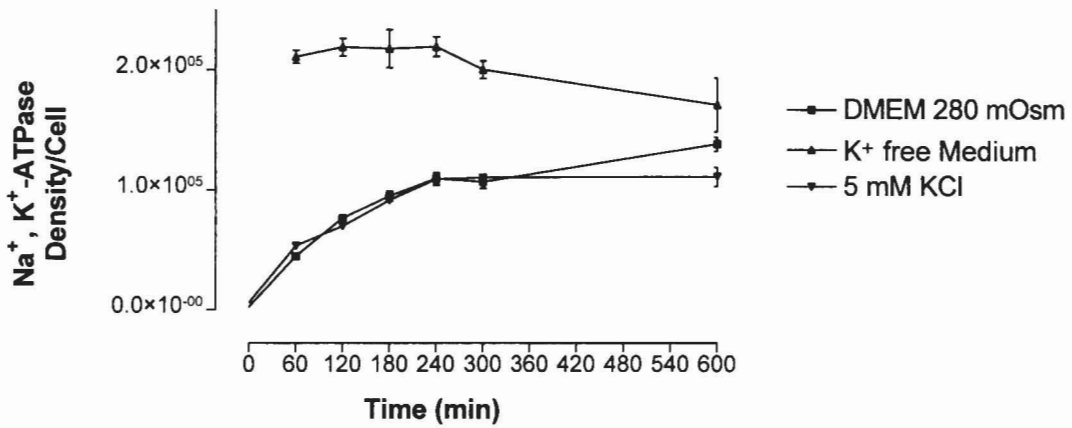
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C



D



affected areas of articular cartilage and this further exacerbates the ionic and osmotic changes to the environment of chondrocytes. Chondrocytes may adapt to these progressive changes by alterations to Na^+ , K^+ -ATPase density and maintain the normal intracellular $\text{Na}^+:\text{K}^+$ ratio possibly preventing further changes to matrix metabolism. If adaptive responses are not initiated, this may be the start of a progressive failure of these cells to regulate their composition and hence matrix turnover. Any direct involvement of the Na^+ , K^+ -ATPase in cartilage pathology needs to be considered in context, incorporating the contribution of other membrane transport systems involved in the regulation of cell volume, ionic milieu, intracellular Ca^{2+} and intracellular pH.

Intracellular calcium homeostasis in cartilage

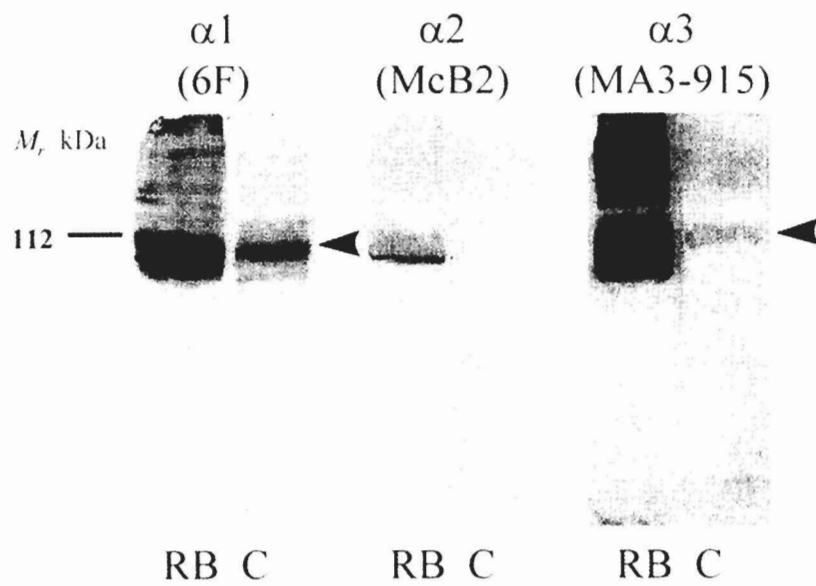
A general feature of the most living cells is the extremely low free $[\text{Ca}^{2+}]$ maintained in the cytosol (Carafoli, 1991). All cells, including chondrocytes, need to regulate the intracellular concentration of Ca^{2+} in their cytosol (Borke et al., 1987, 1989) and most mammalian cells maintain an intracellular $[\text{Ca}^{2+}]$ of approximately 100 nM or lower. Thus there is a 10,000 fold difference in $[\text{Ca}^{2+}]$ across the plasma membrane and intracellular $[\text{Ca}^{2+}]$ must be carefully regulated since it acts as a potent second messenger capable of initiating a large number of intracellular events (Berridge, 1997). Calcium plays a central role in the regulation of many forms of cellular activity and it has been implicated in many physiological processes including cell proliferation and cell death. In cartilage there is the additional physiological requirement of controlled extracellular and intracellular calcium homeostasis required for localized matrix mineralization. To establish very low intracellular $[\text{Ca}^{2+}]$ and to maintain this against Ca^{2+} influx through voltage and stretch activated Ca^{2+} channels and non-specific leakage, a typical cell may possess two systems for Ca^{2+} extrusion, the active Ca^{2+} -ATPase and a coupled transporter, the $\text{Na}^+ \times \text{Ca}^{2+}$ -exchanger. In many cells, the Ca^{2+} -ATPase performs the vital physiological function of Ca^{2+} extrusion (Carafoli, 1991). The Ca^{2+} -ATPase is a high affinity, low-capacity Ca^{2+} extrusion mechanism that is ATP- and Mg^{2+} -dependent, present in the plasma membrane of all eukaryotic cells (Stys et al., 1995). The Ca^{2+} -ATPase is highly dependent on calmodulin (Carafoli 1991), has a high affinity for Ca^{2+} , and is inhibited by vanadate. In contrast, the $\text{Na}^+ \times \text{Ca}^{2+}$ -exchanger is the Ca^{2+} efflux mechanism which plays a

significant role in excitation-contraction coupling in cardiac and skeletal muscle (Philipson et al., 1988; Philipson, 1990). The $\text{Na}^+ \times \text{Ca}^{2+}$ -exchanger plays a critical role in diverse cellular processes in a variety of tissues including heart, nerve and kidney (Lee et al., 1994). This transporter catalyzes a reversible, electrogenic exchange, with a stoichiometry of $3\text{Na}^+:\text{Ca}^{2+}$, which is inhibited by La^{3+} and other divalent cations. It is regulated by intracellular Ca^{2+} , Na^+ and ATP concentrations. If the $\text{Na}^+ \times \text{Ca}^{2+}$ -exchanger was present in chondrocytes, it would undoubtedly be energized by the steep inward gradient of Na^+ established by the Na^+ , K^+ -ATPase and hence depend on an optimal activity of the latter.

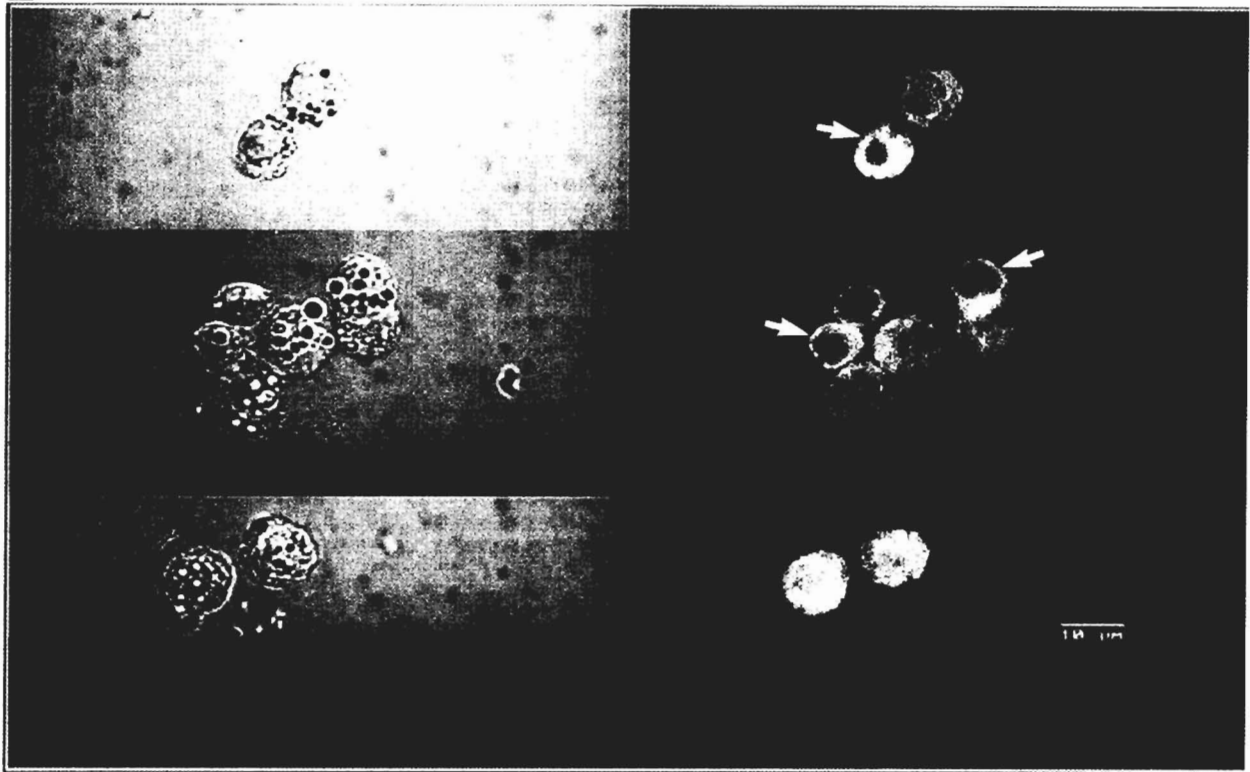
There is relatively little published information on Ca^{2+} homeostasis in cartilage or the regulation of intracellular Ca^{2+} in chondrocytes. Early studies on epiphyseal chondrocytes by Zanetti and co-workers (1982) suggested a key role for the Ca^{2+} -ATPase in calcium extrusion following stimulation by the ionophore A23187. Mechanisms for calcium entry remained unexplored for almost a decade until evidence emerged for two types of Ca^{2+} channel; the first type is blocked by La^{3+} but does not appear to be voltage-gated or sensitive to verapamil (Grandolfo et al., 1992) and the second is reported to be an N-type voltage-sensitive calcium channel (Zuscik et al., 1997). Agonists such as histamine cause a rise in intracellular $[\text{Ca}^{2+}]$ that appears to be the result of Ca^{2+} influx (Horwitz and Harvey, 1995; Horwitz et al., 1996). Thus, it seems certain that Ca^{2+} channels are present in chondrocytes and the studies of Wright and co-workers (1996) suggest that some of the Ca^{2+} channels are stretch activated and blocked by gadolinium ions (Gd^{3+} ; specific inhibitor of stretch activated channels). This raises the very exciting possibility that stretch activated Ca^{2+} channels play an important physiological role following mechanical loading and may be one of the first steps of an intracellular second messenger cascade. This notion is supported by recent studies performed by Yellowley and co-workers (1997) who have evidence for fluid flow-induced intracellular Ca^{2+} mobilization in bovine articular chondrocytes. Thus, the compression or fluid flow-induced influx of Ca^{2+} , possibly through mechanosensitive and voltage sensitive Ca^{2+} channels results in a rise in intracellular $[\text{Ca}^{2+}]$ and may contribute to the mechanism by which mechanical loads are transduced by chondrocytes. The physiological importance of calcium channels and other known ion channels in chondrocytes will be discussed in depth separately in a later section.

Fig. 5. Expression of Na^+ , K^+ -ATPase α isoforms in isolated bovine articular chondrocytes. **A.** Western blot analysis of crude rat brain homogenate (RB) and lysates of isolated bovine chondrocytes using monoclonal isoform specific antibodies indicates that $\alpha 1$, $\alpha 2$ and $\alpha 3$ are expressed in brain whereas chondrocytes only express $\alpha 1$ and $\alpha 3$, $\alpha 1$ being more abundant than $\alpha 3$. There is no detectable expression of the $\alpha 2$ isoform in chondrocytes. **B.** Confocal laser microscopy reveals abundant expression of the $\alpha 1$ isoform in isolated, fixed chondrocytes. The panels on the left represent phase contrast images of groups of chondrocytes examined by immunofluorescence using a monoclonal $\alpha 1$ specific antibody and a FITC-conjugated anti-mouse IgG. In the panels on the right arrows indicate the plasma membrane location of the Na^+ , K^+ -ATPase in chondrocytes. **C.** Controls where the primary antibody has been omitted. Bars: 10 μm approx.

A



B



C



Intracellular Ca^{2+} ions that have gained access to the cytosol may either be pumped or transported out of the cell or alternatively sequestered in intracellular stores (Berridge, 1997). Preliminary evidence suggests that the Mg^{2+} -dependent plasma membrane Ca^{2+} -ATPase (PMCA) is abundantly present in chondrocytes (Fig. 6). This finding and the apparent absence of the cardiac isoform of the $\text{Na}^+ \times \text{Ca}^{2+}$ -exchanger protein suggests that the plasma membrane Ca^{2+} -ATPase is the principal active mechanism for Ca^{2+} extrusion in chondrocytes. The Ca^{2+} -ATPase is likely to be the PMCA1 isoform (plasma membrane Ca^{2+} -ATPase 1) also found in erythrocytes and kidney cells (Borke et al., 1987, 1989). Although the cardiac $\text{Na}^+ \times \text{Ca}^{2+}$ -exchanger protein does not appear to be expressed in chondrocytes, the presence of other isoforms or splice variants cannot be excluded. The possibility of an $\text{Na}^+ \times \text{Ca}^{2+}$ -exchanger protein cannot be exclusively ruled out until further physiological, biochemical, immunohistochemical and molecular studies are carried out. The current

information on Ca^{2+} transport in chondrocytes is summarized in Figure 7.

Transport mechanisms involved in pH regulation in chondrocytes

The fixed negative charge of cartilage extracellular matrix attracts protons in addition to cations resulting in an unusually acidic extracellular environment (pH 6.9-7.1; Table 1). The tissue pH may fall further by static joint loading when fluid is expressed from the matrix raising the local FCD. Cartilage is avascular and the low partial pressure of oxygen implies that chondrocyte metabolism is predominantly anaerobic. Large amounts of lactate are produced by anaerobic metabolism (glycolysis) and long periods are required for diffusion of these acidic molecules across the cartilage matrix and absorption by the synovial membrane. The accumulating lactate further exacerbates the low pH in cartilage (Stockwell, 1991). Chondrocytes have a resting intracellular pH (pH_i) of approximately 7.1 units and changes to pH_i have been shown to modify the synthesis of matrix macromolecules in bovine articular chondrocytes (Wilkins and Hall, 1992). It has also been demonstrated that extracellular pH (pH_o) regulates the synthesis of matrix macromolecules (Wilkins and Hall, 1995). Extremes of extracellular acidity result in a reduction in the pH_i of isolated articular chondrocytes and physiological manipulations resulting in intracellular acidosis inhibits matrix synthesis in cartilage (Wilkins and Hall, 1995). Therefore, pH regulation is important to the health and turnover of cartilaginous tissues.

In most cells, pH_i regulation is achieved by parallel and functionally independent acid and base extrusion pathways. The physiological pH of a typical cell is maintained at around 7.2-7.3 pH units by plasma membrane transport proteins that either extrude acid (protons; H^+) or base (bicarbonate; HCO_3^-) (Alper, 1994). Amiloride sensitive Na^+/H^+ -exchangers and proton-extruding ATPases are involved in the process of acid extrusion whereas stilbene-sensitive anion dependent systems are involved in base extrusion and operate in parallel with the acid extruders to regulate pH_i . Base extruders include members of the erythrocyte Band-3 related anion exchange (AE) family (Alper, 1991, 1994).

Na^+ dependent pH regulation by cation exchange

The Na^+/H^+ -exchanger or cation exchanger is one of the best-characterized intracellular pH regulators (Orlowski and Grinstein, 1997; Wakabayashi et al., 1997). The Na^+/H^+ -exchanger is involved in multiple cellular functions in addition to its primary role, the regulation of intracellular pH. These include the control of cell volume and trans-epithelial ion transport. It is involved in the exchange of Na^+ and H^+ ; Na^+ ions move down their steep concentration gradient into the cell via NHE proteins and are exchanged for protons ejected

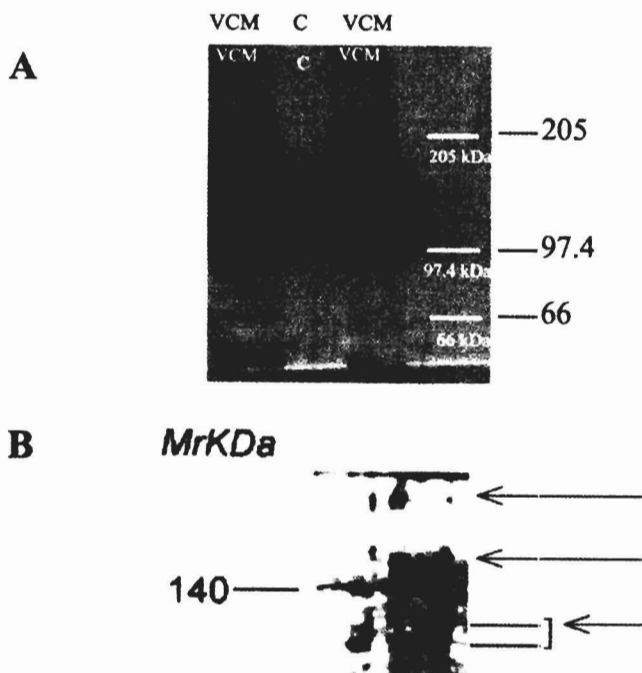


Fig. 6. Absence of the $\text{Na}^+ \times \text{Ca}^{2+}$ -exchanger and the expression of the plasma membrane Ca^{2+} -ATPase in isolated chondrocytes. **A.** Western blot analysis of chondrocyte and ventricular myocyte lysates using a monoclonal antibody against the $\text{Na}^+ \times \text{Ca}^{2+}$ -exchanger demonstrates the absence of this exchanger in chondrocytes (lane C) in contrast with ventricular cardiac myocytes (positive control, lane VCM, isolated according to Powell et al., 1980) where it is abundantly expressed (Philipson et al., 1988). **B.** Analysis of plasma membrane Ca^{2+} -ATPase (PMCA1 isoform) expression in isolated bovine articular chondrocytes. Western blot analysis using monoclonal antibody 5F10 (Borke et al., 1988) results in staining of a band at approximately 140 kDa and lower molecular weight bands that may represent proteolytic fragments of the Ca^{2+} -ATPase in bovine chondrocyte lysates. The bands above 140 kDa may represent Ca^{2+} -ATPase aggregates.

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from within the cytoplasm. The mode of exchange is electroneutral with a stoichiometry of 1:1. These transporters are regulated by a remarkably wide variety of stimuli that can modulate their expression level and activity (Bianchini and Pouyssegur, 1994). The first cloned human Na^+/H^+ -exchanger which is now called

NHE1 was reported by Sardet et al. (1989). The kinetic, immunological and pharmacological data initially available suggested that multiple isoforms of NHE were present in different cells and tissues (Clark and Limbird, 1991). Since then four more members of the gene family (NHE2-5) have been identified and characterized by cloning (Klanke et al., 1995) and they define a new gene family of vertebrate transporters. These isoforms share the same overall structure but exhibit differences with respect to amiloride sensitivity, cellular localization, kinetic variables, regulation by various stimuli (i.e. growth factors) and plasma membrane targeting in polarized epithelial cells. NHE-1 is commonly referred to as the "housekeeping" isoform since it appears to be ubiquitously expressed (Tse et al., 1994) where it most likely maintains cytosolic pH and cellular volume. It is found in high concentrations in the basolateral membrane of epithelia. It is said to be "smart", that is to say its activity is enhanced when pH_i falls. It is inhibited by the loop diuretic amiloride and its analogue ethylisopropyl amiloride (EIPA). It is stimulated by phosphorylation (Counillon and Pouyssegur, 1995) and activated by cell shrinkage (Grinstein et al., 1992; Demarex and Grinstein, 1994). NHE isoforms 2, 3 and 4 have similar properties and are likely to be Na^+ -absorbers or H^+ -secretors in the apical membrane of epithelial cells. However, their tissue specific expression is primarily limited to kidney, stomach and intestines. They may be stimulated or inhibited by phosphorylation. In general all NHE proteins are amiloride-sensitive except NHE 3 which is amiloride-resistant, but sensitive to EIPA.

Na^+/H^+ exchange mechanisms in chondrocytes

Physiological inhibitor and ion substitution studies have demonstrated that pH_i regulation in freshly isolated chondrocytes is principally mediated by the acid extruding amiloride sensitive Na^+/H^+ -exchanger (Wilkins et al., 1996). Many characteristics of this exchanger, for example its sensitivity to amiloride ($K_{0.5} = 5 \mu\text{M}$; Hall et al., 1996), activation by cell shrinkage (Wilkins et al., 1995) and sensitivity to phorbol esters (Hall et al., 1996) suggest a physiological and functional role for the NHE1 isoform of this exchanger. Measurements of acid equivalent efflux from acid-loaded cells have confirmed the dominance of NHE mechanisms over anion dependent systems in chondrocytes. Studies by Wilkins et al. (1996) have confirmed that isolated bovine articular chondrocytes possess more than one form of Na^+/H^+ -exchanger; using monoclonal antibodies against various isoforms of the $\text{Na}^+ \times \text{H}^+$ -exchanger it has become apparent that in chondrocytes this system is composed of two distinct isoforms, NHE1 and NHE3, the regulation of which are significantly different. Although there is no information on NHEs in human cartilage the finding that NHE3 is expressed in bovine chondrocytes confirms that NHE1 is not the only Na^+/H^+ -exchanger in these cells. The presence of two

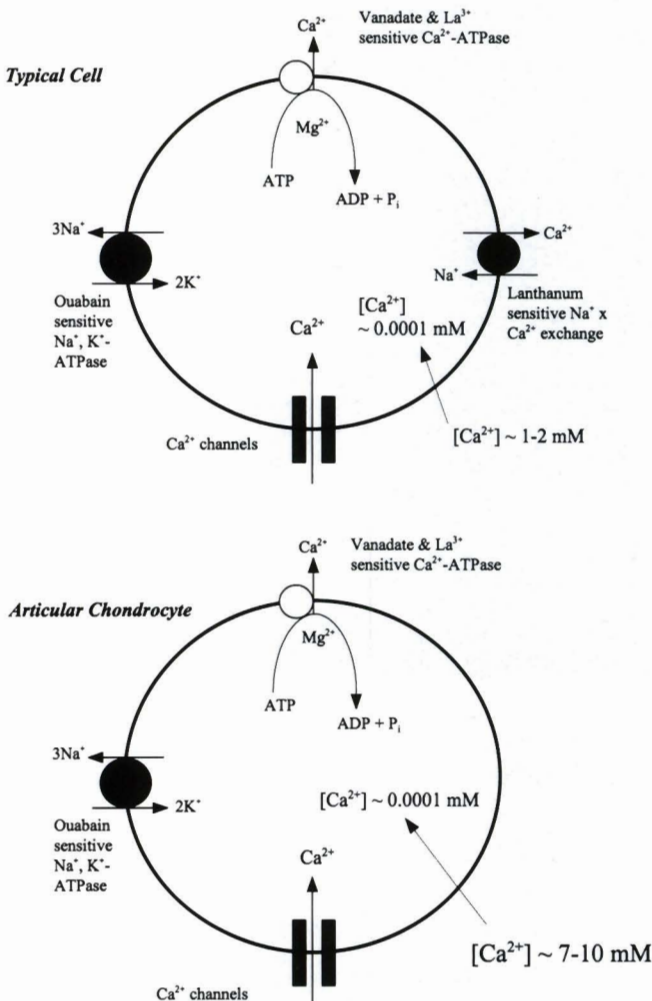


Fig. 7. This figure illustrates and summarizes the current understanding of Ca^{2+} transport in chondrocytes and compares the $[\text{Ca}^{2+}]_i$ regulatory mechanisms of articular chondrocytes with a typical neuronal, skeletal or renal cell. In most typical mammalian cells the Ca^{2+} -ATPase works in tandem with the $\text{Na}^+ \times \text{Ca}^{2+}$ -exchanger to maintain a low intracellular $[\text{Ca}^{2+}]_i$ in the order of $1 \times 10^{-4} \text{ M}$. The situation is different in chondrocytes, where it appears that the $\text{Na}^+ \times \text{Ca}^{2+}$ -exchanger is not present. In its place, the Ca^{2+} -ATPase functions to extrude Ca^{2+} from the cytosol. The Ca^{2+} -ATPase is likely to be the PMCA1 isoform (plasma membrane Ca^{2+} -ATPase 1) also found in erythrocytes and kidney cells. However, the possibility of specific expression of an unknown $\text{Na}^+ \times \text{Ca}^{2+}$ -exchange mechanism (novel or previously undetected isoform) cannot be ruled out. La^{3+} sensitive Ca^{2+} -channels (voltage-sensitive and insensitive) are expressed in chondrocytes as well as the cell types above (Grandolfo et al., 1992). Arrows with dotted lines represent passive diffusion or non-specific leakage.

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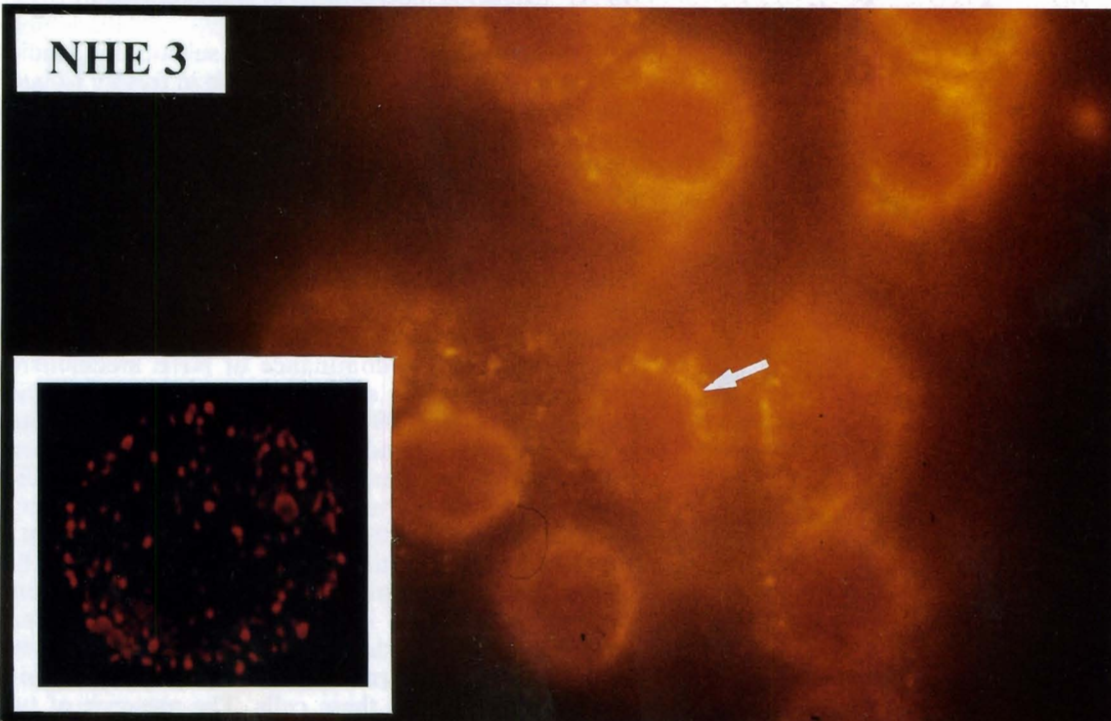
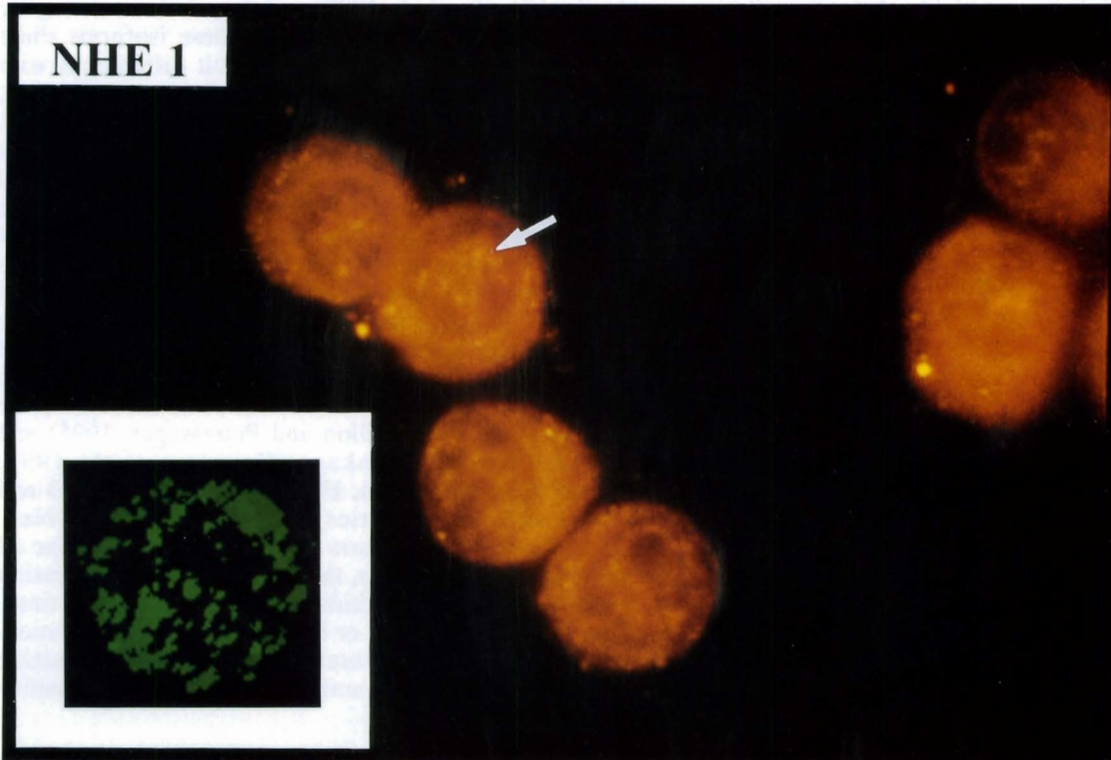
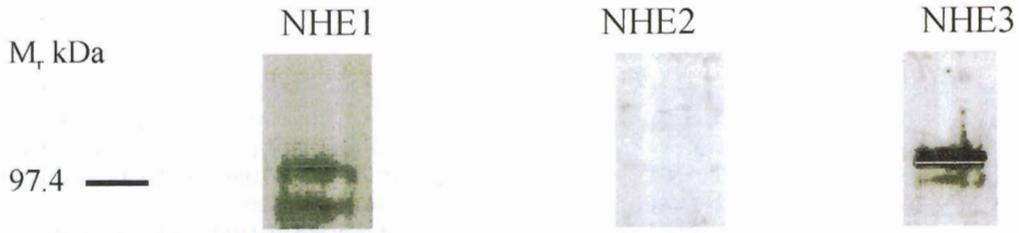


Fig. 8. Abundant expression of NHE1 and NHE3 in chondrocytes as assessed by Western blot analysis and immunofluorescence microscopy. NHE expression demonstrated by immunofluorescence in paraformaldehyde fixed, SDS permeabilised chondrocytes (according to Brown et al., 1996). The immunofluorescence micrographs demonstrate NHE1 and NHE3 expression in isolated bovine chondrocytes; the inserts are laser confocal micrographs of chondrocytes immunostained with the same isoform specific antibodies and thus provide a more detailed 3-dimensional view of NHE 1 and NHE3 expression. NHE1 staining is distinctly dense and patchy suggesting high level expression in the plasma membrane compared with NHE3 which was more punctate but less dense. There was no fluorescent staining observed with the NHE2 specific antibodies (results not shown) suggesting its lack of expression in chondrocytes.

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isoforms of Na^+/H^+ -exchange in chondrocytes (Fig. 8) is likely to be a cartilage specific adaptation to the unusually acidic extracellular matrix of cartilage (Sah et al., 1991; Wilkins et al., 1996). Furthermore, the presence of NHE1 and NHE3 in chondrocytes raises the possibility of independent regulatory mechanisms. The expression of the NHE3 isoform has been shown to be sensitive to growth factors and serum (Tse et al., 1994; Wakabayashi et al., 1997). The partition co-efficient of serum and growth factors in intact, healthy cartilage is low; in other words, growth factors do not easily penetrate the matrix and gain access to chondrocytes. Therefore, the expression of NHE3 in degenerate cartilage may be elevated since the extracellular matrix has been compromised and the exposed chondrocytes are more likely to come into contact with growth factors and cytokines.

Anion exchange and other pH regulatory mechanisms in chondrocytes

Other experiments performed on avian chondrocytes maintained in monolayer culture have indicated that proton-extruding ATPases and base extruding HCO_3^- -dependent mechanisms may also exist under certain conditions (Dascalu et al., 1993). Recent studies performed by Golding and co-workers (1997) add considerable weight to this opposing view. Their studies suggest that band-3 related anion dependent systems are present in isolated chondrocytes. The anion exchanger AE2 has been identified in chondrocytes at the transcriptional and translational levels (Golding et al., 1997). However, in chondrocytes, this AE2 transporter may not participate in base extrusion but rather may be involved in the transport of sulphate ions since this isoform of anion exchange has been demonstrated to function as a rapid sulphate transporter at low pH (Sekler et al., 1995). Immunofluorescence confocal studies suggest that the AE2 protein appears to be concentrated in intracellular membranes of chondrocytes (S. Golding et al., unpublished observations) and related osteoblasts (Mobasher et al., 1998) and the intracellular staining observed is characteristic of the Golgi complex in both cases. An alternative explanation for the presence of proton-extruding ATPases and anion

dependent mechanisms is that prolonged culturing of chondrocytes in monolayers may cause their de-differentiation to the fibroblastic phenotype (Benya and Schaffer, 1982) where all the above pH regulatory systems operate.

Overall, these results suggest that chondrocytes exploit the steep inward Na^+ gradients that exist across the plasma membrane by expressing the amiloride sensitive acid extruding Na^+/H^+ -exchangers to regulate pH_i . Under certain conditions acid extruding ATPase proton pumps and base extruding HCO_3^- -dependent mechanisms operate in tandem with at least two isoforms of the Na^+/H^+ -exchanger (NHE1 and NHE3) to regulate pH_i . The differential sensitivity of the NHE isoforms to $[\text{H}^+]$, cell volume and growth factors may provide an additional level of pH_i regulation. Further work is required to determine the exact role of NHE mechanisms in cartilage pathologies, particularly the up- or down-regulation of NHE isoforms in rheumatoid and osteoarthritis.

Ion channels in chondrocytes

The current information on ion channels in chondrocytes is very limited. However, there has been growing interest in cartilage electrophysiology and the results of these recent developments is briefly summarized here. It is important to mention that the evidence for the presence of ion channels in chondrocytes has been obtained largely by means of physiological and electrophysiological techniques. With the recent development of isoform specific antibodies against ion channel protein subunits more information will become available about their expression and tissue specific localization and possible involvement in degenerate and pathological cartilage.

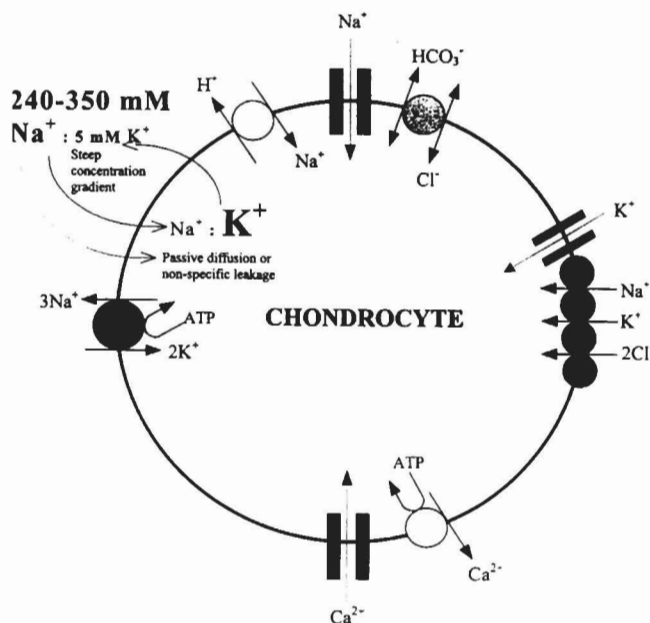
There is evidence for tetrodotoxin-sensitive sodium channels which would serve as one of at least three major Na^+ entry mechanism in chondrocytes (Wright et al., 1992; see Fig. 9). There is no information about the molecular composition of these sodium channels. Calcium activated potassium channels have been reported in chondrocytes (Grandolfo et al., 1990, 1992). One type of potassium channel found in chondrocytes is of high conductance (~ 200 pS) and the other of low

Table 3. Ion exchangers and co-transporter systems in chondrocytes.

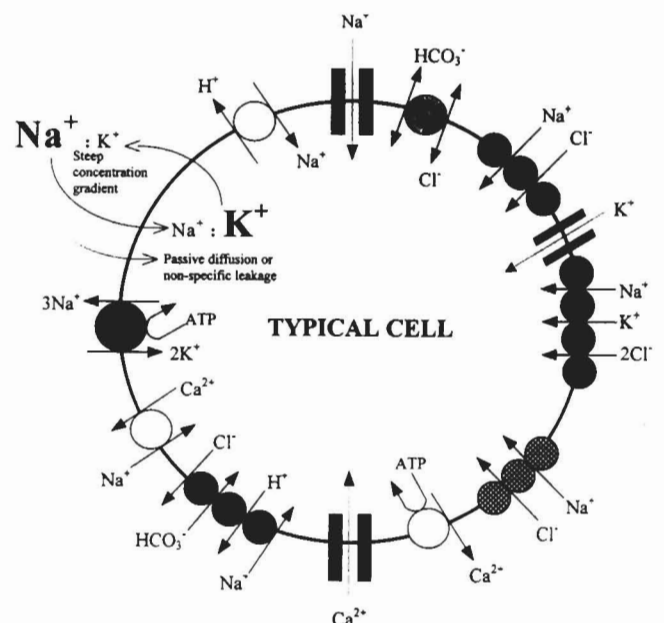
	PROPERTIES	REFERENCE
$\text{Na}^+ \times \text{H}^+$ exchange	Two isoforms, NHE1 and NHE3; NHE1 ubiquitous "housekeeping" NHE1 isoform abundantly expressed in chondrocytes, inhibited by amiloride, activated by cell shrinkage, stimulated by phosphorylation and growth factors; NHE3 is amiloride resistant, EIPA sensitive and growth factor inducible.	Dascalu et al., 1993; Wilkins et al., 1996; Hall, et al., 1996
$\text{Cl}^- \times \text{HCO}_3^-$ Band 3-like "anion" exchange	Does not participate in $\text{Cl}^- \times \text{HCO}_3^-$ and pH regulation (possibly modified for sulphate transport in chondrocytes); found mainly in intracellular membranes of the Golgi apparatus; specialized function as an "organellar" sulphate transporter for extracellular matrix synthesis.	Wilkins and Hall, 1992; Golding et al., 1997
$\text{Na}^+ / \text{K}^+ / 2\text{Cl}^-$ co-transport	Bumetanide sensitive, activated by cell shrinkage regulatory volume increase in isolated and <i>in situ</i> chondrocytes.	Errington and Hall, 1995; Errington et al., 1997

conductance (~20 pS). Patch clamp evidence suggests that a rise in intracellular calcium increases the open probability of potassium channels and elevates K^+ currents in chondrocytes. The open probability of the potassium channels and K^+ currents also increase after experimental elevation of extracellular $[Ca^{2+}]$ and treatment with the ionophore A23187. There is evidence for a Ca^{2+} entry pathway blocked by La^{3+} although it does not appear to be voltage-gated or -sensitive to verapamil or dihydropyridines (Grandolfo et al., 1992). In contrast, in growth plate chondrocytes voltage-sensitive calcium channels have been recently demonstrated (Zuscik et al., 1997). Characterization of this calcium channel using pharmacological inhibitors has revealed the possible involvement of N-type channels, ruling out the presence of L-type and T-type channel activities. There is no information on the subunit composition of this voltage-sensitive calcium channel.

However, since it has been shown to have N-type channel activity, it would be expected to consist of a large pore-forming α subunit (isoforms $\alpha 1B$ and $\alpha 2$), a regulatory β subunit and a smaller δ subunit (Catterall, 1995). Since growth plate chondrocytes are involved with the process of endochondral bone formation and calcification, N-type voltage-sensitive calcium channels may facilitate Ca^{2+} influx into chondrocytes to provide a source of this ion for Ca^{2+} signaling and matrix vesicle loading and subsequent matrix calcification (Zuscik et al., 1997). The collagen binding protein annexin V, which was originally called anchorin II, has been found to exhibit calcium channel activity in addition to collagen binding. Annexin V is abundantly expressed in the plasma membrane of chondrocytes and has been shown to play a major role in matrix vesicle-initiated cartilage calcification as a collagen-regulated calcium channel (von der Mark and Mollenhauer, 1997).



Principal pumps: 1) Na^+/K^+ -ATPase, inhibited by cardiac glycosides i.e. ouabain; isoforms $\alpha 1$ and $\alpha 3$ (Mobasheri et al., 1997a,b); 2) Ca^{2+} -ATPase, inhibited by vanadate, PMCA1 isoform. **Principal ion exchangers and transporters:** 1) $Na^+ \times H^+$ -exchange, inhibited by amiloride, EIPA; isoforms NHE1 and NHE3 (Wilkins et al., 1996; this study); 2) $Na^+/K^+/2Cl^-$ -co-transporter, inhibited by loop diuretics, bumetanide and furosemide (Errington and Hall, 1995, 1997); 3) other Na^+ -dependent transport mechanisms (i.e. Na^+ /glucose symport and Na^+/P_i symport) not yet identified. **Principal channels:** 1) Na^+ channels, TTX-sensitive, pressure induced depolarization, isoforms not yet characterized (Wright et al., 1992); 2) K^+ channels, high and low conductance calcium-activated (Grandolfo et al., 1992; Vittur et al., 1994); 3) Ca^{2+} channels, Lanthanum-sensitive, verapamil-insensitive Ca^{2+} entry (Grandolfo et al., 1990, 1992); 4) voltage-sensitive (N-type) calcium channels (Zuscik et al., 1997).



Principal pumps and transporters: 1) Na^+, K^+ -ATPase, inhibited by cardiac glycosides (ouabain, digoxin); 2) $Na^+ \times H^+$ exchange, inhibited by amiloride and its analogues (EIPA); 3) $Na^+/K^+/2Cl^-$ -co-transporter inhibited by loop diuretics, bumetanide and furosemide; 4) Na^+/Cl^- -co-transporter inhibited by bumetanide; 5) Na^+/Cl^- -co-transporter inhibited by thiazide; 6) $Na^+ \times Ca^{2+}$ -exchanger inhibited by La^{3+} ; 7) Na^+/HCO_3^- -co-transporter. **Principal channels:** 1) Na^+ channels; 2) K^+ channels; 3) Ca^{2+} channels; (Na^+/HCO_3^- -co-transporter, $Na^+ \times H^+$ exchange and also participate in pH_i regulation; for clarity, Na^+ /glucose symport has been excluded).

Fig. 9. Membrane transport processes identified in chondrocytes. The principal known ion channels and transport systems (pumps and exchangers) in chondrocytes are illustrated and compared with a typical cell.

Annexin V represents another calcium entry pathway in chondrocytes which may be involved in mechanotransduction. Interestingly, annexin V may have important pathophysiological roles. It has been shown to inhibit the procoagulant and proinflammatory activities of apoptotic cells (Reutelingsperger and van Heerde, 1997). Fluorescent annexin V binding assays are currently being used to detect apoptotic cells and distinguish between cellular apoptosis and necrosis (Zhang et al., 1997). In the early stages of apoptosis specific changes occur at the cell surface (Vermees et al., 1995). One of these plasma membrane alterations is the translocation of phosphatidylserine (PS) from the inner side of the plasma membrane to the outer layer, by which PS becomes exposed at the external surface of the cell. Annexin V has a high, natural affinity for PS and hence it can be used as a sensitive probe for PS exposure upon the cell membrane. Consequently, Annexin V assays offer the possibility of detecting early phases of apoptosis in cartilage before the loss of membrane integrity and permits measurements of the kinetics of apoptotic death in relation to the cell cycle.

Discussion

There is limited information on the fundamental aspects of cartilage cell physiology in which roles of ion transport mechanisms must be important in maintaining tissue integrity. In this review some of the known membrane transport systems of chondrocytes have been discussed in the context of their physiological importance in cartilage.

Sodium is the most abundant monovalent cation in cartilage and there appear to be at least four pathways for Na^+ entry into chondrocytes. The ion channels, co-transporters and exchangers involved are summarized in Figure 9. There are two isoforms of $\text{Na}^+ \times \text{H}^+$ exchange expressed in chondrocytes, namely NHE1 and NHE3 (Wilkins et al., 1996). They are involved in Na^+ -dependent pH regulation and their density is relatively high, particularly that of NHE1 as judged by the dense and patchy immunofluorescence staining of NHE1. At least one form of the bumetanide sensitive $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ -co-transporter is expressed in chondrocytes (Errington and Hall, 1995; Errington et al., 1997). This transporter is involved in the process of regulatory volume increase as incubation of chondrocytes with bumetanide (a specific inhibitor of the $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ -co-transporter) inhibits this volume regulation. The $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ -co-transporter acts as a major Na^+ entry mechanism in chondrocytes (along with K^+ and Cl^-) particularly following the hypertonic shock effect of increasing extracellular $[\text{Na}^+]$. The $\text{Na}^+ \times \text{Ca}^{2+}$ exchange does not appear to be expressed in chondrocytes and thus it is unlikely to serve as a Na^+ entry mechanism. Tetrodotoxin sensitive Na^+ channels have been reported in chondrocytes (Wright et al., 1992) and together with very low passive, non-specific Na^+ leakage (Stein, 1990) account for the remaining Na^+ entry pathways. The Na^+ ,

K^+ -ATPase catalyses the extrusion of the Na^+ ions that gain access to the chondrocyte via the mechanisms described above and help to maintain the necessarily low physiological $\text{Na}^+:\text{K}^+$ ratio required for cell function.

The Ca^{2+} -ATPase (PMCA1 isoform; Shull and Greeb, 1988a,b) has been identified in chondrocytes. In the absence of a $\text{Na}^+ \times \text{Ca}^{2+}$ -exchanger, this Ca^{2+} -ATPase most likely represents the major mechanism for Ca^{2+} extrusion from chondrocytes. The presence of the AE2 isoform of anion exchange in the plasma membrane of chondrocytes has been confirmed by the RT-PCR, Western analysis and immunofluorescence confocal microscopy (Golding et al., 1997). Its predominant subcellular localization suggests that it may be engaged in "organellar" sulfate transport (Ruetz et al., 1993; Sekler et al., 1995) and its presence in lower quantities in the plasma membrane may account for the apparent lack of $\text{Cl}^- \times \text{HCO}_3^-$ activity in chondrocytes (Wilkins and Hall, 1992). In the plasma membrane AE2 may function as a modified sulphate transporter in parallel with DDST (diastrophic dysplasia sulfate transporter; Rossi et al., 1996).

Changes to the physical and chemical environment of cartilage routinely occur during static and dynamic joint loading as fluid is expressed from the matrix. These events alter the ionic composition of cartilage matrix, which in turn alters the intracellular composition of ions and activates plasma membrane transport systems. The activation of these systems is the chondrocyte's homeostatic response to the altered ionic environment that if left unchallenged may result in deleterious alterations in matrix biosynthesis. Drastic alterations to the usual ionic and osmotic environment generally tend to depress matrix synthesis (Urban and Hall, 1992). Therefore, it is possible to hypothesize that during the initial stages of osteoarthritis (where cartilage swells and proteoglycans are lost from the matrix of compromised cartilage) the ionic and osmotic environment of the chondrocyte is altered resulting in a modulation of matrix metabolism. During the initial pathological stages chondrocytes may be able to keep up with the ionic and osmotic changes to their environment by adaptive alterations to the density and pattern of ion transporter isoform distribution thus maintaining their ionic homeostasis. However, in the long-term sustained and progressive changes to the matrix and the ionic environment may not be tolerated by chondrocytes, which by that stage may be unable to initiate matrix repair mechanisms. With the identification of major ion transport pathways in chondrocytes from normal cartilage, it remains to be seen if the density and isoform distribution of transporters will change in osteoarthritic and rheumatoid cartilage. The information thus gained may contribute to the basic understanding of cartilage pathology and enhance the approach for pharmacological intervention.

In summary, there remains much work that needs to be done to build a complete picture of the ion transport mechanisms present in chondrocytes. Furthermore, these

transport mechanisms may not be identical in chondrocytes from the various zones of cartilage or indeed different types of cartilage (articular, hyaline, calcified). The work done thus far indicates that a range of different transport mechanisms are expressed by chondrocytes that are well placed to take advantage of the unique ionic make-up of cartilage and maintain the physiology of chondrocytes but whose particular characteristics warrant further investigation.

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