Korean J Biol Sci 4: 361-366, 2000

Protein Kinase Cα Is Involved in the Cell Condensation During Chondrogenesis *In Vitro*

Young Bin Lim, Shin-Sung Kang[1], Young-Sup Lee[2], and Jong Kyung Sonn*

Department of Biology, Teachers' College, [1]Department of Biology and [2]Department of Biochemistry, College of Natural Sciences, Kyungpook National University, Taegu 702-701, Korea

Key Words:
Chondrogenesis
Cell condensation
Protein kinase C isoforms

In order to investigate the role of protein kinase C (PKC) in chondrogenic differentiation, we examined the localization of PKC isoforms in a limb bud micromass culture system. PKCα is specifically localized in the regions which would become cartilage nodules, while PKCλ/ι and ζ display widespread distribution in the whole culture. Distribution of PKCα change along with promotion or inhibition of chondrogenesis by lysophosphatidylcholine or phorbol 12-myristate 13-acetate. On the other hand, localization of PKCλ/ι or ζ was not changed by the modulation of chondrogenesis. Peanut agglutinin binding protein which is associated with cell aggregation during chondrogenesis was present in the cell condensation regions and its expression in those regions was influenced by PKC activity. Expression of fibronectin and N-cadherin in the cell condensing area were also affected by modulation of PKC activity. These results suggest involvement of PKCα in the cell condensation, possibly through regulating expression of fibronectin and N-cadherin.

Chondrogenesis is one of distinct events occurring in the early embryonic development. In the beginning of limb cartilage differentiation, prechondrogenic mesenchymal cells are closely juxtaposed and become condensed. The condensation is a multi-step process, including cell recruitment, cell to cell contact, and concomitant changes in cell shape which are necessary to trigger the chondrogenic differentiation. The condensation is followed by expression of genes for cartilage-specific matrix such as sulfated proteoglycan, type II and IX collagen (Kosher et al., 1986a; 1986b; Nah et al., 1988; Kulyk et al., 1991). When limb mesenchymal cells are placed in high-density micromass cultures, the cells first undergo distinct aggregation, and later the aggregates become cartilage nodules (Solursh et al., 1978).

Accumulating evidence indicates that protein kinase C (PKC) plays an important role in chondrogenic differentiation. Since the relationship between PKC and chondrogenesis was reported using phorbol 12-myristate 13-acetate (PMA), an activator of PKC (Garrison et al., 1987; Kulyk and Reichert, 1992), activity of PKC was measured in the cells undergoing chondrogenesis and found to be closely related to chondrogenesis (Sonn and Solursh, 1993).

PKC is composed of at least 11 isoforms that can be divided into three groups on the basis of their structure and their cofactors for enzyme activation (Nishizuka, 1995). Conventional PKCs (cPKC) such as α, βI, βII and γ require Ca^{2+}, phosphatidylserine, and diacylglycerol or phorbol ester for their activation. Novel PKCs (nPKC) such as δ, ε, η, θ, and μ are activated by phosphatidylserine and diacylglycerol or phosphatidylethanolamine and are independent of Ca^{2+}. Atypical PKCs (aPKC) such as ζ and λ/ι need only phosphatidylserine as an activating factor. The different PKC isoforms exhibit distinct tissue distribution (for review, see Liu and Heckman, 1998) and play important roles in the various cellular processes, including cell differentiation (for review, see Goodnight et al., 1994). Presence of various PKC isoforms in chondrogenic competent cells and chondrocyte was demonstrated by immunohistochemistry (Bareggi et al., 1994) and Western blot assay (Choi et al., 1995). Among these, an increase in the expression and activity of PKCα was most evident, suggesting an important role for PKCα in the chondrogenic differentiation (Yang et al., 1998; Lee et al., 1998). In addition, it has been proposed that PKC might regulate chondrogenesis by modulating expression of integrin and fibronectin (FN) (Chang et al., 1998). All the above studies were based on Western blot assay. Since there are two different cell regions, condensing or chondrogenic and uncondensing or non-chondrogenic, in the cultures of stage 23/24 chick embryo wing mesenchyme, expression of signaling molecules, extracellular matrix component, or cell adhesion molecule by Western blot is the sum of the two different regions. Therefore, information about the localization of various proteins in the micromass

* To whom correspondence should be addressed.
Tel: 82-53-950-5915, Fax: 82-53-950-6809
E-mail: sonnjk@knu.ac.kr

culture is necessary to identify the factors which are related to chondrogenesis.

The present paper reports on the temporal and spatial distribution of PKC isoforms in the two different regions during chondrogenesis of chick embryo limb mesenchymal cells *in vitro*. We further examined the involvement of PKC in the cell condensation.

Materials and Methods

Cell culture

Micromass cultures of chick wing bud mesenchymal cells were performed as described by Ahrens et al., (1977). Briefly, wing buds were removed from stage 23/24 chick embryos and incubated for 10 min at 37℃ in 0.1% trypsin-collagenase in Ca^{2+}/Mg^{2+}-free saline G. Following mechanical dissociation of cells, cell suspension was centrifuged and the cellular pellet was resuspended in F12 medium containing 10% fetal bovine serum and filtered through two layers of No 20 Nitex to remove cell clumps. Cell number was counted with a hemacytometer and cell density was adjusted to 2×10^7 cells/ml. Ten microliter of cell suspension was plated on 35-mm Corning tissue culture dishes. After 1 hr incubation at 37℃ to allow the cells to attach, 2 ml of F12 medium with 10% fetal bovine serum was supplied to the cultures. Cultures were fed every 24 h by a complete change of medium.

Antibodies

The mouse anti-mouse PKCα, λ/ι, and ζ monoclonal antibodies were obtained from Transduction Lab. (Lexington, KY, USA). The II-II6B3 mouse anti-avian type II collagen monoclonal antibody was from the Developmental Studies Hybridoma Bank (University of Iowa, Iowa City, IA, USA) and mouse anti-avian FN monoclonal antibody and rabbit anti-chick N-cadherin polyclonal antibody were from Sigma (St. Louis, MO, USA).

Immunocytochemistry

After the appropriate time, the cultures were fixed for 10 min at room temperature in a freshly prepared 4% paraformaldehyde in phosphate-buffered saline (PBS). Following several washes with PBS, cultures were permeabilized by exposure to 0.2% Triton X-100/PBS at room temperature for 5 min. Cells were incubated with 5% normal horse serum for 20 min and primary antibodies were applied to the cultures for 30 min at room temperature. The cultures were then washed several times with PBS and incubated for 1 h with biotinylated horse anti-mouse or -rabbit IgG (Vectastain ABC kit; Vector Laboratories, Burlingame, CA, USA). After washing several times with PBS, the cultures were treated with avidin-biotin horseradish peroxidase complex (Vectastain ABC kit), washed with PBS, developed with diaminobenzidine/H_2O_2 solution, and mounted in Crystal mount (Biomeda Corp, Foster City, CA, USA).

In case of peanut agglutinin (PNA) staining, the fixed cultures were incubated with biotinylated PNA for 30 min. PNA was visualized by the same procedure as the above.

Western blot assay

Cultures were lysed and scraped in RIPA buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM PMSF, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin, 1 mM NaF, 1 mM Na_3VO_4, 0.25% sodium deoxycholate and 1% NP-40). The cell lysates were centrifuged and the amount of protein in the supernatant was measured using a Bio-Rad DC Protein Assay kit (Bio-Rad, CA, USA). Proteins were analyzed by 10% SDS-PAGE and transferred onto nitrocellulose (BA 85, Schleicher & Schuell, Keene, NH, USA). The membranes were blocked in 3% non-fat dry milk for 1 h and then probed with anti-PKC α, λ/ι, or ζ. Horseradish peroxidase conjugated anti-mouse IgG was used as a secondary antibody. The immunoreactive proteins were visualized with the ECL detection kit (Amersham, Buckinghamshire, UK).

Results

Progression and extent of chondrogenic differentiation was monitored by immunocytochemical localization of cartilage-specific type II collagen (Fig. 1A). On day 2, numerous discrete type II collagen stained regions were detectable and the intensity of staining was considerably increased on day 3. The cartilage nodules were scattered in various distinct regions of the culture and were separated from one another by a considerable amount of nonstaining internodular areas or non-chondrogenic regions.

The localization of PKCα showed similar pattern to that of type II collagen in that PKCα was present in restricted regions and staining became increased as chondrogenesis proceeded (Fig. 1A). Comparison of bright light and phase contrast photomicrographs of PKCα staining at higher magnification revealed that the PKCα-stained cells were under cellular condensation on day 2 and formed nodules on day 3 (Fig. 1B). On the other hand, PKCλ/ι had widespread and diffuse distribution throughout the culture on day 2 and this pattern was not changed on day 3. Higher magnification photomicrograph showed that PKCλ/ι was present both in chondrogenic and non-chondrogenic regions. PKCζ had diffuse distribution pattern and was present both in chondrogenic and non-chondrogenic regions on day 2, but was lost in nodules on day 3 (Fig. 1).

These PKC isoforms-specific distribution patterns occurring during chondrogenesis were also observed when chondrogenesis was modulated. PKC has been

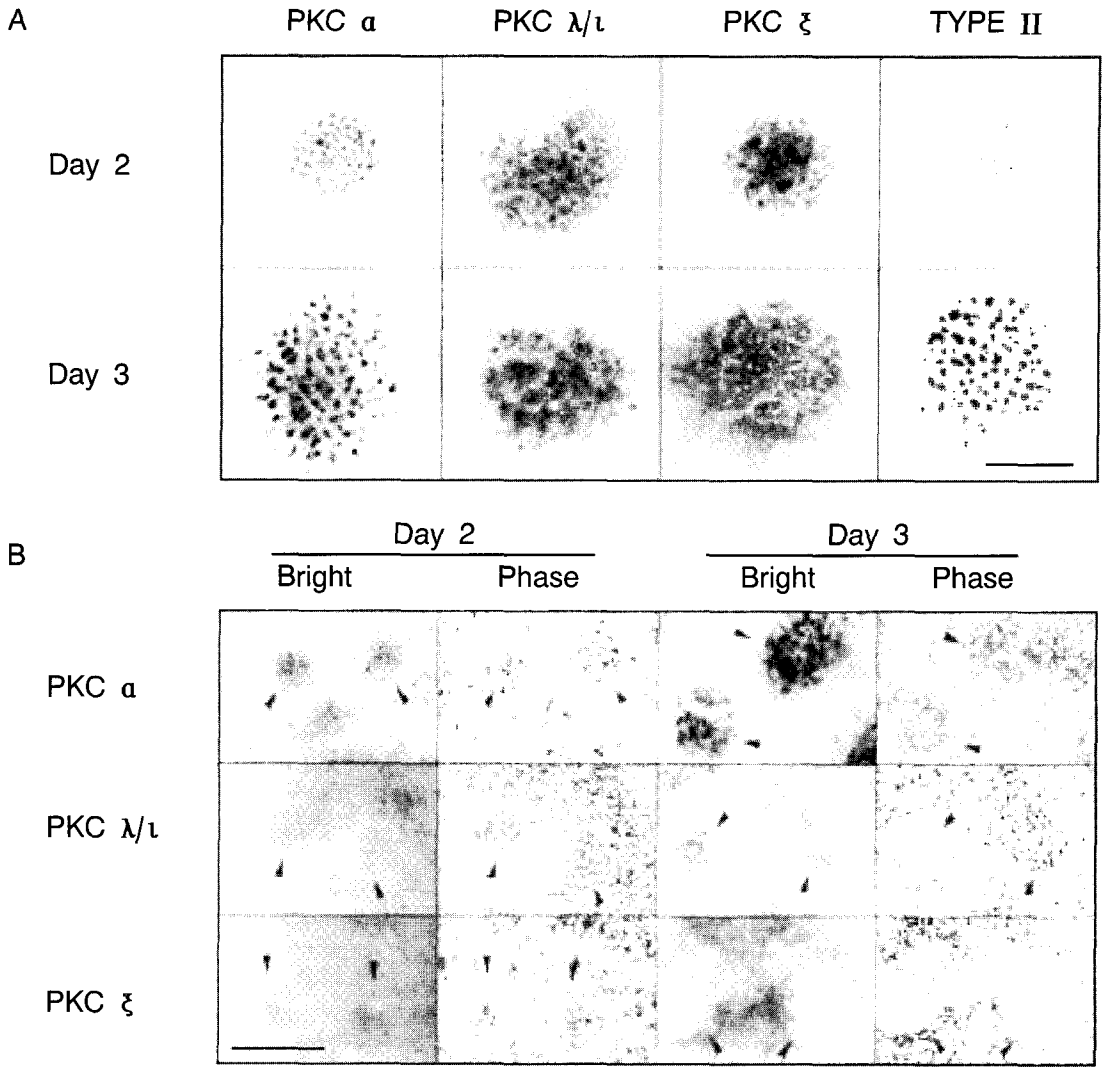

Fig. 1. Immunocytochemical localization of PKC isoforms and type II collagen in chick wing bud mesenchymal cells *in vitro*. A, Wing bud mesenchymal cells cultured at the indicated times were fixed and stained for PKCα, λ/ι, ζ, and type II collagen (Type II). B, Bright light (Bright) and corresponding phase (Phase) photomicrographs of cultures stained for PKCα, λ/ι, and ζ at higher magnification. Arrowheads indicate the regions where cellular condensation occurs. Scale bars=100 μm(B) and 500 μm(A).

known to affect chondrogenic differentiation (Lee et al., 1998; Yang et al., 1998). Treatment of lysophosphatidylcholine (LPC), a lipid responsible for sustained activation of PKC (Lee et al., 1998), promoted chondrogenic differentiation, while PMA, which is a PKC activator but down regulates PKC by prolonged treatment, inhibited chondrogenesis as determined by type II collagen staining (Fig. 2A). PKCα staining was dramatically increased in nodular regions by LPC treatment but almost completely disappeared by PMA. However, distribution of PKCλ/ι or ζ was not changed by LPC and still present in PMA-treated cultures.

Western blot assay of total cell extract showed a parallel increase in the expression of PKCα along with culture period and constant expression of PKCλ/ι and ζ throughout the culture period (Fig. 2B). Changes in the amount of total PKCα by LPC or PMA treatment in the Western blot assay behaved similar to those of PKCα staining in nodular regions while the amount of PKCλ/ι or ζ expression was not influenced either by LPC or PMA (Fig. 2).

In order to examine the physiological role of PKC in cell condensation, cultures treated with LPC or PMA were stained with PNA which is known to be a specific marker for precartilage aggregates (Aulthouse and Solursh, 1987). As shown in Fig. 3, many PNA-positive spots were detectable at condensation stage, and LPC increased the number and intensity of PNA stained spots, supporting that PKC enhances cellular condensation. The weak but detectable staining of PNA in PMA-treated cultures is probably due to aggregates of non-chondrogenic cells.

Expression of FN, one of the extracellular matrix components and N-cadherin, a cell adhesion molecule,

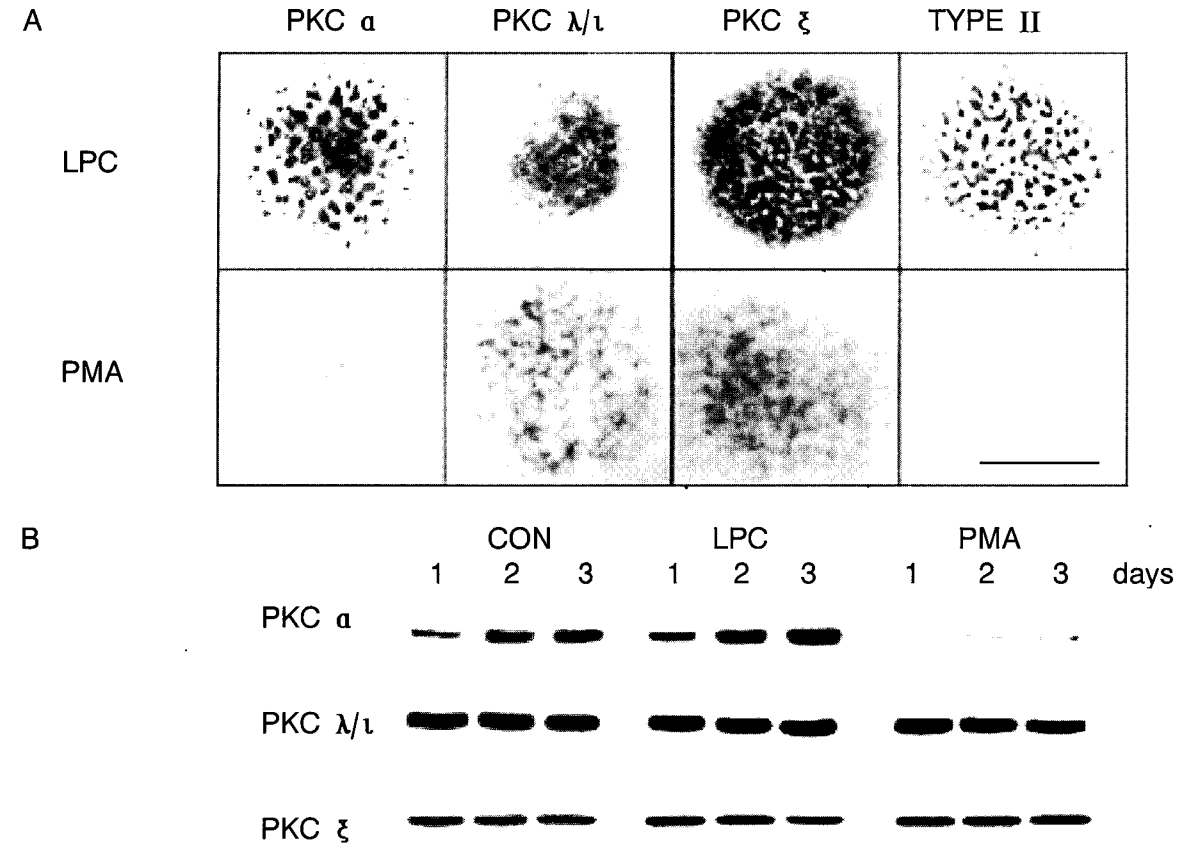

Fig. 2. The effects of LPC and PMA on the distribution and expression of PKC isoforms. A, Wing bud mesenchymal cells were cultured in the presence of 1 μM LPC or 0.1 μM PMA for 3 days and stained for PKCα, λ/ι, ζ, and type II collagen. B, Cell extracts from LPC- or PMA-treated cultures were separated on SDS-PAGE and transferred on nitrocellulose membrane. PKCα, λ/ι, or ζ was detected after incubation with corresponding antibodies following standard protocol for ECL system (Amersham). Scale bar=500 μm.

were examined. Numerous FN and N-cadherin staining regions were detectable and those regions consisted of cells under going condensation based on higher magnification photomicrograph (Fig. 4). Activation of PKC by LPC dramatically increased both FN and N-cadherin staining. Interestingly, FN was still present in PMA treated cultures.

Discussion

Two experimental approaches such as pharmacological treatment and Western blot assay have been used to explore the involvement of PKC in chondrogenic differentiation of chick limb mesenchyme. Modulation of PKC activity with PKC inhibitors or activator influences chondrogenesis (Choi et al., 1995; Chang et al., 1998; Lee et al., 1998; Yang et al., 1998). Among the PKC isoforms expressed in micromass cultures of chick limb mesenchyme, expression or activity of PKCα was correlated with the state of chondrogenic differentiation, while that of PKCλ/ι or ζ remained constant (Lee et al., 1998). It should be noted that in the micromass cultures where whole wing buds of stage 23/24 chick embryos are used, chondrogenic differentiation is triggered by cell condensation in restricted regions rather than whole culture area. Therefore, protein samples prepared for Western blot analysis are derived not only from chondrogenic cells but also from non-chondrogenic cells.

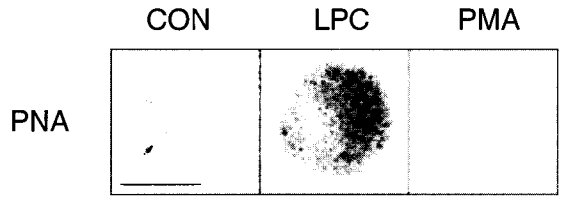

Fig. 3. Effects of LPC and PMA on the cell condensation during chondrogenesis *in vitro*. Wing bud mesenchymal cells were cultured in the absence or presence of LPC or PMA for 2 days and stained with PNA as described in 'Materials and Methods'. Arrowhead indicates a PNA-positive spot. Scale bar=250 μm.

In this study, we employed immunostaining for PKC isoforms to define the cells which are responsible for the changes in PKC expression during chondrogenesis of chick limb mesenchymal cells. Coincidence of sites for PKCα expression and cellular condensation indicates that the increase in PKCα expression observed in Western blot assay during chondrogenesis by others (Choi et al., 1995) and in this study (Fig. 2) is made by the chondrogenic cells suggesting that PKCα is involved in cell condensation.

A widespread and diffuse distribution of PKCλ/ι or ζ indicates that expression of PKCλ/ι and ζ from West-

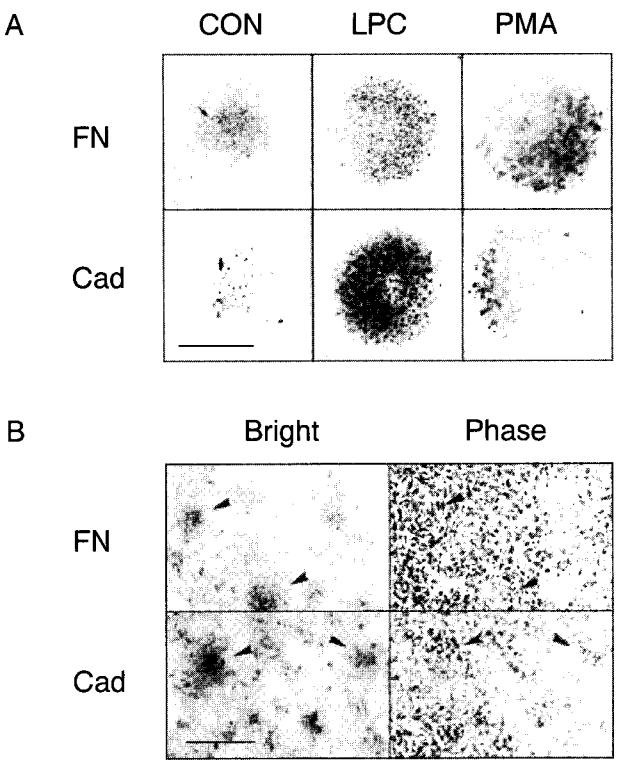

Fig. 4. Effects of LPC and PMA on the distribution of fibronectin and N-cadherin. A, Wing bud mesenchymal cells were cultured in the absence or presence of LPC or PMA for 2 days and stained for fibronectin (FN) or N-cadherin (Cad). Arrow head indicates one of FN-positive spots. B, Bright light (Bright) and corresponding phase (Phase) photomicrographs of the spots stained with anti-FN or -N-cadherin antibody at higher magnification. Arrowheads indicate the regions where cellular condensation occurs. Scale bars=100 μm(B) and 500 μm(A).

ern blot assay was made by both chondrogenic and non-chondrogenic cells. The presence of PKCλ/ι or ζ in the sites where cellular condensation occurs leaves a room for the possibility that they may be involved in cellular condensation. If PKCλ/ι and ζ participate in cellular condensation, their expression in chondrogenic sites should be correlated with the state of chondrogenesis. However, expression of the two PKC isoforms in chondrogenic sites was not influenced by LPC which enhanced chondrogenesis. Furthermore, considerable amounts of PKCλ/ι and ζ were present in PMA-treated cultures where chondrogenic region is hard to be found suggesting that PKCλ/ι and ζ are not involved in the cellular condensation. There is an interesting finding that PKCζ, which is present in most cells on day 2 of culture, became lost in nodule areas on day 3. Even though it is not likely that PKCζ is in close relation to chondrogenesis based on our results, additional study is needed to explore the relationship between PKCζ and chondrogenic differentiation.

In order to further investigate the involvement of PKC in the cellular condensation, the effect of modulation of PKC activity on the cellular condensation was examined using PNA staining. PNA is known to be a blastema marker and has been used to detect condensation (Dunlop and Hall, 1995; Miyake et al., 1996). The concurrent changes in the number of PMA-stained regions with the state of chondrogenesis clearly demonstrate that PKC plays a promoting role in cellular condensation (Fig. 4).

FN is present in the cells undergoing precartilage condensation in developing chick limb bud (Tomasek et al., 1982) and cultures of limb bud (Frenz et al., 1989), and is lost in differentiated area (Tavella et al., 1997). In addition, FN gene is maximally expressed at the early period of chondrogenesis in chick embryo primary culture (Kulyk, et al., 1989) and during early chondrocyte differentiation (Tavella et al., 1997) and is decreased thereafter. On day 2, there are many FN-stained spots in the whole cultures and the cells in the FN-stained areas are under cellular condensation as determined by phase contrast microscope, which is consistent with the previous finding (Frenz et al., 1989). FN also has been known to have physiological role in cellular condensation. Treatment of FN to the micromass cultures of chick embryo mesenchyme inhibits chondrogenesis (Swalla and Solursh, 1984), and treatment of antibody against the amino-terminal heparin-binding domain of FN and oligopeptide gly-arg-gly reduced the cell condensation (Frenz et al., 1989). The findings that LPC increased the number of FN-stained dots and induced a deep staining of FN suggest that enhancement of chondrogenesis by LPC is accomplished by the acceleration of cellular condensation through increased expression of FN.

N-cadherin is maximally expressed in the period of active cellular condensation (Oberlender and Tuan, 1994a, 1994b; Tavella et al., 1994) and is functionally required in mediating the cell-cell interactions among mesenchymal cells (Oberlender and Tuan, 1994a). There is a report that 1,25-dihydroxyvitamin D3 affects expression of N-cadherin during the chondrogenesis of chick limb bud mesenchymal cells (Tsonis et al., 1994). In this study, N-cadherin was found to be present in the areas of cellular condensation. In addition, activation of PKC by LPC caused the N-cadherin-stained dots markedly deeper than those in control cultures. These results show that PKC promotes chondrogenesis through enhancement of N-cadherin expression in the region of cellular condensation.

Interestingly, considerable amounts of FN and N-cadherin were present in the PMA treated cultures. Because there are few chondrogenic regions in the PMA treated cultures, FN and N-cadherin are expressed by non-chondrogenic cells. It has been reported that over-expression of FN in human fibrosarcoma adopted a more flattened morphology and suppressed the ability of the tumor cells to proliferate in soft agar (Akamatsu et al., 1996). PMA increased relative abundance of rat embryo E-cadherin mRNA and disrupted the morphology of rat embryonic visceral yolk sac (Chen and Hales, 1994). Therefore, it seems that the increase in the expression of FN and N-cadherin in the PMA treated cultures has negative effects on the

chondrogenesis. However, there remains a possibility that the increase in the expression of FN and N-cadherin is a result of an inhibitory effect of PMA on chondrogenesis.

In summary, we have carried out immunostaining of PKC isoforms, PNA binding protein, FN, and N-cadherin during the differentiation of chick limb mesenchyme. The immunostaining method revealed the localization of various factors in the early stage of chondrogenesis, which could not be observed by Western blot assay in previous studies. This study shows that PKCα is localized in cells which undergo cartilage differentiation and that modulation of PKC activity affects cellular condensation and expression of FN and N-cadherin in the condensing area. Taken together, these results suggest that PKCα plays a role in chondrogenesis by influencing cellular condensation through the control of FN and N-cadherin expression in chondrogenic regions.

Acknowledgements

This work was supported by grants from the Korea Ministry of Education through Research Fund (BSRI-97-4402) and from Korea Science and Engineering Foundation (971-0507-039-2).

References

Ahrens PB, Solursh M, and Reiter RS (1977) Stage-related capacity for limb chondrogenesis in cell culture. *Dev Biol* 60: 69-82.

Akamatsu H, Ichihara-Tanaka K, Ozono K, Kamiike W, Matsuda H, and Sekiguchi K (1996) Suppression of transformed phenotypes of human fibrosarcoma cells by overexpression of recombinant fibronectin. *Cancer Res* 56: 4541-4546.

Aulthouse AL and Solursh M (1987) The detection of a precartilage, blastema-specific marker. *Dev Biol* 120: 377-84.

Bareggi R, Martelli AM, Grill V, Sandrucci MA, Zweyer M, and Narducci P (1994) Protein kinase C (PKC) isoenzymes exhibit specific expression in the vertebral column of human fetuses. *Boll Soc Ital Biol Sper* 71: 83-90.

Chang SH, Oh CD, Yang MS, Kang SS, Lee YS, Sonn JK, and Chun JS (1998) Protein kinase C regulates chondrogenesis of mesenchymes via mitogen-activated protein kinase signaling. *J Biol Chem* 273: 19213-19219.

Chen B and Hales BF (1994) 12-O-tetradecanoyl-phorbol-13-acetate-induced rat embryo malformations *in vitro* are associated with an increased relative abundance of embryonic E-cadherin mRNA. *Teratology* 50: 302-310.

Choi B, Chun JS, Lee YS, Sonn JK, and Kang SS (1995) Expression of protein kinase C isozymes that are required for chondrogenesis of chick limb bud mesenchymal cells. *Biochem Biophys Res Commun* 216: 1034-1040.

Dunlop LL and Hall BK (1995) Relationships between cellular condensation, preosteoblast formation and epithelial-mesenchymal interactions in initiation of osteogenesis. *Int J Dev Biol* 39: 357-71.

Garrison JC, Pettit GR, and Uyeki EM (1987) Effect of phorbol and bryostatin I on chondrogenic expression of chick limb bud, *in vitro*. *Life Sci* 41: 2055-2061.

Goodnight J, Mischak H, and Mushinski J (1994) Selective involvement of protein kinase C isozymes in differentiation and neoplastic transformation. *Adv Cancer Res* 64: 159-209.

Frenz DA, Jaikaria NS, and Newman SA (1989) The mechanism of precartilage mesenchymal condensation: a major role for interaction of the cell surface with the amino-terminal heparin-binding domain of fibronectin. *Dev Biol* 136: 97-103.

Kosher RA, Gay SW, Kamaniz JR, Kulyk WM, Rodgers BJ, Sai S, Tanaka T, and Tanzer ML (1986a) Cartilage proteoglycan core protein gene expression during limb cartilage differentiation. *Dev Biol* 118: 112-117.

Kosher RA, Kulyk WM, and Gay SW (1986b) Collagen gene expression during limb cartilage differentiation. *J Cell Biol* 102: 1151-1156.

Kulyk WM, Coelho CN, and Kosher RA (1991) Type IX collagen gene expression during limb cartilage differentiation. *Matrix* 11: 282-288.

Kulyk WM and Reichert C (1992) Staurosporine, a protein kinase inhibitor, stimulates cartilage differentiation by embryonic facial mesenchyme. *J Craniofac Genet Dev Biol* 12: 90-97.

Kulyk WM, Upholt WB, and Kosher RA (1989) Fibronectin gene expression during limb cartilage differentiation. *Development* 106: 449-455.

Lee SR, Lee Y-S, Chun J-S, Sonn JK, and Kang S-S (1998) Lysophosphatidylcholine enhances chondrogenesis by the modulation of protein kinase C isoform expression. *Korean J Biol Sci* 2: 229-232.

Liu WS and Heckman CA (1998) The sevenfold way of PKC regulation. *Cell Signal* 10: 529-542.

Miyake T, Cameron AM, and Hall BK (1996) Stage-specific onset of condensation and matrix deposition for Meckel's and other first arch cartilages in inbred C57BL/6 mice. *J Craniofac Genet Dev Biol* 16: 32-47.

Nah HD, Rodgers BJ, Kulyk WM, Kream BE, Kosher RA, and Upholt WB (1988) In situ hybridization analysis of the expression of the type II collagen in the developing chicken limb bud. *Collagen Rel Res* 8: 277-294.

Nishizuka Y (1995) Protein kinase C and lipid signaling for sustained cellular responses. *FASEB J* 9: 484-496.

Oberlender SA and Tuan RS (1994a) Expression and functional involvement of N-cadherin in embryonic limb chondrogenesis. *Development* 120: 177-187.

Oberlender SA and Tuan RS (1994b) Spatiotemporal profile of N-cadherin expression in the developing limb mesenchyme. *Cell Adhes Commun* 2: 521-537.

Sonn JK and Solursh M (1993) Activity of protein kinase C during the differentiation of chick limb bud mesenchymal cells. *Differentiation* 53: 155-62.

Solursh M, Ahrens PB, and Reiter RS (1978) A tissue culture analysis of the steps in limb chondrogenesis. *In Vitro* 14: 51-61.

Swalla BJ and Solursh M (1984) Inhibition of limb chondrogenesis by fibronectin. *Differentiation* 26: 42-48.

Tomasek JJ, Mazurkiewicz JE, and Newman SA (1982) Nonuniform distribution of fibronectin during avian limb development. *Dev Biol* 90: 118-126.

Tsonis PA, Del Rio-Tsonis K, Millan JL, and Wheelock MJ (1994) Expression of N-cadherin and alkaline phosphatase in chick limb bud mesenchymal cells: regulation by 1,25-dihydroxyvitamin D3 or TGF-beta 1. *Exp Cell Res* 213: 433-437.

Tavella S, Bellese G, Castagnola P, Martin I, Piccini D, Doliana R, Colombatti A, Cancedda R, and Tacchetti C (1997) Regulated expression of fibronectin, laminin and related integrin receptors during the early chondrocyte differentiation. *J Cell Sci* 110: 2261-2270.

Tavella S, Raffo P, Tacchetti C, Cancedda R, and Castagnola P (1994) N-CAM and N-cadherin expression during *in vitro* chondrogenesis. *Exp Cell Res* 215: 354-362.

Yang MS, Chang SH, Sonn JK, Lee YS, Kang SS, Park TK, and Chun JS (1998) Regulation of chondrogenic differentiation of mesenchymes by protein kinase C alpha. *Mol Cells* 8: 266-271.

[Received September 4, 2000; accepted November 8, 2000]