Journal of Cellular Biochemistry 69:81–86 (1998)

# Parathyroid Hormone Effect on Cell-to-Cell Communication in Stromal and Osteoblastic Cells

**R. Massas**[1] **and D. Benayahu**[2]*

[1]Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel
[2]Department of Cell Biology and Histology, Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel

**Abstract** We characterized the formation and regulation of the gap junction in calvarial osteoblasts and in a series of subtypes from marrow stromal cells. The stromal cells included osteogenic, chondro-osteogenic, and endothelial cells. The cell coupling was measured by using fluorescence dye injected into single cells, and its migration to neighboring cells was measured. The functional coupling of cells was highly expressed by the osteoblastic cells. This process is mediated through fast changes in intracellular $Ca^{+2}$ levels. Calcium ionophore (A 23187) demonstrated an uncoupling effect on the cells. In addition, the exposure of the cells to the parathyroid hormone increased the formation of the gap junction complex; the highest level was demonstrated in the osteoblastic cells. J. Cell. Biochem. 69:81–86, 1998. 

**Key words:** cell communication; osteoblasts, stromal cells

In the bone marrow, there are multipotential stem cells of the stroma (MSC) that are nonhematopoietic. The MSC provide the important microenvironment for both regulations of differentiation of hemopoietic and skeletal cells. The stroma compartment contains various cell types: fibroblast, endothelial, adipocyte, and osteogenic cells [Owen, 1988; Aubin et al., 1992]. The stroma cells are intimately involved with the bone formation and function in close proximity to the matrix and their progenitors in the marrow. The function of the stromal cell is based on cell–cell communication and secretion of soluble factors. Morphological studies have shown that gap junctions are channels that span the transmembrane space that exists between bone cells [Shen et al., 1986; Mackie and Tucker, 1992; Schiller et al., 1992; Civitelli et al., 1993; Steinberg et al., 1994; Yamaguchi et al., 1994; Donahuse et al., 1995] and stromal cells [Dorshkind et al., 1993; Rosendaal et al., 1994]. The gap junctions mediate the intracellular exchange of regulatory ions and small molecules that allow metabolic cooperation between adjacent cells that control the cell differentiation and growth. These junction complexes are formed by at least 12 proteins of the connexine family. The major gap junction protein connexin43 (Cx43) has been identified in normal and transformed rat osteoblastic cells [Schiller et al., 1992] and in the mouse osteoblastlike MC3T3-E1 cell line [Yamaguchi et al., 1994]. Another junction protein, connexin45 (Cx45), has been identified in osteoblastic cells derived from human bone marrow stroma, in trabecular bone osteoblasts, in the human osteogenic sarcoma cell line SaOS-2 [Civitelli et al., 1993], and in the rat osteoblastlike osteosarcoma cell line UMR 106-01 [Steinberg et al., 1994]. Unlike Cx43, Cx45 allows the passage of only small ions in UMR-106 [Steinberg et al., 1994] and has low levels of expression in human osteoblastic cells [Civitelli et al., 1993]. Cx43 has been shown to be expressed among stromal cells [Dorshkind et al., 1993] and between stromal and hemopoietic cells [Rosendaal et al., 1994], which may be an additional recognition mechanism between these cell types. The existence of gap junctions has been demonstrated, and the modulation by hormones has been studied [Shen et al., 1986; Schiller et al., 1992; Donahuse et al., 1995].

*Correspondence to: Dafna Benayahu, Ph.D., Department of Cell Biology and Histology, Sackler School of Medicine, Tel Aviv University, Tel Aviv, 69978 Israel. E-mail: dafnab@post.tau.ac.il

Received 10 September 1997; Accepted 19 November 1997

Because of the structural complexity of bone tissue, it is difficult to analyze the intercellular communication at the organ tissue level. Therefore, to study intercellular communication, we used in vitro cell cultures as our model system. In the present study, we focused on cell–cell interactions between calvarial osteoblastic cells and cells of the stroma compartment. The stromal cells includes different cell types that are represented by the MBA series of cells. These cells are different in their morphology, growth rate, production and distribution of extracellular matrix proteins, and levels of enzymatic activities (ALK-P and CD10) [Indig et al., 1990; Benayahu et al., 1994a, 1995; Fried and Benayahu, 1996]. In addition, the responsiveness of cells to growth factors and to bone-seeking hormones was studied [Benayahu et al., 1994a, 1995; Fried and Benayahu, 1996]. These cells were studied for their function in a support microenvironment for lymphohematopoietic differentiation and for the ability to form bone [Benayahu et al., 1989, 1992, 1994b–d; Fried et al., 1993; Zipori et al., 1984, 1985a,b]. The hormonal regulation of direct intercellular communication in different osteogenic or non-osteogenic cells in cultures demands a thorough study of the regulation mechanisms of cell–cell communication. Thus, we have chosen in the present study to examine the effect of parathyroid hormone (PTH) on intercellular communication in different types of cells. The differential effects of PTH on osteogenic and nonosteogenic cells have been discussed previously [Benayahu et al., 1989, 1991, 1995; Massas, 1995]. Thus, the present study discusses the cellular interactions between specific cell types, and these events may shed some light on the cellular interactions that take place in the skeletal–hemopoietic microenvironment.

## MATERIALS AND METHODS

### Cell Tissue Culture

Primary cultures of calvaria-derived cells were harvested from newborn rats. The cells prepared by an isolation technique based on the ability of bone cells to migrate from bone tissue onto glass. Stripped parietal bones were fragmented and transferred onto glass coverslips (either 22 × 22 mm or 10.5 × 22 mm) that were placed in a 35-mm Petri dish (Falcon, England) containing 2 ml of Minimum Essential Medium (Biological Industries, BetHaemek, Israel) supplemented with 10% fetal calf serum (FCS; Biological Industries). After 5–7 days, an extended outgrowth zone of cells surrounded the bone explants. At this phase of growth, the cells were in contact with each other but did not reach a completely confluent state. Bone-marrow-derived stromal cell lines, MBA series [Benayahu et al., 1989, 1991; Fried et al., 1993] stock cells were seeded onto tissue culture plates in high-glucose Dulbecco's Modified Eagle Medium supplemented with 10% FCS.

Stock cultures were incubated at 37°C in a humidified atmosphere of 10% $CO_2$ in air, and the culture medium was replaced every 3 days. For experiments, the cells were investigated for their response to hormones when they were in contact with each other but did not reach complete confluence.

### Modulators In Vitro

The hormone 1-34-PTH (Sigma, St. Louis, MO) was dissolved in 1% sodium acetate, and the stock kept at -20°C. The calcium ionophore A23187 (Sigma) was dissolved in distilled water.

### Measurement of Intercellular Communication

The intercellular communication through gap junctions was measured by a method based on the microinjection by a conventional micropipette (unpolished) of a fluorescent dye, Lucifer Yellow (LY) CH (Sigma), into a single cell in a cell monolayer. Micropipettes were filled at the tips with 4% (w/v) aqueous LY CH [Stewart, 1978]. The cultured cells grown on the coverslip (22 × 22 mm) were transferred to the experimental chamber and perfused with 4-(2-hydroxyethl)-1-piperazineethanesulfonic acid (Hepes) buffer containing 145 mM NaCl, 5 mM KCl, 2mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM glucose, and 10 mM Hepes, pH 7.3. After cell implantation by a microelectrode, hyperpolarizing pulses (20 nA) were applied for 20 s to eject the dye from the pipette into the cell. Spreading of the dye from the impaled cell into its neighboring cells was assayed under epifluorescence illumination by counting the number of fluorescent cells surrounding the microinjected cell 2 min after the voltage pulses. The results are given in terms of mean ± S.E.M. (n = number of independent microinjection experiments).

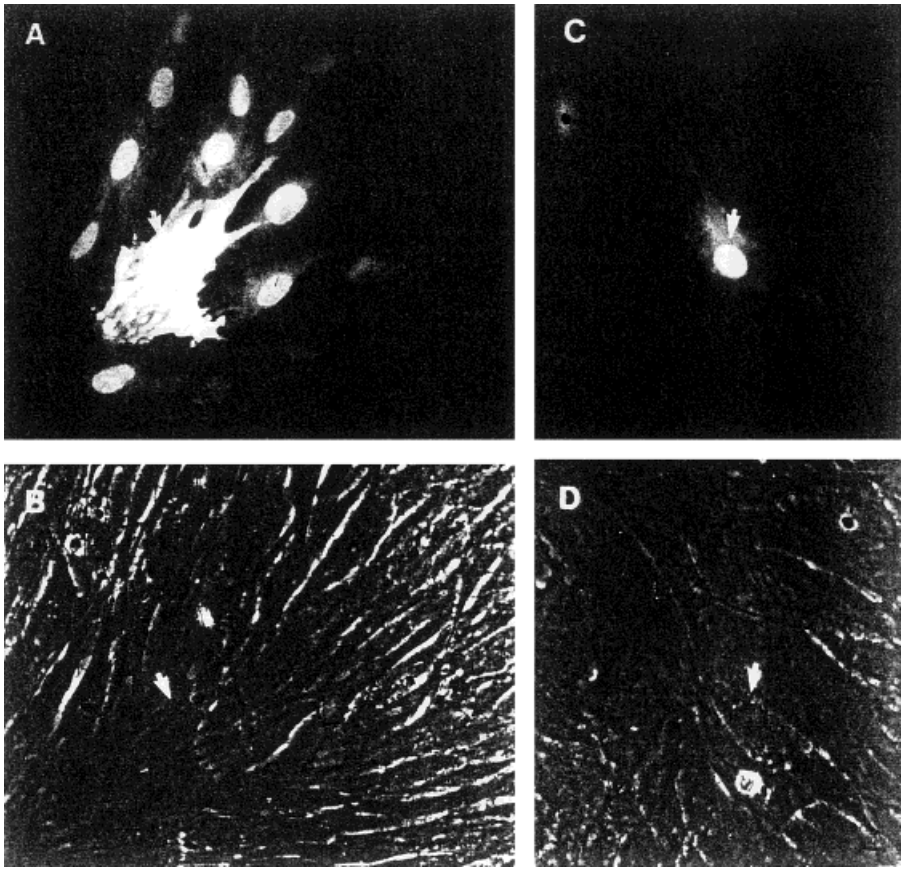


**Fig. 1.** Morphology of monolayer cells grown in tissue culture visualized by an inverted microscope. **A:** The cellular communication by gap junctions was demonstrated by a method based on the microinjection of a fluorescent dye, Lucifer Yellow (LY) CH, into a single cell (arrow) with a conventional micropipette. Two minutes after the injection, the spreading of the dye to neighboring cells is visible. **B:** Parallel to fluorescence staining, the monolayer of cells is visible. Calcium ionophore A-23187 caused uncoupling of the gap junction between cells after preincubation to the LY microinjection. **C:** Uncoupling of cells. **D:** Cell monolayer.

### Statistical Analysis

The data were summarized as standard error of the mean (S.E.M), after being analyzed with a double-sided *t*-test to study the significance of the differences in the number of coupled cells.

## RESULTS

### Cell-to-Cell Communication Presence Between Different Cells Types

Primary calvaria cells and MBA stromal cells were used in the present study. The MBA cells include the MBA-15 osteoblastic cell and its clonal lines MBA-15.6 and MBA-15.4, the MBA-1-chondro-osteoblastic cell and MBA-1.2, and MBA-2 endothelial cells. Independent measurements were performed to quantify the basal level of cell communication as the number of coupled cells following microinjection of LY. The dye injection into a single cell of the monolayer resulted in transfer of the dye to neighboring cells. A quick response based on coupling by the gap junction complex demonstrated that the fluorescence dye had spread to neighboring cells (Fig. 1A). The quantitation of this coupling phenomenon is summarized in Figure 2. When the primary calvarial cells were measured, the cell coupling resulted in $35 \pm 6$ (in 56 different experiments, $n = 56$) of neighboring coupled cells within 2 min after dye injection (Fig. 2). The MBA-15 cells had $21 \pm 5$ ($n = 61$) coupled cells, and MBA-15.6 and MBA-15.4 measured $15 \pm 4$ ($n = 57$) and $11 \pm 3$ ($n = 54$), respectively. Lower levels were measured in the MBA-1 ($5 \pm 2$, $n = 52$), MBA-1.2 ($3 \pm 2$, $n = 55$), and the MBA-2 ($6 \pm 2$, $n = 53$) cells (Fig. 2). These results demonstrated the highest basal level of junction formation by the osteoblastic

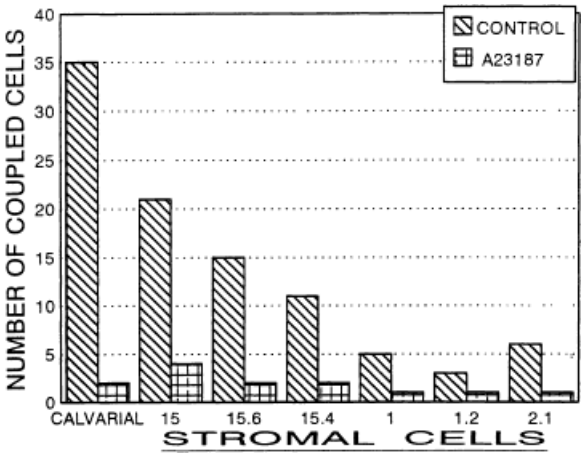


**Fig. 2.** Gap junction formation measured between calvarial cells and stromal cell lines. The intercellular communication through gap junctions was measured after microinjection of a fluorescent dye into a single cell (as shown in Fig. 1). Two minutes after injection into single cells, the spreading of the dye to neighboring cells was counted. Calcium ionophore A-23187 caused uncoupling of the gap junction between cells after preincubation to the LY microinjection. The results are expressed as mean ± S.E.M. of 55–65 microinjections for each cell type.

cells from calvaria and at the stromal compartment.

### Effect of Calcium Ionophore A23187

Calcium was shown to cause dependent reduction of the permeability of the junction. We examined the effect of calcium ionophore A23187 ($10^{-6}$ M) on the formation of coupled junctions in these cells. Preincubation of the cells with A23187 for 30 min caused uncoupling, and dye was unable to transfer to neighboring cells (Fig. 1C). The quantitation of decrease in the number of coupled cells of all cell populations is summarized in Figure 2. The results indicate that $Ca^{2+}$ plays a major role in the process of gap junction formation.

### PTH Effect on Modulation of Cell–Cell Communication

Preincubation of tested cells with PTH ($10^{-7}$ M) for 30 min and then microinjection of LY resulted in an increase in the number of coupled cells in all cell populations studied, but at different levels (Fig. 3). The osteoblastic cells had high levels of coupled cells in response to PTH, which occurred in calvaria cells (51 ± 8, n = 60) and MBA-15 cells (32 ± 10, n = 54).

## DISCUSSION

The importance of the existence of gap junctions in the marrow was shown and increased during forced stem cell division that was based on the ability to transfer direct regulatory molecules [Rosendaal et al., 1994]. The bone marrow is responsible for two fundamental processes: the production of mature blood cells from the hemopoietic stem cells and the support of bone formation. The stromal cells play an important role in regulation of these processes and in the creation of the special microenvironment. The role and function of these cells lie in the complex network of the extracellular matrix with growth factors and cytokines. The function of the stromal system is based on cell-to-cell communication and the existence of junction complexes. In normal marrow, there is a certain latent gap junction network. An increase in gap junction has been observed following the stress of chemotherapy, which has been linked to hemopoietic stem cell renewal and proliferation [Rosendaal et al., 1994]. In vitro, hemopoiesis is regulated in long-term culture of marrow stromal cells [Dexter et al., 1977]. In this type of culture, the first interaction observed is mediated through cell-to-cell of the hemopoietic to the stromal cells. Numerous studies have concluded that gap junctions exist primarily between stromal cells. It has been

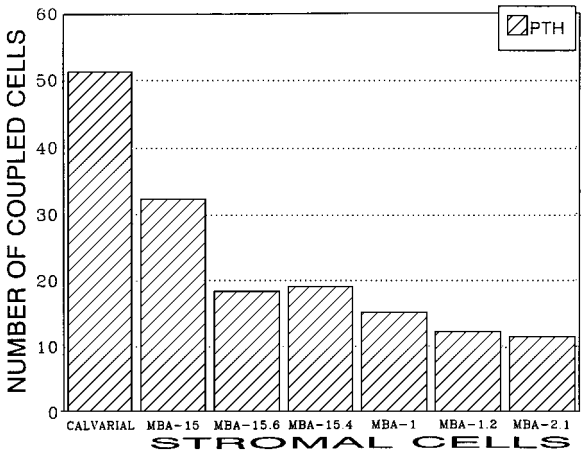


**Fig. 3.** Effect of PTH on the formation of a gap junction between calvarial cells and stromal cell lines was measured. Spreading of the dye from the impaled cell into its neighboring cells was assayed under epifluorescence illumination by counting the number of fluorescent cells surrounding the microinjected cell after preincubation with PTH ($10^{-7}$ M) for a period of 30 min. After each preincubation, the intercellular coupling was determined. The results are expressed as mean ± S.E.M. of 55–65 microinjections for each cell type.

suggested that the interaction among stromal cells is not among all stromal cells but dependent on cell differentiation status [Umezawa et al., 1990]. There is no information on the type of cellular interaction among the different cell subtypes. Therefore, we conducted the present study, and we were able to demonstrate the capacity to form gap junction between differentiated stromal cells. For this reason, we used the MBA series of cells that represent different cell types: osteogenic, osteochondrigenic, and endothelial that were compared with primary calvarial cells. Transfer of fluorescent LY illustrated the functional gap junctions. Our results demonstrate that the osteoblastic subpopulations as represented by the MBA-15 stromal cells and the primary calvaria cells express the higher level of gap junctions, whereas other stromal cell types are lower in the capacity to form the junction complex. Hormones and growth factors control cell–cell communication. We also have shown that the osteoblastic cells respond to the addition of PTH to the culture media by the increased number of coupled osteoblastic cells. Few reports have discussed the effect of PTH or other effects on bone cells [Shen et al., 1986; Schiller et al., 1992; Donahuse et al., 1995]. PTH affected cell shape change and did not affect the number of gap junctions in fetal calvarial cells, whereas UMR-106 cells demonstrated that long exposure to PTH is needed to obtain an increased level of cell–cell communication. Others have shown that exposure of primary bone cultures to PTH is sufficient to cause increased intercellular coupling but not in osteoblastic cell lines ROS 17/2.8, MC3T3-E1, and SAOS-2.

The present study has demonstrated high levels of cellular gap junction complexes formed by cells of the osteoblastic lineage. PTH increased the cellular complexes and gap junction after modulation. The results emphasize the importance of the quick response of the cells of the osteoblastic lineage to PTH as measured by the increase in the coupling of the cells, which may also explain the process of the osteoblastic cells as they function in situ.

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