Intercellular calcium signalling between chondrocytes and synovial cells in co-culture

Paola D'ANDREA¹, Alessandra CALABRESE and Micaela GRANDOLFO

Dipartimento di Biochimica, Biofisica e Chimica delle Macromolecole, Università di Trieste, via Licio Giorgieri 1, I-34127 Trieste, Italy

Intercellular communication allows the co-ordination of cell metabolism between tissues as well as sensitivity to extracellular stimuli. Paracrine stimulation and cell-to-cell coupling through gap junctions induce the formation of complex cellular networks that favour the intercellular exchange of nutrients and second messengers. Heterologous intercellular communication was studied in co-cultures of articular chondrocytes and HIG-82 synovial cells by measuring mechanically induced cytosolic changes in Ca^{2+} ion levels by digital fluorescence video imaging. In confluent co-cultures, mechanical stimulation induced intercellular Ca^{2+} waves that propagated to both cell types with

similar kinetics. Intercellular wave spreading was inhibited by 18 α -glycyrrhetic acid and by treatments inhibiting the activation of purinoreceptors, suggesting that intercellular signalling between these two cell types occurs both through gap junctions and ATP-mediated paracrine stimulation. In rheumatoid arthritis the formation of the synovial pannus induces structural changes at the chondrosynovial junction, where chondrocyte and synovial cells come into close apposition: these results provide the first evidence for direct intercellular communication between these two cell types.

INTRODUCTION

Intercellular communication confers on tissues the ability to co-ordinate several cellular functions, such as the regulation of cell volume, the intracellular ionic composition and cell metabolism [1]. Cell-to-cell communication through gap junctions represents the pathway for direct intercellular exchange of small cytosolic constituents that diffuse through the intercellular channels composed of the multimeric assembly of connexins, a family of closely related proteins. Besides allowing homologous communication between the same cells in a tissue, gap junctions can couple also different cell types [2,3] owing to the molecular compatibility of the connexins expressed [4]. In some cases, such as in neuroglial cells, heterologous communication results in an asymmetrical coupling that allows a unidirectional flux of metabolites, suggesting the potential for a hierarchy of command between connected cells [5].

An alternative pathway supporting intercellular communication is provided by ATP secretion activating nucleotide receptors on target cells [6,7]. In hepatocytes both gap junctions [8] and paracrine stimulation [7] can support cell-to-cell signalling, suggesting that the two mechanisms might co-operate in controlling tissue homeostasis and in co-ordinating cell responses against changes in the extracellular environment.

Homologous and heterologous intercellular communication is critically involved in the development of cartilage during differentiation [9]; in adult articular cartilage, in contrast, chondrocytes exist as individual cells embedded in the extracellular matrix, and direct cell-to-cell communication is normally absent. Chondrocytes extracted from adult articular cartilage, however, retain the ability to form functional gap junctions in culture, as demonstrated by the expression of connexin 43 [10] and by the ability to develop co-ordinated pathways of intercellular Ca^{2+} oscillations and waves when stimulated by different means [11,12].

Some articular pathologies, such as rheumatoid arthritis, are known to induce an unscheduled remodelling of cartilage and the degradation of extracellular matrix, thus allowing chondrocytes

to interact directly with the infiltrating inflammatory cells [13]. With the formation of synovial pannus, which accompanies the advanced stages of disease, proliferating synovial cells penetrate the cartilage, thereby increasing the rate of tissue degradation [13]. The role of cadherins in the attachment of synovial cells to articular chondrocytes has been recently elucidated [14,15]; given the permissive role played by cadherins in the establishment of gap junction-mediated communication [16], the possibility that heterologous gap junctions might be functionally expressed at some stages of pannus formation was intriguing.

We have recently observed that in chondrocytes and HIG-82 synovial cells, mechanical stimulation induced intercellular Ca^{2+} waves [16a] that were inhibited by 18 α -glycyrrhetic acid (18-GA), an inhibitor of gap junction-mediated communication [17], suggesting that direct cell-to-cell coupling mediates intercellular Ca^{2+} signalling in both cell types. In the present video-imaging study we present evidence for a complex interplay between gap junction-mediated intercellular communication and paracrine stimulation underlying cell-to-cell signalling. In fura 2-loaded cells grown in co-culture, mechanical stimulation induced propagated Ca^{2+} waves that spread bidirectionally to both cell types. The spreading distance of the intercellular waves was decreased by 18-GA, indicating heterologous cell-to-cell coupling. However, intercellular communication was also inhibited by suramin, a competitive inhibitor of purinoreceptors [18], by the ATP/ADPase apyrase and by purinoreceptor desensitization, demonstrating the involvement of ATP-mediated paracrine stimulation in intercellular communication. The possible implications of a heterologous chondro-synovial network for cartilage pathology are discussed.

MATERIALS AND METHODS

Chondrocyte and HIG-82 cultures

Articular cartilage was obtained from the ends of long bones of 3–4 kg rabbits. The tissue was rinsed in PBS [137 mM

Abbreviation used: 18-GA, 18 α -glycyrrhetic acid.

¹ To whom correspondence should be addressed.

Figure 1 Chondrocytes and synovial cell morphology is not affected by co-culture

(A) Phase-contrast micrograph of confluent articular chondrocytes with typical polygonal morphology (magnification $\times 300$). (B) Phase-contrast micrograph of confluent HIG-82 synovial cells characterized by a spindle-shaped morphology (magnification $\times 300$). (C) Phase-contrast micrograph of co-culture of chondrocyte and HIG-82 synovial cells (magnification $\times 300$). (D) Higher-magnification ($\times 600$) micrograph showing the boundary region between chondrocytes and HIG-82 synovial cells. Scale bar, 30 μm .

NaCl/2.7 mM KCl/8.1 mM Na_2PO_4 /1.5 mM KH_2PO_4 /0.9 mM CaCl_2 /0.49 mM MgCl_2 (pH 7.4)] and finely chopped in the same medium. Tissue fragments were digested with collagenase (250 units/ml, CLC type II; Worthington, Freehold, NJ, U.S.A.) for 4–5 h at 37 °C under continuous mild agitation. Chondrocytes were plated at a density of 5×10^5 cells/cm² on Cell-Tak (Becton Dickinson, Bedford, MA, U.S.A.) coated coverslips in Ham's F-12 medium supplemented with 10% (v/v) fetal calf serum and 2 mM L-glutamine and cultured at 37 °C in a humidified air/CO₂ (19:1) atmosphere for 5–6 days, until semiconfluent monolayers of at least 20–30 cells were observed. HIG-82 synoviocytes (obtained from ATCC; CRL-1832) were cultured in Ham's F-12 medium supplemented with 10% (v/v) fetal calf serum and 2 mM L-glutamine. When confluent, cells were subcultured at a 1:10 ratio. Co-cultures were obtained by seeding HIG-82 cells on coverslips carrying chondrocytes seeded 5–6 days previously: synovial cells attached and grew on the portion of coverslips that had not been coated with Cell-Tak. Confluent co-cultures were usually obtained 4–5 days after HIG-82 seeding.

Ca²⁺ imaging

Cells were loaded at room temperature with fura 2 (5 μM), dissolved in 20% (1:2) Pluronic (Molecular Probes) and added

to the culture medium. After 60 min the loading solution was removed and the cells were washed three times with KRH buffer [25 mM Hepes/NaOH, pH 7.4/125 mM NaCl/5 mM KCl/1 mM MgSO_4 /0.7 mM KH_2PO_4 /2 mM CaCl_2 /6 mM glucose]. Video-microscopy and Ca²⁺ measurements were performed at room temperature. The digital fluorescence-imaging microscopy system is built around a Zeiss inverted Axiovert 100 TV microscope. Cells were excited at 340 and 380 nm (or 360 nm) by a modified Jasco CAM-230 dual-wavelength microfluorimeter. Fluorescence images, collected through a Zeiss oil immersion 40 $\times$ 1.8 numerical aperture objective, were captured by a low-light-level charge-coupled device camera (Hamamatsu Photonics) and fed into a digital image processor developed in the laboratory. Video frames were then digitized, integrated and processed off-line to convert fluorescence data in Ca²⁺ maps (340/380 nm excitation wavelength ratio method).

Mechanical stimulation

Mechanical stimulation of single cells in subconfluent monolayers was performed by deforming the cell surface briefly with a micropipette. A fire-polished glass micropipette (approx. 1 μm tip diameter) was positioned over a single cell by a micromanipulator

Figure 2 For legend see p. 684

(Narishige, Tokyo, Japan). The pipette was briefly deflected downwards manually, to distort the membrane transiently.

RESULTS

The established HIG-82 synovial cell line, in which cells retain both the morphology and the differentiation markers of synoviocytes [18], was preferred to a primary synoviocyte culture owing to its homogeneous composition in synovial fibroblasts.

It had been reported that culturing chondrocytes on synovial fibroblasts promotes a change in shape and dedifferentiation of the chondrocytes [14]. We therefore designed a co-culture system that allowed the two cell types to grow separately until confluence, when the different cell layers came into close proximity. As shown in Figure 1, co-culture (Figure 1C) did not appreciably affect the morphology of either chondrocytes or synovial cells, which both retained their characteristic shape and dimensions compared with homotypic cultures (Figures 1A and 1B). Moreover, the different shapes of the two cell types facilitated the identification of the cells involved by Ca^{2+} waves.

Articular chondrocytes in culture are known to be phenotypically unstable. In cells cultured for 7–12 days, stimulation with extracellular ATP (1–100 μM) induces an increase in cytosolic Ca^{2+} concentration in 100% of the cell population [19]. We noticed that on prolonged culturing (more than 18 days) the number of responsive cells tended to decrease significantly (15–18%), possibly owing to a progressive loss of membrane receptors (P. D'Andrea, V. Paschini and M. Grandolfo, unpublished work). Such an altered phenotype was never observed in our short-term co-cultures, where chondrocytes retained their full sensitivity to agonist stimulation (see below), suggesting that the co-culture did not induce chondrocyte dedifferentiation.

When mechanical stimulation, obtained by briefly distorting the plasma membrane with a fire-polished glass micropipette, was performed on fura 2-loaded co-cultures of chondrocytes and synovial cells, it induced communicated Ca^{2+} waves that involved both cell types, independently of the type of stimulated cell. In Figure 2(A), the video-imaging recording shows a sequence of eight images collected at different times after the mechanical stimulation of a synovial cell (arrow). The Ca^{2+} wave propagated radially, subsequently involving 11 synovial cells and 10 chondrocytes. The temporal analysis, recorded from the cells labelled with different colours, is reported in Figure 2(B). In Figures 2(C) and 2(D) an analogous result is shown, obtained by stimulating a single chondrocyte (arrow) in the same cell layer after recovery for 10 min. In this case the stimulation induced an intercellular wave involving seven chondrocytes and eight synoviocytes. Overall, intercellular Ca^{2+} waves involved 20 ± 7 cells (mean $\pm$ S.D.) and proceeded with a constant wave speed of approx. 20–25 $\mu\text{m/s}$ for both cell types ($n = 31$).

We evaluated the role of gap junctions in sustaining the spreading of the intercellular wave by treating the cells with 18-GA, an inhibitor that has been demonstrated not to interfere with intracellular Ca^{2+} homeostasis [11,20]. Figure 3 shows that

18-GA (20 μM) decreased the number of cells involved by the intercellular Ca^{2+} waves (10 ± 6 cells; $n = 15$). Complete inhibition of the intercellular spreading, however, could be attained only by treating the cells both with 18-GA and with suramin ($n = 10$), a competitive antagonist of P_2 purinoreceptors [6], suggesting that ATP-mediated paracrine stimulation is involved in intercellular Ca^{2+} signalling. In both chondrocytes and HIG-82 synovial cells, extracellular ATP activates polyphosphoinositide hydrolysis and $\text{Ins}(1,4,5)\text{P}_3$ -mediated intracellular Ca^{2+} release [16a]; in co-cultures, suramin (100 μM) totally prevented the Ca^{2+} response induced by 1–10 μM ATP (results not shown).

When mechanical stimulation was performed in the presence of suramin alone the number of cells involved by the Ca^{2+} wave was consistently decreased with respect to controls, independently of the type of stimulated cell (5 ± 1 cells, 3 ± 1 chondrocytes and 2 ± 1 synovial cells; $n = 16$) (Figure 4). In the presence of suramin, 18-GA totally blocked the intercellular Ca^{2+} spreading ($n = 10$) (Figure 4).

To investigate further the role of ATP in intercellular signalling, we examined the effect of desensitization of ATP receptors on Ca^{2+} waves. Perfusion with 10 μM ATP induced an increase in Ca^{2+} in virtually all chondrocytes and HIG-82 cells. Repetitive stimulation induced receptor desensitization, as demonstrated by the lack of Ca^{2+} response (Figure 5): under these conditions mechanical stimulation induced a Ca^{2+} wave that was propagated to only 4 ± 1 cells (2.2 ± 1 chondrocytes and 2 ± 0.6 synovial cells, $n = 10$). The possibility that the repetitive ATP stimulation depleted the intracellular Ca^{2+} stores was ruled out by inducing Ca^{2+} increases with bradykinin (1 μM), an agonist known to activate the mobilization of $\text{Ins}(1,4,5)\text{P}_3$ -dependent Ca^{2+} stores [21].

The involvement of ATP in mediating intercellular signalling between chondrocytes and HIG-82 synovial cells was next examined by treating the cells with the ATP/ADPase apyrase, which was expected to decrease the concentration of the active nucleotide(s). As expected, apyrase reversibly decreased the number of responding cells (9 ± 6 ; $n = 9$) (Figure 6); after the apyrase had been washed out, mechanical stimulation induced Ca^{2+} waves involving a number of cells similar to controls (17 ± 7 ; $n = 9$) (Figure 6). Taken together, these findings support the conclusion that ATP-mediated paracrine stimulation contributes to intercellular signalling between chondrocytes and HIG-82 synovial cells.

DISCUSSION

Articular cartilage is a tissue designed to withstand compression during joint movement and, *in vivo*, is subjected to a wide range of mechanical loading forces. Mechanosensitivity has been demonstrated to influence chondrocyte metabolism and cartilage homeostasis, inducing cytoskeletal changes and proteoglycan synthesis both *in vitro* and *in vivo* [22,23]. Similarly to other cell systems, a mechanical stimulus arriving at the cell surface is

Figure 2 Mechanically induced Ca^{2+} waves propagate bidirectionally between chondrocytes and HIG-82 cells in co-culture

(A) Communicated Ca^{2+} wave in response to mechanical stimulation of a single synoviocyte. Panel 0: image of a confluent co-culture at rest (0 s). The position of the micropipette is indicated by the arrow. Panels 1–7 and 10: pseudo-colour images of the same field of cells at sequential time points (seconds) after mechanical stimulation. A wave of increased Ca^{2+} is communicated radially from the stimulated synoviocyte to ten synoviocytes and nine chondrocytes. Intensities of fluorescence ratio values are indicated by the calibration bar at the left, where the lowest values are coded blue. Scale bar, 30 μm . (B) Time course of the same experiment corresponding to the cells selected in the left panel. HIG-82 cells are labelled with diamonds, chondrocytes with asterisks. The vertical scale in the right panel is the fluorescence (340/380 nm excitation wavelengths). (C) Propagated Ca^{2+} wave after mechanical stimulation of a single chondrocyte. Panel 0: image of the same field as in (A), after 10 min of recovery. The position of the micropipette is indicated by the arrow. Panels 1–15: pseudo-colour images collected at sequential time points (seconds) after mechanical stimulation. The intercellular Ca^{2+} wave is communicated to seven chondrocytes and six synoviocytes. (D) Time course of the same experiment corresponding to the cells selected in the left panel; the vertical scale in the right panel is the fluorescence (340/380 nm excitation wavelengths).

Figure 3 Mechanically induced Ca^{2+} waves depend on gap junctions and paracrine stimulation

(A) Monochrome photo showing the boundary between chondrocytes and synovial cells. (B) Pseudo-colour images showing the development of Ca^{2+} waves induced by mechanical stimulation (panels are labelled in seconds after stimulation). Control (top panels): under control condition, stimulation of a single HIG-82 synovial cell (arrow) induced a wave that spread radially involving 12 synovial cells and 10 chondrocytes. Middle panels: in the presence of 18-GA (20 M, 15 min), stimulation of an adjacent chondrocyte (arrow) induced a wave that involved three chondrocytes and six synovial cells. Bottom panels: in the presence of both 18-GA (20 μM) and suramin (100 μM , 15 min), mechanical stimulation of the same chondrocyte (arrow) induced a Ca^{2+} response in the stimulated cell, but failed to evoke propagated Ca^{2+} waves. Scale bar, 30 μm .

Figure 4 Intercellular Ca^{2+} waves are inhibited by suramin

Mechanical stimulation of a single HIG-82 synovial cell (arrow) induced a propagated wave involving six synovial cells (diamonds) and six chondrocytes (asterisks). After 15 min of recovery, cell treatment with suramin ($100 \mu\text{M}$) decreased the number of cells (five) recruited by a second stimulation (arrow). After further recovery for 15 min, a third stimulation in the presence of both suramin and 18-GA ($20 \mu\text{M}$) led to a Ca^{2+} increase in the stimulated cell but did not induce an intercellular Ca^{2+} wave. The positions of the cells with respect to the point of stimulation are shown in the drawing.

Figure 5 Purinoreceptor desensitization inhibits intercellular wave spreading

Mechanical stimulation of a single chondrocyte (arrow) induced a propagated wave involving four synovial cells (diamonds) and six chondrocytes (asterisks). After 10 min of recovery, cell stimulation with extracellular ATP ($10 \mu\text{M}$) induced Ca^{2+} increases in all chondrocytes and HIG-82 synovial cells. Repetitive stimulations at 1 min intervals led to a progressive decrease in the response amplitude, indicating receptor desensitization. A second mechanical stimulation (arrow) induced a Ca^{2+} increase that involved four neighbouring cells. Bradykinin (BK; $1 \mu\text{M}$) induced a Ca^{2+} rise in eight out of ten cells, demonstrating that the Ca^{2+} signalling machinery was not desensitized. The positions of the cells with respect to the point of stimulation are shown in the drawing.

transduced into an intracellular Ca^{2+} increase that depends on both Ca^{2+} influx and on Ca^{2+} release from intracellular stores [12]. In cultured chondrocytes and HIG-82 cells, the expression of functional gap junctions allows the signal to be propagated to unstimulated cells, leading to the amplification of localized extracellular stimulus [16a]. The evidence that both gap junctions and paracrine stimulation mediate heterologous Ca^{2+} signalling between chondrocytes and synovial cells is intriguing and suggests that homologous and heterologous cell-cell interactions might be involved not only in chondrogenic differentiation but also in some joint pathologies. In homotypic chondrocytes and HIG-82 cultures, gap junctions entirely account for intercellular com-

munication [16a]. The evidence that in co-cultured cells extracellular ATP contributes to intercellular Ca^{2+} signalling suggests that the heterotypic interactions between these two cell types induce significant changes in the expression of crucial phenotypes. Nucleotide release via vesicular exocytosis has been demonstrated in several systems, including mechanically stimulated fibroblasts [24] and endothelial cells [25], indicating that mechanical loading represents an effective stimulus for ATP secretion. Interestingly, interleukin 1, secreted by both primary synoviocytes and HIG-82 cells, is known to enhance the sensitivity of articular chondrocytes to extracellular ATP [26], an agonist effective in increasing the rate of cartilage matrix degradation [27].

Figure 6 Apyrase reversibly inhibits intercellular wave spreading

Mechanical stimulation of a single chondrocyte (arrow) induced a propagated wave involving both five synovial cells (diamonds) and four chondrocytes (asterisks). Apyrase addition (10 units/ml) slightly increased the fluorescence signal, owing to apyrase autofluorescence. Cell stimulation in the presence of apyrase induced a wave involving three cells. After apyrase had been washed out, mechanical stimulation of a neighbouring chondrocyte induced a Ca^{2+} wave involving eight cells.

Heterotypic interactions between chondrocytes and synovial cells are known to occur in rheumatoid arthritis, a chronic inflammation of synovial tissue [13]. As part of the synovial tissue reaction, proliferating synovial cells penetrate cartilage in the form of a pannus, causing cartilage destruction [13]. Synovial pannus formation is initiated with the recognition and adhesion of synovial cells to chondrocytes and to cartilage matrix, mediated by cadherins and integrins [14,15]. The evidence that these two cell types *in vitro* develop pathways allowing direct and indirect intercellular communication suggests that synovial pannus might evolve as a heterologous cell network, ultimately resulting in an increased tissue sensitivity to changes in the extracellular environment. By allowing the intercellular diffusion of second messengers, gap junctions provide a mechanism by which the network response can be greater than the sum of the individual cells [28]; in contrast, ATP-mediated paracrine stimulation provides an additional pathway for integrating and amplifying the information.

In articular chondrocytes, the cytosolic Ca^{2+} concentration modulates differentially the expression of several inducible genes involved in the inflammatory response. Increased Ca^{2+} levels induce the expression of cyclo-oxygenase II [29] and inhibit the expression of NO synthase [30], ultimately increasing overall cell sensitivity to the proliferative stimuli present in the synovial fluid. The development of synovial pannus is associated with a continuous tissue overgrowth [13], a phenomenon that might be related to the formation of the chondro-synovial network, where intercellular Ca^{2+} signalling would strengthen the actions of the agonists present in the inflamed synovial fluid.

The present results provide the first evidence of a complex

mechanism of intercellular communication between chondrocytes and synovial cells *in vitro*: although further work is needed to ascertain the phenotype expression *in vivo*, the possibility of defining the synovial pannus as a heterologous cellular network would expand our understanding of cartilage pathology and the design of novel pharmacological strategies.

We thank Franco Vittur for helpful discussions, Adriano Bianchi for technical assistance and the Foundation Carlo and Dirce Callerio for providing laboratory facilities. The work was supported by MURST, Università di Trieste, Italy.

REFERENCES

- Bruzzone, R., White, T. W. and Paul, D. L. (1996) *Eur. J. Biochem.* **238**, 1–27
- Oliani, S. M., Girol, A. P. and Smith, R. L. (1995) *Acta Anat.* **154**, 267–271
- Rook, M. B., van Ginneken, A. C., de-Jonge, B., el-Aoumari, A., Gros, D. and Jongsma, H. J. (1992) *Am. J. Physiol.* **263**, C959–C977
- White, T. W. and Bruzzone, R. (1996) *J. Bioenerg. Biomembr.* **28**, 339–349
- Robinson, S. R., Hampson, E. C., Munro, M. N. and Vaney, D. I. (1993) *Science* **262**, 1072–1074
- Osipchuk, Y. and Cahalan, M. (1992) *Nature (London)* **359**, 241–244
- Schlosser, S. F., Burgstahler, A. D. and Nathanson, M. H. (1996) *Proc. Natl. Acad. Sci. U.S.A.* **93**, 9948–9953
- Robb-Gaspers, L. D. and Thomas, A. P. (1995) *J. Biol. Chem.* **270**, 8102–8107
- Ede, D. A. (1983) in *Cartilage* (Hall, B., ed.), pp. 143–185, Academic Press, New York
- Donahue, H., Guilak, F., Vander Moolen, M. A., McLeod, K. J., Rubin, C. T., Grande, D. A. and Brink, P. R. (1995) *J. Bone Miner. Res.* **10**, 1359–1364
- D'Andrea, P. and Vittur, F. (1996) *Cell Calcium* **20**, 389–397
- D'Andrea, P. and Vittur, F. (1997) *FEBS Lett.* **400**, 58–64
- Harris, Jr., E. D. (1985) in *Textbook of Rheumatology* (Kelley, W. N., Harris, Jr. E. D., Ruddy, S. and Sledge, C. B., eds.), pp. 886–914, W. B. Saunders, Philadelphia
- Ramachandrala, A., Tiku, K. and Tiku, M. L. (1992) *J. Cell Sci.* **101**, 859–871
- Ishikawa, H., Hirata, S., Nishibayashi, Y., Imura, S., Kubo, H. and Ohno, O. (1994) *Clin. Orthoped. Rel. Res.* **300**, 297–303
- Mege, R.-M., Matsuzaki, F., Gallin, W. J., Goldberg, J. I., Cunningham, B. A. and Edelman, G. M. (1988) *Proc. Natl. Acad. Sci. U.S.A.* **85**, 7274–7278
- Grandolfo, M., Calabrese, A. and D'Andrea, P. (1998) *J. Bone Miner. Res.*, in the press
- Davidson, J. S., Baumgarten, I. M. and Harley, E. H. (1986) *Biochem. Biophys. Res. Commun.* **134**, 29–36
- Sung, K., Mendelow, D., Georgescu, H. I. and Evans, C. H. (1988) *Biochim. Biophys. Acta* **971**, 148–156
- D'Andrea, P. and Vittur, F. (1996) *J. Bone Miner. Res.* **11**, 946–954
- Venance, L., Stella, N., Glowinski, J. and Giaume, C. (1997) *J. Neurosci.* **17**, 1981–1992
- D'Andrea, P. and Vittur, F. (1995) *Biochem. Biophys. Res. Commun.* **215**, 129–135
- Parkkinen, J. J., Lammi, M. J., Inkinen, R., Jortikka, M., Tammi, M., Virtanen, I. and Helminen, H. J. (1995) *J. Orthoped. Res.* **13**, 495–502
- Smith, R. L., Donlon, B. S., Gupta, M. K., Mohtai, M., Das, P., Carter, D. R., Crooke, J., Gibbons, G., Hutchinson, N. and Schurman, D. J. (1995) *J. Orthoped. Res.* **13**, 824–831
- Grierson, J. P. and Meldolesi, J. (1995) *J. Biol. Chem.* **270**, 4451–4456
- Milner, P., Bodin, P., Loesch, A. and Burnstock, G. (1990) *Biochem. Biophys. Res. Commun.* **170**, 649–656
- Caswell, A. M., Leong, W. S. and Russell, R. G. G. (1992) *Biochim. Biophys. Acta* **1137**, 52–58
- Leong, W. S., Russell, R. G. G. and Caswell, A. M. (1994) *Biochim. Biophys. Acta* **1201**, 298–304
- Vander Molen, M. A., Rubin, C. T., McLeod, K. J., McCauley, L. K. and Donahue, H. J. (1996) *J. Biol. Chem.* **271**, 12165–12171
- Geng, Y., Bianco, F. J., Cornelissen, M. and Lotz, M. (1995) *J. Immunol.* **155**, 796–801
- Geng, Y. and Lotz, M. (1995) *J. Cell Biol.* **129**, 1651–1657