

β 1-integrins co-localize with Na, K-ATPase, epithelial sodium channels (ENaC) and voltage activated calcium channels (VACC) in mechanoreceptor complexes of mouse limb-bud chondrocytes

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Summary. Interactions between chondrocytes and their extracellular matrix are partly mediated by β 1-integrin receptors. Recent studies have shown that β 1-integrins co-localize with a variety of cytoskeletal complexes, signaling proteins and growth factor receptors. Since mechanosensitive ion channels and integrins have been proposed to participate in skeletal mechanotransduction, in this study, we investigated the possible co-localization of β 1-integrins with two ion channels and a P-type ATPase in mouse limb-bud chondrocytes. The α subunits of Na, K-ATPase, the epithelial sodium channel (ENaC) and the voltage activated calcium channel (VACC) were immunostained in organoid cultures derived from limb-buds of 12-day-old mice using well-characterized antibodies. Indirect immunofluorescence revealed abundant expression of β 1-integrins and each of the selected systems in limb-bud chondrocytes. Two-fluorochrome immunostaining demonstrated that β 1-integrin, Na, K-ATPase, ENaC and VACC co-localize in chondrocytes. Co-immunoprecipitation experiments revealed co-localization and association of integrins with ENaC, VACC and Na, K-ATPase. Cellular responses and signaling cascades initiated by the influx of calcium or sodium through putative mechanosensitive channels may be regulated more effectively if such channels were organized around integrins with receptors, kinases and cytoskeletal complexes clustered about them. The close proximity of ATPase ion pumps such as Na, K-ATPase to chondrocyte mechanoreceptor complexes could facilitate rapid homeostatic responses to the ionic perturbations brought about by activation of mechanically gated cation channels and efficiently regulate the intracellular milieu of chondrocytes.

Key words: β 1-integrin, Na, K-ATPase, ENaC, voltage activated calcium channel (VACC), chondrocyte mechanoreceptor complex, co-localization

Introduction

Molecular interactions between chondrocytes and extracellular matrix (ECM) proteins such as type II collagen and fibronectin influence the proliferation and phenotypic differentiation of the cells and morphogenesis of cartilage tissue (von der Mark et al., 1977). Integrins are cell-surface receptors that mediate cell attachment to ECM components and act as molecular conduits for inside-out and outside-in signal transduction in focal adhesion contact sites (Ruoslahti and Pierschbacher, 1987). The ECM in articular cartilage is not only a mechanical framework, but is also important for chondrocyte differentiation, phenotypic stabilization and promotion of chondrocyte survival in the matrix. The integrin-mediated adhesion of chondrocytes to the ECM prevents apoptosis and is important for the continued survival of these cells (Hirsch et al., 1997). Integrins are heterodimers of specific combinations of α and β subunits that form cell attachment and mechanoreceptor complexes (Wang et al., 1993; Ingber, 1997; Svoboda, 1998). Interactions between the ECM and chondrocytes are partly mediated by the β 1-integrin subfamily of receptors; integrin-mediated adhesion of chondrocytes to type II collagen leads to phosphorylation of cytoskeletal and signaling proteins in focal adhesion sites (Shakibaei, 1995; Shakibaei et al., 1999). Interactions of plasma membrane integrins, associated proteins in mechanoreceptor complexes and subcellular signaling components are an important component of the mechanotransduction pathway (Mobasher and Martín-Vasallo, 1999; Lee et al., 2000; Salter et al., 2001; Shakibaei and Mobasher, 2001; Mobasher et al., 2002a).

It is becoming increasingly apparent that integrins are not passive cell adhesion molecules solely concerned with linking the ECM and the cellular cytoskeleton; integrins also collaborate with a variety of extracellular receptors involved in intracellular biochemical signaling pathways and proteolytic enzymes concerned with cartilage degradation in the ECM. Recent studies have suggested that integrins co-localize with a variety of proteins in chondrocytes including the insulin-like growth factor-I receptor (Shakibaei et al., 1999), caveolins (Schwab et al., 2000), matrix metalloproteases (Schulze-Tanzil et al., 2001) and urokinase-type plasminogen activator receptor (Schwab et al., 2001). Integrins have long been proposed as components of mechanosensory focal adhesion complexes containing calcium-selective ion channels that are involved in transduction of mechanical stimuli in bone, cartilage and other cell types (Pickard, 1994; Duncan, 1995; Salter et al., 1997). There is evidence from other cell systems that integrins (particularly α 5 β 1 integrin), integrin-linked tyrosine kinases and focal adhesion proteins such as focal adhesion kinase (FAK) regulate L-type calcium channel activity (Wu et al., 2001). Calcium currents mediated by L-type calcium channels have been shown to be integrin-dependent (Chan et al., 2001) and integrins directly modulate the release of calcium from intracellular stores and trigger cytoplasmic calcium waves that propagate throughout the cells bringing about changes in gene expression. It has been proposed that such interactions may represent a novel mechanism for control of calcium influx in vascular smooth muscle and other cell types (Wu et al., 1998, 2001).

Collaboration between integrins, ion channels and ion pumps in chondrocytes may be a pre-requisite event preceding mechanotransduction, cytoskeletal reorganization and more fundamental activities such as chondrocyte differentiation and survival. Since stretch-activated ion channels and transporters have been proposed to play a role in mechanotransduction in chondrocytes (Mobasheri et al., 2002a,b), in this study, we investigated the possible co-localization of integrins with two ion channels and a P-type ATPase pump that have been previously identified in chondrocytes (Mobasheri et al., 1998; Trujillo et al., 1999a,b). A preliminary form of this paper has been published in abstract form (Shakibaei and Mobasheri, 2001).

Materials and methods

Antibodies

Pan antibody against the α subunit of the voltage activated calcium channel (VACC) was purchased from Alomone Labs (Jerusalem, Israel). This polyclonal antiserum which recognizes all α_1 subunits was raised against a purified peptide (C)DNFDYLTRDWSILGPHHLD corresponding to residues 1382-1400 of the α_{1S} subunit of VACC (CP¹³⁸²⁻¹⁴⁰⁰) and affinity purified by column chromatography using immobilized CP¹³⁸²⁻¹⁴⁰⁰

(Striessnig et al., 1990a,b; Westenbroek et al., 1992a,b). The polyclonal antibody against the α subunit of the epithelial sodium channel (ENaC) was a gift from Dr. C.M. Canessa (Department of Cellular and Molecular Physiology, Yale University, New Haven, USA) (Duc et al., 1994). This antibody has been used successfully in previous studies to detect α ENaC expression in human chondrocytes and human osteoblasts (Trujillo et al., 1999b; Mobasheri and Martín-Vasallo, 1999; Mobasheri et al., 2001). The antibodies to Na, K-ATPase (monoclonal antibodies α 6F and α 5) were gifts from Dr. D.M. Fambrough (Johns Hopkins University, Baltimore, USA) (Takeyasu et al., 1988; Lebovitz et al., 1989). The antibodies α 5 and α 6F to Na,K-ATPase were also purchased from the Developmental Studies Hybridoma Bank (DSHB <http://www.uiowa.edu/~dshbwww/>) which is under the auspices of the NICHD and is maintained by The University of Iowa, Department of Biological Sciences, Iowa City, IA 52242, USA. A polyclonal antibody to β 1-integrin was obtained from Chemicon International (Temecula, CA, USA). A monoclonal antibody to β 1-integrin was obtained from Coulter (Hialeah, Florida, USA). Texas red-coupled goat anti-rabbit IgG and FITC-coupled goat anti-mouse IgG were obtained from Dianova (Hamburg, Germany). Secondary antibody conjugated to alkaline phosphatase was purchased from Boehringer Mannheim (Germany).

Cartilage organoid culture

A detailed description of the culture technique for the cartilage culture used for the following experiments has been published by Zimmermann et al. (1992) and Shakibaei et al. (1993). Upper and lower limb-buds of 12-day-old mouse embryos were excised, rinsed with Hanks' solution and shaken for 20 min in 0.2% dispase (Boehringer Mannheim, Mannheim, Germany) in Ca^{2+} - and Mg^{2+} -free solution. After the addition of medium, the suspension was homogenized by pipetting and separated from non-dissociated tissue fragments by filtration through a nylon mesh with a pore diameter of 20 μm . For sedimentation of the cells, the suspension was centrifuged at 6000g. Aliquots of 10 μl of this cell suspension (cell density: approximately 2×10^6 cells) were placed onto a membrane filter at the medium/air interface. The cells were grown in an incubator under 5% CO_2 at 37 °C. The medium (Ham's F-12/DMEM: 50/50, 10% FCS, 1% amino acids, 1% L-glutamine, 50 units/ml streptomycin, 50 units/ml penicillin, and 2.5 $\mu\text{g/ml}$ amphotericin B, 25 $\mu\text{g/ml}$ ascorbic acid) was changed every 3 days.

Transmission electron microscopy

Cultures were fixed in 1% glutaraldehyde plus 1% tannic acid in 0.1 M phosphate buffer, pH 7.4, and post-fixed in 1% OsO_4 in phosphate buffer. After rinsing and dehydration in ethanol, the preparations were embedded in Epon (Plano, Marburg, Germany) and cut with an

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Ultracut E ultramicrotome (Reichert) and then the sections were stained with 2% uranyl acetate/lead citrate. The specimens were examined with a transmission electron microscope (Zeiss EM 10).

Immunoprecipitation

Immunoprecipitation was carried out as previously described (Shakibaei et al., 1999). Briefly, organoid cultures were extracted via lysis buffer (50 mM Tris-HCl pH 7.2, 150 mM NaCl, 1% Triton X-100, 1 mM sodium orthovanadate, 50 mM sodium pyrophosphate, 100 mM sodium fluoride, 0.01% aprotinin, 4 μg/ml pepstatin A, 1 μg/ml leupeptin, 1 mM phenylmethyl-sulphonylfluoride (PMSF) on ice for 30 min. The cell extract was centrifuged at 13000 rpm for 30 min and cell debris was removed. Prior to immunoprecipitation, the samples were first pre-cleared by incubation with 25 μl normal rabbit IgG and *Staphylococcus aureus* (*S. aureus*) cells (Sigma, Munich, Germany). Samples were incubated with primary antibodies against β1 integrins (antibodies were diluted 1:100 in a wash buffer consisting of 0.1% Tween 20, 150 mM NaCl, 50 mM TRIS-HCl pH 7.2, 1 mM CaCl₂, 1 mM MgCl₂, 1 mM PMSF) for 2 h at 4 °C, followed by *S. aureus* cells, and the suspensions were shaken for 1 h at 4 °C. Control immunoprecipitations were performed by incubating the samples with normal (non-immune) rabbit IgG-serum alone. *S. aureus* cells were washed 5 times with wash buffer and once with 50 mM TRIS-HCl pH 7.2. To examine whether integrins can interact with αNa, K-ATPase, αENaC and the α subunit of VACC in chondrocytes, co-immunoprecipitation assays were performed. After immunoprecipitation with anti-β1 integrin antibodies, the samples were probed by immunoblotting with antibodies to αNa, K-ATPase, αENaC and αVACC.

Western blot analysis

Samples were separated by SDS-PAGE (7.5% and 10% gels) under reducing conditions. Proteins were transferred onto nitrocellulose, blocked with 5% (w/v) skimmed milk powder in PBS/0.1% Tween 20 overnight at 4 °C and incubated with primary antibodies (anti-αNa, K-ATPase, α subunit of VACC and αENaC) diluted in blocking buffer for 1 h at RT. Membranes were washed in blocking buffer and then incubated with alkaline phosphatase-conjugated secondary antibody diluted in blocking buffer for 30 min at RT. After that membranes were washed in blocking buffer and finally in 0.1 M TRIS, pH 9.5, containing 0.05 M MgCl₂, and 0.1M NaCl. Specific binding was detected using nitroblue tetrazolium and 5-bromo-4-chloro-3-indoylphosphate (p-toluidine salt; Pierce, Rockford, USA) as substrates. Protein determination was performed using the bicinchoninic acid system (Pierce, Rockford, USA).

Immunofluorescence

The seven-day-old organoid cultures were immersed

in O.C.T. embedding material (Bayer, Munich, Germany) and immediately frozen in liquid nitrogen. For double immunolabelling 8 μm-thick sections were fixed in methanol and rinsed in PBS for 3x5 min. Sections probed with monoclonal antibody to Na, K-ATPase were also probed with polyclonal antibodies to β1-integrin. Cryosections exposed to polyclonal antibodies to ENaC and VACC were co-incubated with monoclonal antibody to β1-integrin. Incubation with the primary antibodies (anti-αNa, K-ATPase, anti-αENaC and anti-αVACC antibodies, diluted 1:50 in PBS) was for 1 h at RT followed by secondary antibody (either goat-anti-rabbit or goat-anti-mouse immunoglobulin conjugated to FITC or TRITC, diluted 1:50 in PBS) for 1 h at RT. Washing with PBS followed each step and the sections were examined under a fluorescence microscope (Axiophot 100, Zeiss, Germany).

Results

Transmission electron microscopy

Mesenchymal cells isolated from limb-buds of mouse embryos cultivated in organoid culture initially aggregated into blastema-like units. After three days, a cartilage-specific matrix developed in these blastemata. After a culture period of 3-7 days, typical cartilage nodules of variable size could be found. The chondrocytes had oval shapes and produced an extracellular matrix (Fig. 1). The cell membranes exhibited numerous small projections into the extracellular matrix and were in direct contact with the surrounding matrix. The cytoplasm contained a well-developed rough endoplasmic reticulum, a large Golgi apparatus and a large round nucleus (Zimmermann et al., 1992; Shakibaei et al., 1993, 1995).

Integrins co-immunoprecipitate with Na, K-ATPase, ENaC and VACC

To examine a possible association of β1-integrins with Na, K-ATPase, ENaC and VACC in chondrocytes, we performed co-immunoprecipitation assays in the organoid cultures. Immunoprecipitation of cell lysates with antibodies raised against β1-integrin followed by immunoblotting with antibodies to the selected transport systems demonstrated whether integrins co-localized with any of them. The cells were lysed, cleared and immunoprecipitated with antibodies to β1-integrin. The β1-integrin immunoprecipitates were then immunoblotted with antibodies to the α subunits of Na, K-ATPase, ENaC and VACC (using pan α antibodies). Immunoprecipitation experiments provided evidence for co-localization and association of β1-integrins with the α subunits of ENaC, VACC and Na, K-ATPase (Fig. 2). We deliberately used pan antibodies capable of detecting all the known α subunits of these three membrane proteins in the fully denatured forms in SDS gels. There was no evidence of non-specific immunoreactivity in the Western blots of β1 integrin immunoprecipitates and no

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proteolytic degradation of protein bands occurred. A 110 kDa protein corresponding to the α subunit of Na, K-ATPase was found in β 1-integrin immunoprecipitates but not in control immunoprecipitates (Fig. 2A). A highly glycosylated (90 kDa) α ENaC subunit also co-immunoprecipitated with β 1-integrin (Fig. 2B). A large molecular weight protein migrating around 195-200 kDa corresponding to the α subunit of VACC was also found in β 1-integrin immunoprecipitates (Fig. 2C). This protein was not detectable in control immunoprecipitates. Immunoprecipitation with control antibody (non-immune antiserum) did not produce any specific bands in control experiments (lanes labelled C in Fig. 2). Taken together, these findings demonstrate that Na, K-ATPase, ENaC and VACC associate with β 1-integrin adhesion complexes in the plasma membrane of mouse limb-bud chondrocytes.

β 1-integrin co-localizes with Na, K-ATPase, ENaC and VACC in organoid cultures

To demonstrate the expression and co-localization of β 1-integrin with the transport systems, we used double immunofluorescence (indirect method) using FITC conjugated secondary antibodies to localize β 1-integrin immunostaining and TRITC conjugated secondary antibodies to localize the α subunits of Na, K-ATPase, ENaC and VACC. Immunofluorescence microscopy revealed cell-specific expression of β 1-integrin in all organoid culture cells (Fig. 3). The α subunits of Na, K-ATPase, ENaC and VACC were also abundantly expressed in mouse limb-bud chondrocytes (Fig. 3). Both antigens produced similar immunostaining patterns when examined under low and high power magnifications. Most of the immunostaining observed with antibodies to the transport systems was on the cell surface; there was some intracellular, cytoplasmic

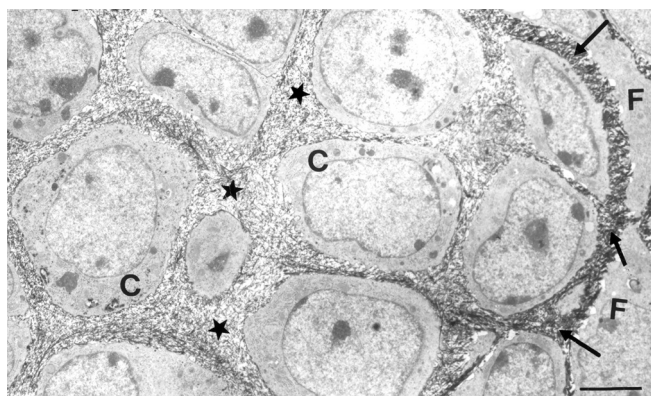
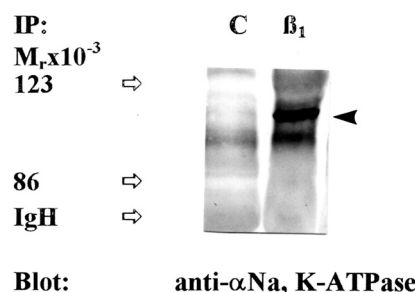


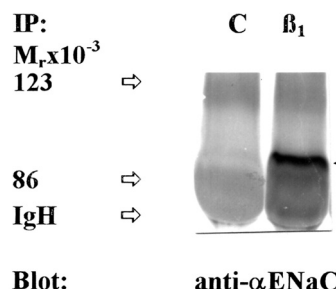
Fig. 1. Electron micrograph of high-density organoid culture from mouse limb bud mesenchymal cells after a cultivation period of 7 days. The transitional zone of perichondrium/cartilage is shown. The flat fibroblast-like cells (F) of the perichondrium are embedded in a dense mass of thick collagen fibrils (as indicated by arrows). Typical chondrocytes (C) are embedded in the cartilage matrix (as indicated by stars). Bar: 2 μ m

staining which is typically seen in these cells with subunits of Na, K-ATPase and ENaC (Mobasheri, 1999; Mobasheri and Martín-Vasallo, 1999). There was no non-specific immunostaining in the ECM (indicated by

A.



B.



C.

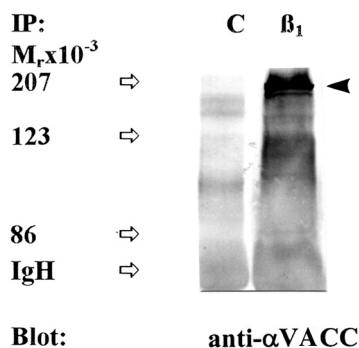


Fig. 2. Co-immunoprecipitation assays and immunoblotting of β 1-integrin, α Na, K-ATPase, α ENaC and α VACC. **A.** Immunoprecipitation with anti β 1-integrin antibody followed by immunoblotting with anti- α Na, K-ATPase antibody. **B.** Immunoprecipitation with anti β 1-integrin antibody followed by immunoblotting with anti- α ENaC antibody. **C.** Immunoprecipitation with anti β 1-integrin antibody followed by immunoblotting with anti- α VACC antibody. In each panel the left hand Western blot lanes labeled C indicate control immunoprecipitates using non-immune control anti-rabbit immunoglobulin antibody. Visualization was by nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate (p-toluidine salt). Molecular weight markers are indicated in kilodaltons.

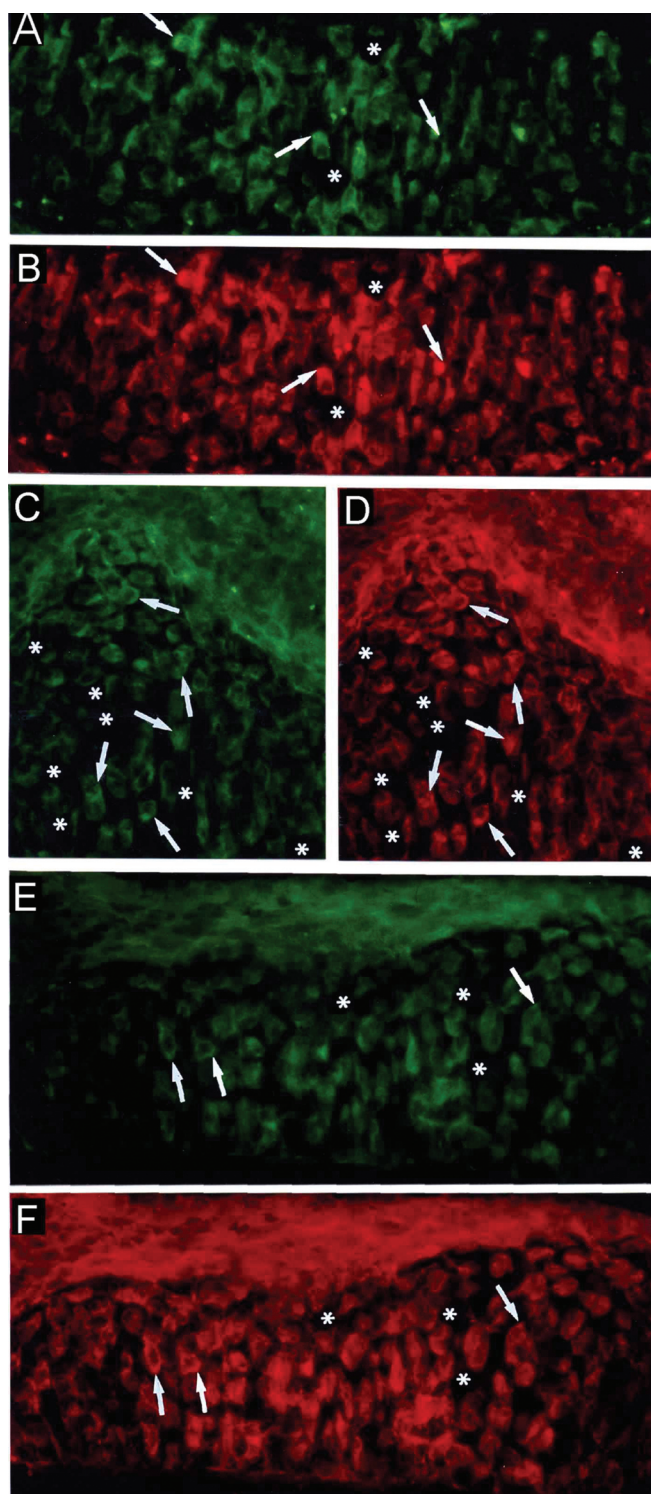


Fig. 3. Immunofluorescence micrographs demonstrating the expression and co-localization of β 1-integrin (**A**, **C** and **E**) and the α subunits of Na, K-ATPase (**B**), ENaC (**D**) and VACC (**F**) in mouse limb-bud chondrocytes grown in organoid culture. Note the high specificity of the cellular immunostaining observed in each case (confined to chondrocytes as indicated by arrows) and the lack of immunostaining in the ECM (indicated by asterisks). Original magnification $\times 100$

asterisks in Fig. 3). Two-fluorochrome immunostaining revealed positive correlation between β 1-integrins, VACC and Na, K-ATPase expression. ENaC and β 1-integrins were also found to co-localize on the cell surface and non-specific staining was not observed in the ECM.

Discussion

The major results of this study indicate that β 1-integrins in chondrocyte adhesion complexes co-localize with two ion channels that have recently been proposed to function as mechanosensitive components of chondrocyte mechanoreceptors (Mobasheri et al., 2002a) and a P-type ion transporting ATPase pump responsible for maintaining intracellular Na^+ and K^+ concentrations and cell homeostasis (Mobasheri et al., 1997a,b). This conclusion is based on the following experimental observations: (i) Indirect immunofluorescence revealed abundant expression of β 1-integrins and all the selected transport systems in mouse limb-bud chondrocytes. Previous observations in bovine chondrocytes suggest an abundance of Na, K-ATPase (Mobasheri et al., 1997a,b, 1998; Mobasheri, 1999) and studies in human chondrocytes have confirmed Na, K-ATPase and α ENaC expression (Trujillo et al., 1999a,b). Although there is evidence for VACC activity in chondrocytes, (N-/L-type voltage activated calcium channels; Guilak et al., 1999; Wang et al., 2000a) this is the first time the expression of the α subunit of the VACC has been convincingly demonstrated by immunochemical methods. (ii) Two fluorochrome immunostaining showed co-localization of β 1-integrins and the three selected transport systems in mouse limb-bud chondrocytes in organoid culture. (iii) Furthermore, we could also demonstrate that in chondrocytes, β 1-integrin co-immunoprecipitates with the α subunits of Na, K-ATPase, ENaC and VACC. This is, to our knowledge, the first description that integrins and ion channels or ion pumps are part of the same complexes in chondrocytes.

For non-innervated mechanosensitive cells such as chondrocytes, mechanotransduction may be resolved into several distinct phases: 1) mechano-coupling, the characteristics of the mechanical force applied to the cell via the ECM, 2) biochemical coupling, or the mechanism through which the mechanical signal is transduced into a subcellular biochemical signal, 3) transmission of signal to the nucleus of the effector cell and 4) the effector cell response (i.e. a change in gene expression; Duncan, 1995). In previous studies of mechanotransduction in chondrocytes and fibroblasts it has been demonstrated that Na^+ ions first enter the cells via stretch activated Na^+ channels (possibly through ENaC or voltage activated sodium channels or VASC), resulting in depolarization of the membrane, which is then followed by an elevation in intracellular Ca^{2+} causing hyperpolarization of the membrane (Wright et al., 1996). This opens Ca^{2+} activated K^+ channels resulting in K^+ efflux, in an effort to return the

membrane potential to its original resting value. The inward Na^+ currents mediated by ENaC and/or VACC depolarize the chondrocyte membrane thus beginning a sequence of channel opening events (i.e. VACC, followed by Ca^{2+} activated K^+ channels) elevating intracellular Ca^{2+} and culminating in the propagation of calcium waves that activate subcellular signaling cascades. It has recently been demonstrated that integrins are involved in the secretion of interleukin-4, which acts in an autocrine manner via cytokine receptors and is also involved in the hyperpolarization of the membrane in the chondrocyte mechanotransduction pathway (Millward-Sadler et al., 1999). It has also been suggested that the mechanotransduction pathway is defective in osteoarthritis and abnormalities in such "chondroprotective mechanotransduction pathways" in osteoarthritis may contribute to disease progression (Salter et al., 2001).

The mechanical tension required for activating ion channels in the plasma membrane is most likely transmitted via mechanoreceptor sites containing β 1-integrin, with physical and functional links to the ECM and the cytoskeleton. Functionally, leverage exerted against such adhesion sites will control ion channels in the surrounding fluid mosaic. Reactions initiated by passage of ions through the mechanosensitive channels may be more effectively regulated if such channels were clustered around the mechanoreceptor complexes and if appropriate receptors, kinases, ion pumps, and some key cytoskeletal anchoring sites were in turn clustered about them (Pickard, 1994). Thus the activity of clusters of

mechanosensitive channels may contribute to control of cytoskeletal architecture and of signaling proteins within their domain. Furthermore, a variety of regulatory proteins and components of the chondrocyte cytoskeleton may contribute to control of mechanosensitive channel activity.

Based on the results presented here, the arguments presented by Pickard (1994) and our own hypothetical considerations we propose a chondrocyte mechanoreceptor model, which incorporates ECM macromolecules (collagen type II), β 1-integrins, ENaC and VACC as putative mechanosensitive ion channels (Fig. 4). These ion channels also co-localize with Na, K-ATPase and with β 1-integrins in the chondrocyte plasma membrane and it is highly likely that the mechanosensitive receptors formed by these proteins functionally interact with the cytoskeleton as they do in other cell types, regulating subcellular signal transduction pathways that allow chondrocytes to perceive their physical environment in the ECM (Wang et al., 2001). Needless to say that in addition to mechanoreception, these multi-functional complexes may also be responsible for generating subcellular signals that maintain the chondrocyte phenotype and prevent chondrocyte apoptosis (Shakibaei et al., 1999, 2001).

There is evidence for association between integrins, focal adhesion proteins and ion channels in other cell types (Kwon et al., 2000; Wang et al., 2000b; Chan et al., 2001; Wu et al., 2001). Furthermore, there are indications that β 1-integrins co-localize with G-protein activated inward rectifier potassium (GIRK) channels (McPhee et al., 1998; Ivanina et al., 2000) and voltage activated potassium channels (Kv1.3) (Levite et al., 2000). In T-lymphocytes Kv1.3 channels and β 1-integrins co-immunoprecipitate suggesting that their physical association underlies their functional cooperation (Levite et al., 2000). However, this is the first time β 1-integrins have been shown to co-localize with an ion channel (VACC, ENaC) or ion pump (Na, K-ATPase) in chondrocytes. Therefore, it is possible that in chondrocytes Na, K-ATPase, ENaC and VACC and other ion transport systems may be functionally and physically linked, directly or via hitherto unidentified accessory or linking proteins, to mechanoreceptor complexes in the chondrocyte membrane containing β 1-integrins associated with ECM components such as collagen type II and the intracellular cytoskeleton. There is one report that suggests the α subunit of Na, K-ATPase is a molecular component of an exocytic pathway for basic fibroblast growth factor (FGF-2) (Florkiewicz et al., 1998) and there are experimental papers that suggest the β 2 subunit of Na, K-ATPase functions as a cell adhesion molecule in glia (Antonicek et al., 1987; Pagliusi et al., 1989). We have previously demonstrated that the β 2 subunit of Na, K-ATPase is expressed in chondrocytes (Mobasher et al., 1997b; Trujillo et al., 1999a). However, there are no reports that experimentally demonstrate an adhesive function for the β 2 subunit in

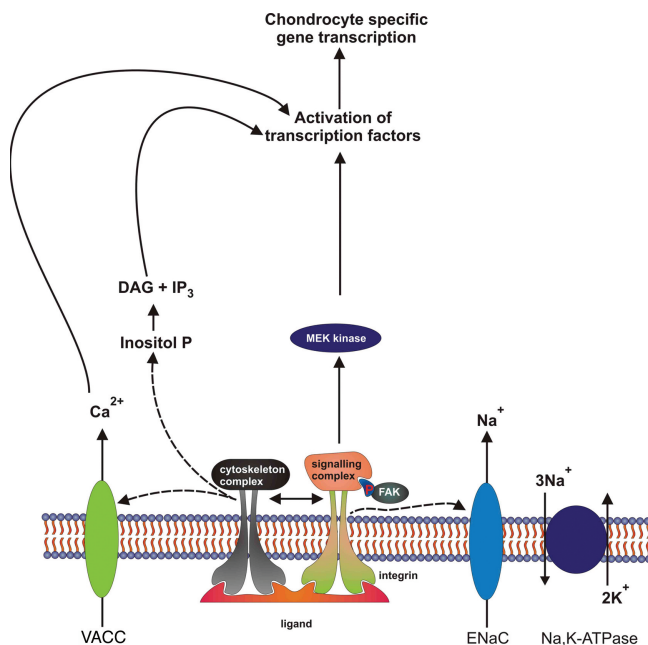


Fig. 4. Proposed model of chondrocyte β 1-integrin/mechanoreceptor complex which implicates the VACC and ENaC ion channels in the chondrocyte mechanotransduction pathway.

β 1-integrins co-localize with ENaC, VACC and Na, K-ATPase

chondrocytes or its participation in any molecular complex or export function in these cells (Mobasheri et al., 2000). It is tempting to speculate that co-localization of these ion transporters with β 1-integrins in close proximity to ECM proteins permits integrins to modulate these systems following mechanical loading, more specifically deformation of the pericellular matrix. Since deformation induced Na^+ and Ca^{2+} currents have been observed in chondrocytes and osteoblasts following stimulation by mechanical load (Sugimoto et al., 1996; Wright et al., 1996; Kizer et al., 1997), the expression and integrin co-localization of VACC and ENaC in chondrocytes makes them ideal candidates as mechanosensitive ion channels. Collaboration between integrins, growth factor receptors, ion channels and ion transporters in chondrocytes may be a pre-requisite event preceding mechanotransduction, cytoskeletal reorganization and more fundamental activities such as chondrocyte differentiation and survival (Mobasheri and Martín-Vasallo, 1999; Mobasheri, 2002; Mobasheri et al., 2002a). Indeed, cellular responses initiated by calcium or sodium influx through putative mechanosensitive channels may be regulated more effectively if such cation channels were organized around integrins with appropriate receptors, kinases, ion pumps and cytoskeletal complexes clustered in the vicinity (Pickard, 1994). Such structural organization would efficiently streamline the downstream signaling pathways by activated ion channels. The exact sequence of events from integrin activation to mechanosensitive ion channel regulation is unknown. Future studies are required to investigate the role of integrin-mediated signaling cascades in the activity and cell surface expression of ion channels (i.e. ENaC, VACC), ATPase ion pumps (i.e. Na, K-ATPase) and other plasma membrane proteins (Mobasheri et al., 2002b) in normal and degenerate articular cartilage.

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