Cell-to-cell communication in the ovarian follicle: developmental and hormonal regulation of the expression of connexin43

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The extensively developed network of cell-to-cell communication in the ovarian follicle is generated by gap junctions. In addition to the transmission of nutrients from the follicular cells to the oocyte, junctional communication in the ovarian follicle mediates the transfer of cAMP, the regulatory signal that maintains the oocyte in meiotic arrest. Luteinizing hormone (LH) interrupts cell-to-cell communication within the ovarian follicle, leading to a decrease in intra-oocyte concentrations of cAMP followed by resumption of meiosis. The developmental and hormonal regulation of the ovarian gap junction protein connexin43 (Cx43) and gene expression throughout folliculogenesis is reviewed in this article. An age-dependent increase in the amount of the Cx43 protein that was accompanied by its phosphorylation in preovulatory follicles has been observed. This protein disappeared after ovulation. The changes in both the amount and phosphorylation state of Cx43 were mimicked by exogenous administration of hormones as follows. Pregnant mare serum gonadotrophin increased Cx43 protein expression with a concurrent induction of its phosphorylation while a further human chorionic gonadotrophin injection resulted in a significant decrease of the protein. Cx43 mRNA showed a similar pattern of expression. In-vitro analysis of isolated ovarian follicles revealed that short time exposure (10 min) to LH stimulates phosphorylation of Cx43 followed by its immediate dephosphorylation, while longer incubations (8 and 24 h) with this hormone result in elimination of the protein. A significant decrease in Cx43 mRNA concentration at 24 h of incubation with LH was observed in these follicles. These results suggest that: (i) the presence of the gap junction protein in the ovary is developmentally regulated; (ii) after sexual maturation, both the amount of the Cx43 ovarian gap junction protein and its phosphorylation state are subjected to regulation by gonadotrophins; (iii) the LH-induced gating mechanism of the gap junctions in rat ovarian follicles is comprised of two steps: the immediate response is represented by a change in the phosphorylation state of the Cx43 protein, and the later response is manifested by a reduction of Cx43 protein concentration, due to attenuation of its gene expression.

Key words: connexin43/folliculogenesis/gap junctions/luteinizing hormone/rat

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Introduction

The growth of the oocyte and its development are strictly dependent upon the supply of nutrients from the follicle cells via gap junctions (Eppig, 1979; Heller *et al.*, 1981; Brower and Schultz, 1982). In addition to transmission of nutrients, junctional communication in the ovarian follicles controls the meiotic status of the oocyte. This action is mediated by the transfer of cAMP, the regulatory signal that maintains the oocyte in meiotic arrest (reviewed by Dekel, 1987; Dekel, 1988a). Resumption of meiosis, also referred to as oocyte maturation, occurs prior to ovulation and is induced by the preovulatory surge of luteinizing hormone (LH). This hormone interrupts cell-to-cell communication within a selected population of antral ovarian follicles, leading to a drop in intra-oocyte concentrations of cAMP followed by oocyte maturation (reviewed by Dekel, 1988b).

Gap junctions are specialized regions in closely opposed membranes of neighbouring cells that mediate cell-to-cell communication (Gilula *et al.*, 1972; Loewenstein, 1981). Several types of gap junction proteins, referred to as connexins, have been identified in different tissues; the somatic cells of the ovarian follicle predominantly express the 43 kDa gap junction protein designated as connexin43 (Cx43; Risek *et al.*, 1990) while the predominant oocyte connexin is apparently Cx37 (Simon *et al.*, 1997). The major involvment of Cx37 in oocyte growth and follicular development has been recently demonstrated in Cx37-deficient mice (Simon *et al.*, 1997); however, its developmental and hormonal regulation in the ovary is largely unknown. On the other hand, the regulation of the ovarian Cx43 protein and gene expression has been extensively studied by us and other laboratories and is summarized in this article.

Developmental regulation of Cx43 expression

The ontogeny of the ovarian gap junctions has been previously demonstrated by morphological examinations. Using transmission electron microscopy, Merk *et al.* (1972) described the initial appearance of gap junctions in 18 day old rat ovarian follicles. Later, more detailed ultrastructural studies (Albertini and Anderson, 1974; Anderson and Albertini, 1976) showed that plaques of gap junctions in rabbit follicle cells are initially detected around the time of antral formation and grow rapidly during expansion of the antrum, attaining their maximal size and frequency in the mature preovulatory follicle.

The use of immunocytochemistry allowed the detection of the gap junction protein, Cx43, at the surface epithelium of rat ovaries as early as at the second postnatal day (Mayerhoffer and Garfield, 1995). The expression of this protein increased in a direct correlation with follicular development and its presence became prominent at ~20 days of age. Developmental analysis of Cx43 protein and gene expression was performed by us recently using immunoblot analysis. Our findings confirmed an age-related increase in Cx43 protein expression (Figure 1; Granot and Dekel, 1997). The increase in the amount of the Cx43

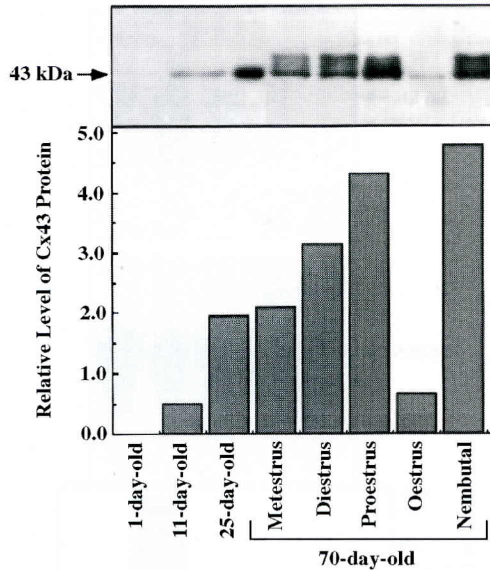

Figure 1. Developmental modulation of connexin (Cx43) protein concentration. Intact ovaries from 1, 11 and 25 day old rats, and individual follicles selected on each day of the oestrous cycle representing specific stages of development, were removed. Membranes were isolated and subjected to sodium dodecyl sulphate–polyacrylamide gel electrophoresis, followed by Western blot analysis using rabbit anti-Cx43 antibodies. Modulation of Cx43 protein concentration was evaluated by densitometric analysis. Reproduced with permission from Granot and Dekel (1997).

protein detected by immunoblotting and immunocytochemistry could account for the enlargement of the gap junction plaques observed in the previously mentioned ultrastructural studies (Albertini and Anderson, 1974; Anderson and Albertini, 1976).

Developmental analysis of the Cx43 mRNA showed a high correlation between the Cx43 protein concentration and its gene expression (Figure 2). The ovarian Cx43 mRNA was not detectable on the first postnatal day. A low concentration of this transcript was observed in ovaries of 11 day old rats followed by its further increase at the age of 25 days (Granot and Dekel, 1997). The correlation between the amounts of the protein and its mRNA indicates that the developmental regulation is at the level of gene expression.

Modulation in Cx43 expression in the mature cycling rat

Analysis of Cx43 protein expression in ovarian follicles of sexually mature rats revealed a substantially higher amount than that in the juvenile rats (Figure 1). This increase was accompanied by the appearance of two additional forms of the protein with a slightly retarded electrophoretic mobility (Granot and Dekel, 1997). The two somewhat larger proteins recognized by the highly specific anti-Cx43 antibodies have been previously shown to represent phosphorylated forms of Cx43 (Granot and Dekel, 1994).

Modifications of the Cx43 gap junction protein and gene in sexually mature

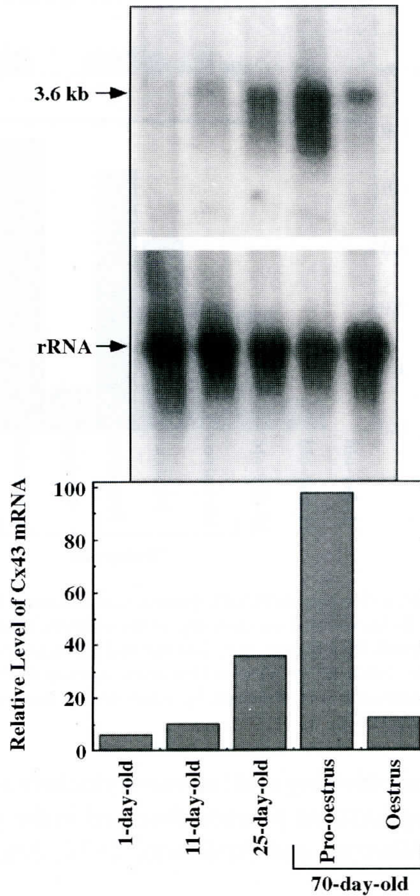

Figure 2. Developmental analysis of connexin (Cx43) gene expression. Intact ovaries from 1, 11 and 25 day old rats and selected follicles from sexually mature rats on pro-oestrus and oestrus were analysed. Total RNA was extracted and the specific Cx43 mRNA was detected by Northern blot analysis using the relevant cDNA probe. Densitometric analysis was performed and normalized according to rRNA. Reproduced with permission from Granot and Dekel (1997).

rats throughout the oestrous cycle have been studied previously by Wiesen and Midgley (1993), using immunocytochemistry and in-situ hybridization. Their study showed a decrease in the amount of Cx43 and its mRNA in the granulosa cells of the large preovulatory follicles at late pro-oestrus and early oestrus. Western blot analysis in our more recent study further demonstrated a gradual increase in Cx43 concentrations between metoestrus and pro-oestrus that was followed by almost total elimination of the protein in young corpora lutea isolated on the oestrous day of the cycle. This sharp decline was specifically related to the LH surge since the abolishment of the hypophyseal LH release by nembutal treatment of pro-oestrous rats, totally blocked the reduction in Cx43 (Figure 1, Granot and Dekel, 1997).

Similar to the results obtained in the protein analysis, we observed an increase in the Cx43 mRNA in large antral follicles of sexually mature animals on pro-

oestrus (Figure 2). This increase in mRNA was followed by a sharp reduction in the amount of the Cx43 transcript in postovulatory follicles on oestrus (Granot and Dekel, 1997).

Hormonal regulation of Cx43 expression (*in vivo*)

The experiments described above demonstrated the modulations of the ovarian Cx43 in large antral follicles upon the transition from the preovulatory to the postovulatory stage. These stages of follicular development are subjected to regulation by gonadotrophins. The effect of gonadotrophins on the ovarian gap junctions was investigated *in vivo* in the commonly used experimental model of sexually immature female rats administered with either pregnant mare's serum gonadotrophin (PMSG) alone, or with PMSG followed by a human chorionic gonadotrophin (HCG) injection. PMSG possesses intrinsic activities of follicle stimulating hormone (FSH) and LH. However, since the granulosa/cumulus cells of ovarian follicles of juvenile animals do not express receptors for LH (Zeleznik *et al.*, 1974), it is unlikely that the effect of this gonadotrophin at this stage of follicle development is related to its LH activity. On the other hand, HCG administered later does bind to the already available granulosa cells LH receptors and elicits biological responses that are similar to that of the pituitary gonadotrophin. Therefore, the use of these two hormones that represent the effects of FSH and LH, respectively, attempts to mimic the sequence of gonadotrophin secretion during the oestrous cycle.

Electron microscopy (Merk *et al.*, 1972) showed an increased number of gap junctions per cell that was induced by exogenous administration of PMSG in rats. An opposite effect has been shown in follicles of rats and rabbits administered with an ovulatory dose of HCG. The injection of this last gonadotrophin led to a significant reduction in the amount of gap junctions in the granulosa cell membranes as well as in the cumulus–oocyte complexes (Larsen *et al.*, 1981, 1986, 1987; Phillips and Dekel, 1991).

HCG has also been shown to affect gap junction permeability. Gilula *et al.* (1978) showed decreasing amounts of electrical coupling in rat cumulus–oocyte complexes after HCG injection. Administration of HCG has also been shown to have a negative effect on metabolic coupling in both sheep and rat ovarian follicles (Moor *et al.*, 1980; Sherizly *et al.*, 1988). The HCG-induced down-regulation observed at the functional level correlates with the decrease in the number of gap junctions observed in the above-mentioned ultrastructural analysis (Larsen *et al.*, 1981, 1986, 1987).

The results of our recent analysis of the Cx43 protein (Granot and Dekel, 1997) agree with these previously reported anatomical and functional examinations. We found that the changes in both the amount and phosphorylation state of Cx43 protein observed at pro-oestrus and oestrus were indeed mimicked by the exogenous administration of the hormones as follows: PMSG substantially increased the Cx43 protein expression with a concurrent induction of its

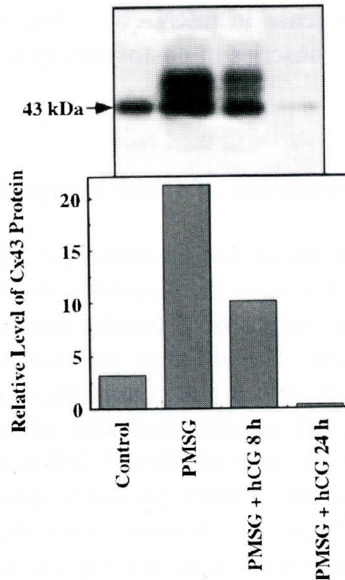

Figure 3. Gonadotrophin-induced modulations of connexin (Cx43) protein in ovarian follicles of juvenile rats. Sexually immature female rats administered with either pregnant mare's serum gonadotrophin (PMSG) alone or with PMSG followed by human chorionic gonadotrophin (HCG) 48 h later were employed. For analysis, individual representative follicles were selected. Membranes were isolated and subjected to sodium dodecyl sulphate–polyacrylamide gel electrophoresis, followed by Western blot analysis using rabbit anti-Cx43 antibodies. Modulation of Cx43 protein concentration was evaluated by densitometric analysis. Reproduced with permission from Granot and Dekel (1997).

phosphorylation while the additional injection of HCG resulted in a decrease of the signal detected at 8 h followed by its elimination at 24 h (Figure 3). The effect of these gonadotrophins was also tested on the Cx43 mRNA (Figure 4). Northern blot analysis revealed that PMSG increased the Cx43 mRNA concentration while the additional HCG injection resulted in a significant decrease in the amount of this transcript.

Hormonal regulation of Cx43 expression and post-translational modification (*in vitro*)

Previous studies demonstrated that the mechanism by which LH induces oocyte maturation consists of interruption of cell-to-cell communication within the ovarian follicle, leading to a drop in intra-oocyte concentrations of cAMP followed by resumption of meiosis (reviewed by Dekel, 1988b). Furthermore, kinetic analysis demonstrated that exposure of isolated follicles to LH for 1 h decreased the metabolic coupling in the cumulus–oocyte complexes to 50% of its initial concentration, while longer exposure to this hormone induced a moderate continuous decrease in coupling (Sherizly *et al.*, 1988). The disappearance of the gap junctions and the reduction in protein expression at later time-points after HCG administration may explain the gradual decrease in gap junction

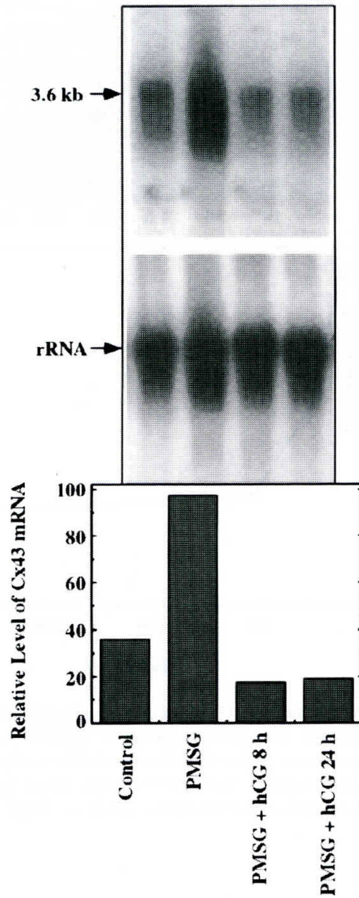

Figure 4. Effect of gonadotrophins on connexin (Cx43) mRNA concentration in ovarian follicles of juvenile rats. Sexually immature female rats administered with either pregnant mare's serum gonadotrophin (PMSG) alone or with PMSG followed human chorionic gonadotrophin (HCG) were employed. For analysis, individual representative follicles were selected. Total RNA was extracted and the specific Cx43 mRNA was detected by Northern blot analysis. Densitometric analysis was performed and normalized according to rRNA. Reported with permission from Granot and Dekel (1997).

permeability demonstrated in our previous study at longer incubations with this hormone (Sherizly *et al.*, 1988). However, these results do not reflect the short-term inhibitory effect of LH on gap junction permeability (Sherizly *et al.*, 1988). We have recently demonstrated the direct in-vitro effect of LH on the Cx43 protein by exposure of isolated rat follicles to the hormone (Figure 5). Western blot analysis of cytoplasmic membranes of freshly isolated follicles revealed that the Cx43 protein is present in multiphosphorylated forms running between 43–45 kDa. Incubation of isolated ovarian follicles with LH for 10 min increased the relative amount of the phosphorylated forms of Cx43 followed by their dephosphorylation at 30 min. Longer exposure (8 h and 24 h) to LH resulted in a total elimination of the protein (Granot and Dekel, 1994). We therefore suggested that the immediate inhibitory effect of LH on gap junction permeability (Sherizly *et al.*, 1988) could result from the changes in the phosphorylation state

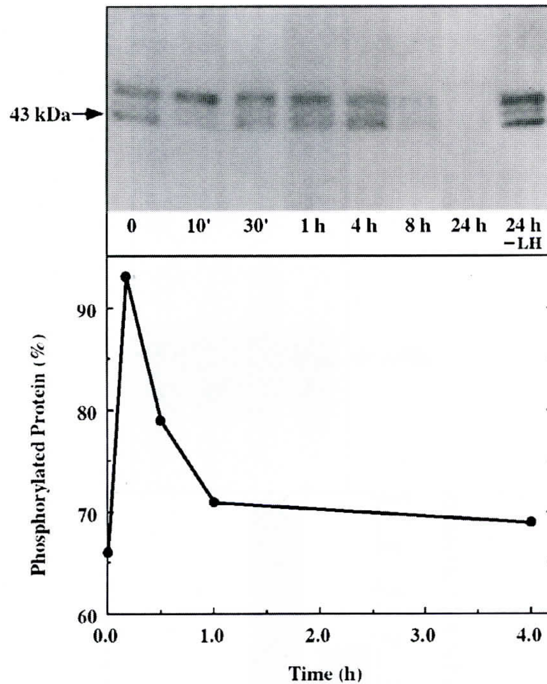

Figure 5. Time course of luteinizing hormone (LH)-induced phosphorylation of connexin (Cx43) in rat ovarian follicles. Follicles were incubated with or without LH (1 mg/ml) for the indicated periods of time. At the end of incubations, membranes were prepared and subjected to sodium dodecyl sulphate–polyacrylamide gel electrophoresis, followed by Western blot analysis using rabbit anti-Cx43 antibodies. Modulation of Cx43 protein was evaluated by densitometric analysis. Reproduced with permission from Granot and Dekel (1994).

of the gap junction protein that occurs shortly after exposure to the hormone (Granot and Dekel, 1994), leading to conformational changes that block the channels.

The idea that hormones can operate a gating mechanism by stimulating post-translational phosphorylation/dephosphorylation reactions of the gap junction protein is of particular interest (Crow *et al.*, 1990; Musil *et al.*, 1990b; Saez *et al.*, 1990; Swenson *et al.*, 1990). Changes in the phosphorylation state of Cx43, that regulate the electrical coupling and modulate the permeability of gap junctions, were previously demonstrated in other mammalian systems such as cardiac cells, fibroblasts and rat liver epithelial cells (De Mello, 1984; Crow *et al.*, 1990, 1992; Filson *et al.*, 1990; Musil *et al.*, 1990b; Swenson *et al.*, 1990; Berthoud *et al.*, 1992; Moreno *et al.*, 1992; Kanemitsu and Lau, 1993).

The possibility that the long-term hormone-induced decrease in the amount of Cx43 protein represents changes in Cx43 gene expression was confirmed by Northern blot analysis (Figure 6). This analysis revealed that exposure of isolated follicles to LH resulted in a decrease of the Cx43 mRNA by 45% after 24 h of incubation.

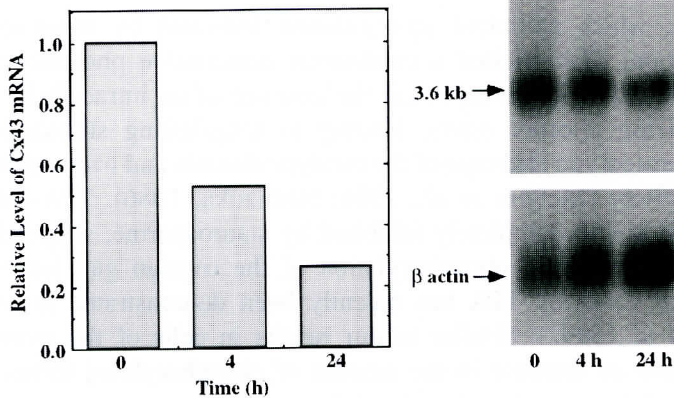

Figure 6. Effect of luteinizing hormone (LH) on connexin (Cx43) mRNA. Follicles were incubated without or with LH (1 mg/ml) for 24 h. At the end of incubations total RNA was extracted and subjected to electrophoresis on agarose gel (1%). Detection of the specific Cx43 transcript was performed by Northern blot analysis using ^{32}P-labelled cDNA probe, T7/T3 a19. A transcript of 3.6 kb was detected by autoradiography. Signal intensities were analysed by densitometry and normalized according to β-actin. Reproduced with permission from Granot and Dekel (1994).

Intracellular signalling involved in Cx43 phosphorylation and expression

The possibility that Cx43 phosphorylation is mediated by the cAMP-dependent protein kinase A (PKA) biochemical pathway was investigated by incubating isolated follicles with forskolin, a potent activator of adenylyl cyclase (Seamon and Daly, 1981). Similar to LH, forskolin induced an immediate increase of the relative intensity of the phosphorylated forms of the Cx43 protein, while longer exposure (24 h) to this agent resulted in elimination of the signal. Maximal phosphorylation of Cx43 in response to forskolin was observed at 30 min of incubation. The ability of forskolin to mimic the effect of LH on Cx43 suggests that, similar to the other biological responses to this gonadotrophin in the ovary, LH-induced phosphorylation of Cx43 is probably mediated by the cAMP-dependent PKA second messenger pathway. This idea agrees with a previous report indicating a negative regulation of gap junction permeability by cAMP-dependent phosphorylation of Cx43 in uterine smooth muscle cells (Cole and Garfield, 1986).

A gonadotrophin-releasing hormone analogue (GnRHa), which does not elevate cAMP in the ovary but is known as a protein kinase C (PKC) modulator, induces uncoupling in rat cumulus–oocyte complexes, followed by oocyte maturation (Dekel *et al.*, 1989). These findings raise the possibility that the cAMP-dependent activation of PKA is not the only biochemical pathway that modulates changes in the ovarian gap junction protein. This idea gains strong support from our demonstrations that similar to LH, GnRHa also exhibited a short-term phosphorylation/dephosphorylation effect on Cx43 protein, with a long-term effect on protein expression. The idea that PKC activation can also mediate down-regulation of communication in the ovarian follicle is further supported by our findings that tumour-promoting phorbol ester (TPA), a known activator of PKC (Castanga *et al.*, 1982), also induced phosphorylation of Cx43. Unlike LH

and GnRHa, which exhibited up-regulation followed by down-regulation of phosphorylation, TPA elicited a continuous constitutive phosphorylation. This mode of TPA action could represent the absence of an intracellular mechanism for metabolizing phorbol esters, leading to long-lasting stimulation of PKC resulting in proteolytic cleavage of the catalytic domain and irreversible activation of kinase activity (Melloni *et al.*, 1986; Nishizuka, 1986). TPA-induced Cx43 phosphorylation was completely inhibited by staurosporine, supporting the idea that PKC participates in phosphorylation of the ovarian gap junction protein. Regulation of Cx43 by TPA has recently been demonstrated in MDCK cells (Berthoud *et al.*, 1992). Similar to our results in cells of the ovarian follicle, TPA stimulated an increase in the amount of phosphorylated forms of Cx43 at the expense of the non-phosphorylated form. Furthermore, a concomitant TPA-induced inhibition of cell-to-cell communication has also been demonstrated in the above system.

Several studies provide evidence indicating that, in addition to stimulation of cAMP production, LH binding to its receptors on the plasma membranes of ovarian cells leads to the generation of another second messenger, diacylglycerol, that activates PKC (Davis *et al.*, 1984; Dimino *et al.*, 1987). This idea was confirmed by demonstrations that the effect of LH on progesterone production by rat granulosa cells is mediated by a dual signalling pathway, that includes both PKA and PKC simultaneously (Gudermann *et al.*, 1992; Morris and Richards, 1993). Our results also suggest that both PKA and PKC can participate in LH-induced Cx43 modulation in rat follicular cells.

The physiological relevance of PKC activation on regulation of cell-to-cell communication in the ovarian follicle has been further investigated by the analysis of the combined effect of staurosporine with either LH or forskolin in our system. Staurosporine is indeed considered as a very potent inhibitor of PKC. However, this compound also exhibits a weak inhibitory action towards PKA (Tamaoki *et al.*, 1986). Therefore, the partial inhibitory effect of staurosporine on the LH-induced phosphorylation of Cx43 demonstrated in our experiments could possibly reflect the interaction of this agent with PKC as well as PKA. This result, therefore, does not provide any definitive information in regard to the possible mediatory role of PKC in the induction of Cx43 phosphorylation by LH. However, the fact that the same concentration of staurosporine failed to affect the action of forskolin in our system does seem to further clarify this issue. Taken together, our results, using the combination of staurosporine with either TPA, LH or forskolin, seem to suggest that similar to the other examples mentioned above, our system is indeed mediated by a dual signalling pathway that includes both PKA and PKC.

Conclusion

In conclusion, taken together, our recent results and previous findings from other laboratories, are compatible with the following sequence of events. At the early

postnatal period the ovarian Cx43 gene expression is gradually elevated, resulting in an age-related increase in the amount of the protein. Follicular development that seems to be gonadotrophin-independent at these early stages (Greenwald and Roy, 1994) is strictly regulated by these hormones at later stages of folliculogenesis. In the large antral follicles, cell-to-cell communication becomes quite intensive due to FSH-induced elevation in Cx43 gene expression and protein translation. This gonadotrophin also stimulates post-translational modifications, manifested as Cx43 phosphorylation that may have a role in regulation of gap junction function. The pro-oestrous LH surge induces a sequence of events that reduces the permeability of the gap junctions. Binding of LH/HCG to its receptors on the ovarian cumulus/granulosa cells stimulates a concomitant activation of PKA as well as PKC. Either directly, or through an as yet unknown cascade of biochemical reactions, these kinases induce phosphorylation of Cx43, resulting in conformational modification of the protein that is immediately manifested by a decreased permeability of the gap junctions. Protein substrate(s) for PKA and/or PKC may further interact with the mRNA to interfere with Cx43 expression, resulting in a significant decrease in the net amount of the gap junction protein at oestrus. Under these circumstances intercellular communication within the ovarian follicle is terminated. Down-regulation of gap junction permeability in rat ovarian follicles has been previously shown to promote oocyte maturation (Piontkewitz and Dekel, 1993).

References

Albertini, D.F. and Anderson, E. (1974) The appearance and structure of intercellular connections during the ontogeny of the rabbit ovarian follicle with particular reference to gap junctions. *J. Cell Biol.*, **63**, 234–250.

Anderson, E. and Albertini, D.F. (1976) Gap junctions between the oocyte and companion follicle cells in the mammalian ovary. *J. Cell Biol.*, **71**, 680–686.

Berthoud, V.M., Ledbetter, M.S., Herzberg, E.L. and Saez, J.C. (1992) Connexin43 in MDCK cells: regulation by a tumor-promoting phorbol ester and Ca^{2+}. *Eur. J. Cell Biol.*, **57**, 40–50.

Brower, P.T. and Schultz, R.M. (1982) Intercellular communication between granulosa cells and mouse oocytes: existence and possible nutritional role during oocyte growth. *Dev. Biol.*, **90**, 144–153.

Castanga, M., Takai, Y., Kaibuchi, K. *et al.* (1982): Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor promoting phorbol esters. *J. Biol. Chem.*, **257**, 7847–7851.

Cole, W.C. and Garfield, R. E. (1986) Evidence for physiological regulation of myometrial gap junction permeability. *Am. J. Physiol.*, **251**, C411–C420.

Crow, D.S., Beyer, E.C., Paul, D.L. *et al.* (1990) Phosphorylation of connexin43 gap junction protein in uninfected and Rous sarcoma virus-transformed mammalian fibroblasts. *Mol. Cell. Biol.*, **10**, 1754–1763.

Crow, D.S., Kurata, W.E. and Lau, A.F. (1992) Phosphorylation of connexin43 in cells containing mutant src oncogenes. *Oncogene*, **7**, 999–1003.

Davis, J.S., Leigh, A.W. and Robert, V.F. (1984) Effects of luteinizing hormone on phosphoinositide metabolism in rat granulosa cells. *J. Biol. Chem.*, **259**, 15028–15034.

Dekel, N. (1987) Interaction between the oocyte and the granulosa cells in the preovulatory follicle. In Armstrong, D.T., Freisen, H.G., Leung, P.C.K. *et al.* (eds), *Endocrinology and Physiology of Reproduction*. Plenum Press, New York, pp. 197–209.

Dekel, N. (1988a) Spatial relationship of follicular cells in the control of meiosis. In Haseltine, F.P. (ed.), *Meiotic Inhibition: Molecular Control of Meiosis*. Alan R.Liss., New York, pp. 87–101.

Dekel, N. (1988b) Regulation of oocyte maturation by cell-to-cell communication. In Piva, F., Bardin, C.W., Forti, G. and Motta, M. (eds), *Cell-to-Cell Communication in Endocrinology*. Serono Symposium Review, Raven Press, New York, Vol. 49, pp. 181–194.

Dekel, N., Aberdam, E., Goren, S. *et al.* (1989) Mechanism of action of GnRH-induced oocyte maturation. *J. Reprod. Fertil.*, **37**, 319–327.

De Mello, W.C. (1984) Effect of intracellular injection of cAMP on the electrical coupling of mammalian cardiac cells. *Biochem. Biophys. Res. Commun.*, **119**, 1001–1007.

Dimino, M.J., Snitzer, J. and Brown, K.M. (1987) Inositol phosphates accumulation in ovarian granulosa after stimulation by luteinizing hormone. *Biol. Reprod.*, **37**, 1129–1134.

Eppig, J.J. (1979) A comparison between oocyte growth in coculture with granulosa cells and oocytes with granulosa cell–oocyte junctional contact maintained *in vitro*. *J. Exp. Zool.*, **209**, 345–353.

Filson, A.J., Azarnia, R., Beyer, E.C. *et al.* (1990) Tyrosine phosphorylation of a gap junction protein correlates with inhibition of cell-to-cell communication. *Cell-Growth-Diff.*, **1**, 661–668.

Gilula, N.B., Reeves, O.R. and Steinbach, A. (1972) Metabolic coupling, ionic coupling, and cell contacts. *Nature*, **235**, 262–265.

Gilula, N.B., Epstein, M.L. and Beers, W.H. (1978) Cell-to-cell communication and ovulation: a study of the cumulus–oocyte complex. *J. Cell. Biol.*, **78**, 58–75.

Granot, I. and Dekel, N. (1994) Phosphorylation and expression of connexin-43 ovarian gap junction protein are regulated by luteinizing hormone. *J. Biol. Chem.*, **269**, 30502–30509.

Granot, I. and Dekel, N. (1997) Developmental expression and regulation of the gap junction protein and transcript in rat ovaries. *Mol. Reprod. Dev.*, **47**, 231-239.

Greenwald, G.S. and Roy, S.K. (1994) Follicular development and its control. In Knobil, E. and Neil, J.D. (eds), *The Physiology of Reproduction*. Raven Press, New York, Vol. 1, pp. 629–724.

Gudermann, T., Birnbaumer, M. and Birnbaumer, L. (1992) Evidence for dual coupling of the murine luteinizing hormone receptor to adenylyl cyclase and phosphoinositide breakdown and Ca^{2+} mobilization. Studies with the cloned murine luteinizing hormone receptor expressed in L cells. *J. Biol. Chem.*, **267**, 4479–4488.

Heller, T., Cahill, D.M. and Schultz, R.M. (1981) Biochemical studies of mammalian oogenesis: metabolic cooperativity between granulosa cells and growing mouse oocytes. *Dev. Biol.*, **84**, 455–464.

Kanemitsu, M.Y. and Lau, A.F. (1993) Epidermal growth factor stimulates the disruption of gap junctional communication and connexin43 phosphorylation independent of 12–0 tetradecanoylphorbol 13-acetate-sensitive protein kinase C: the possible involvement of mitogen-activated protein kinase. *Mol. Biol. Cell*, **4**, 837–848.

Larsen, W.J., Tung, H.N. and Polking, C. (1981) Response of granulosa cell gap junctions to human chorionic gonadotropin (hCG) at ovulation. *Biol. Reprod.*, **25**, 1119–1134.

Larsen, W.J., Wert, S.E. and Brunner, G.D. (1986) A dramatic loss of cumulus cell gap junctions is correlated with germinal vesicle breakdown in rat oocytes. *Dev. Biol.*, **113**, 517–521.

Larsen, W.J., Wert, S.E. and Brunner, G.D. (1987) Differential modulation of follicle gap junction populations at ovulation. *Dev. Biol.*, **122**, 61–71.

Loewenstein, W.R. (1981) Junctional intercellular communication: The cell-to-cell membrane channel. *Physiol. Rev.*, **61**, 829–913.

Mayehoffer, A. and Garfield, R.E. (1995) Immunocytochemical analysis of the expression of gap junction protein connexin 32 in rat ovary. *Mol. Reprod. Dev.*, **41**, 331–338.

Melloni, E., Pontremoli, S., Michetti, M. *et al.* (1986) The involvement of calpain in the activation of protein kinase C in neutrophils stimulated by phorbol myristic acid. *J. Biol. Chem.*, **261**, 4101–4105.

Merk, F.B., Albright, J.T. and Botticelli, C.R. (1972) The fine structure of granulosa cell nexuses in rat ovarian follicles. *Anat. Rec.*, **175**, 107–126.

Moor, R.M., Smith, M.W. and Dawson, R.M.C. (1980) Measurement of intercellular coupling between oocytes and cumulus cells using intracellular markers. *Exp. Cell. Res.*, **126**, 15–29.

Moreno, A.P., Fishman, G.I. and Spray, D.C. (1992) Phosphorylation shifts unitary conductance

and modifies voltage dependent kinetics of human connexin43 gap junction channels. *Biophys. J.*, **62**, 51–53.

Morris, J.K. and Richards, J.S. (1993) Hormone induction of luteinization and prostaglandin endoperoxide synthase-2 involves multiple cellular signaling pathways. *Endocrinology*, **133**, 770–779.

Musil, L.S., Cunningham, B.A., Edelman, G.M. and Goodenough, D.A. (1990) Differential phosphorylation of the gap junction protein connexin43 in junctional communication competent and -deficient cell lines. *J. Cell Biol.*, **111**, 2077–2088.

Nishizuka, Y. (1986) Studies and perspectives of protein kinase C. *Science*, **233**, 305–312.

Phillips, D.M. and Dekel, N. (1991) Maturation of the rat cumulus–oocyte complex: structure and function. *Mol. Reprod. Dev.*, **28**, 297–306.

Piontkewitz, Y. and Dekel, N. (1993) Heptanol an alcanol that blocks gap junctions, induces oocytes maturation. *Endocr. J.*, **1**, 365–372.

Risek, B., Guthrie, S., Kumar, N. and Gilula, N.B. (1990) Modulation of gap junction transcript and protein expression during pregnancy in the rat. *J. Cell Biol.*, **110**, 269 282.

Saez, J.C., Narin, A.C., Czernik, A.J. *et al.* (1990) Phosphorylation of connexin 32, a hepatocyte gap-junction protein, by cAMP-dependent protein kinase, protein kinase C and Ca2+/calmodulin-dependent protein kinase II. *Eur. J. Biochem.*, **192**, 263–273.

Seamon, K. and Daly, J.W. (1981) Activation of adenylate cyclase by the diterpene forskolin does not require the guanine nucleotide regulatory protein. *J. Biol. Chem.*, **256**, 9799–9801.

Sherizly, I., Galiani, D. and Dekel, N. (1988) Regulation of oocyte maturation: communication in the rat cumulus–oocyte complex. *Hum. Reprod.*, **3**, 761–766.

Simon, A.M., Goodenough, D.A., Li, E. and Paul, D. (1997) Female infertility in mice lacking connexin 37. *Nature*, **385**, 525–529.

Swenson, K.I., Piwnica-Worms, H., McNamee, H. and Paul, D.L. (1990) Tyrosine phosphorylation of the gap junction protein connexin43 is required for the pp60v-src-induced inhibition of communication. *Cell. Regul.*, **1**, 989–1002.

Tamaoki, T., Nomoto, H., Takahashi, I. *et al.* (1986) Staurosporine, a potent inhibitor of phospholipid/Ca^{++} dependent protein kinase. *Biochem. Biophys. Res. Commun.*, **135**, 397–402.

Wiesen, J.E.F. and Midgley, A.R. (1993) Changes in expression of connexin 43 gap junction messenger ribonucleic acid and protein during ovarian follicular growth. *Endocrinology*, **133**, 741–746.

Zeleznik, A.J., Midgley, A.R. and Reichert, L.E. Jr (1974) Granulosa cell maturation in the rat: increased binding of human chorionic gonadotropin following treatment with follicle stimulating hormone *in vitro*. *Endocrinology*, **95**, 818–825.