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1-Methyladenine: a starfish oocyte maturation-inducing substance

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1-Methyladenine (1-MeAde) in starfish was the first compound to be identified as an oocyte maturation-inducing substance (MIS) among invertebrates in 1969 by Kanatani and co-workers. In starfish, the ripe ovary contains a huge number of fully grown oocytes of almost equal size. Each oocyte possesses a large nucleus (germinal vesicle, GV), which is arrested in late prophase of the first maturation division. The oocyte is surrounded by a single follicle layer. Such immature oocytes are not fertilisable. Resumption of meiosis in immature oocytes can be induced by 1-MeAde, and the mature oocytes thus become fertilisable (Kanatani *et al.*, 1969; Kanatani, 1985). 1-MeAde is produced by ovarian follicle cells upon stimulation with a gonad-stimulating substance (GSS) released from the radial nerves (Fig. 1).

It has been demonstrated that GSS is a peptide hormone (Kanatani *et al.*, 1971). The action of GSS on 1-MeAde production in follicle cells appears to be mediated by its receptor, G-proteins and adenylyl cyclase (Mita & Nagahama, 1991). These findings suggest that a G-protein coupled (seven transmembrane type) receptor is involved in GSS signal transduction, similarly to the pituitary–gonadal axis in vertebrates.

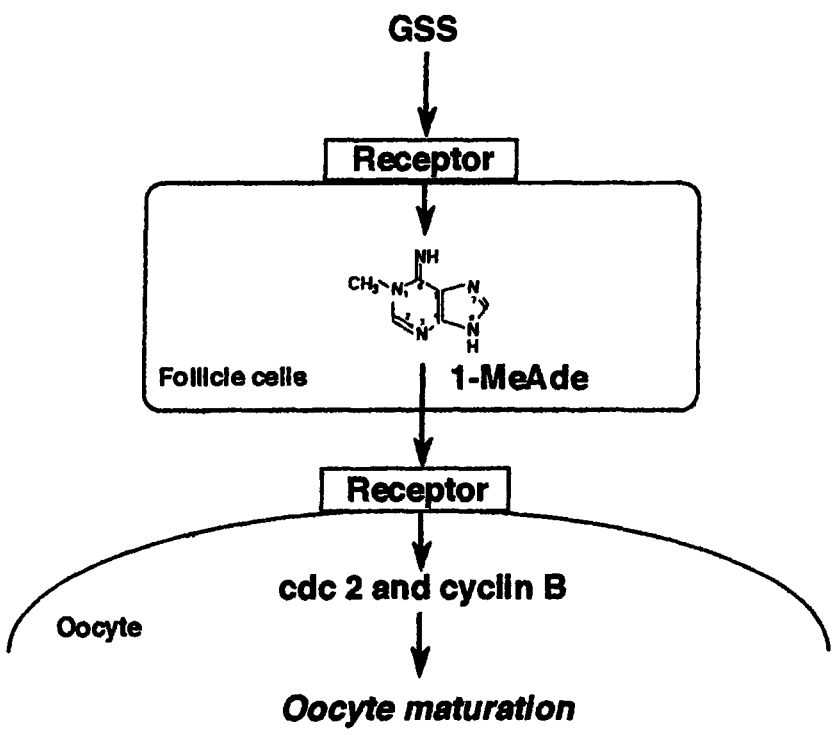


Figure 1 Hormonal mechanisms of starfish oocyte maturation.

Thus, using degenerate probes derived from consensus sequences of the mammalian glycoprotein hormone (GTH and TSH) receptors, cDNA was cloned from mRNA of ovaries of *Asterina pectinifera*. The cDNA showed striking structural homology with members of the glycoprotein hormone receptor family in the transmembrane region, and contained a very large extracellular region. Expression was observed in isolated ovarian follicle cells. Thus, it seems likely that the glycoprotein hormone receptor (GTHR) family gene is related to GSS receptor in ovarian follicle cells. The phylogenic relatedness of the starfish GTHR was also assessed in relation to other vertebrate GTHRs. The analysis showed that the starfish gene diverged before differentiation of the gonadotropin (LH and FSH) and TSH receptors in vertebrates.

In contrast to the situation in starfish, MIS is not an adenine derivative in vertebrates, being 17α, 20β-dihydroxy-4-pregnen-3-one in teleosts (Nagahama & Adachi, 1985) and progesterone in amphibians (Masui & Clarke, 1979). Nevertheless, it is important that not only 1-MeAde but also these steroids in vertebrates are synthesised by ovarian follicle cells under the influence of GSS and pituitary gonadotropins.

Previous *in vitro* studies have shown that upon GSS treatment, isolated follicle cells immediately initiate 1-MeAde production (Mita *et al.*, 1987). Within a few minutes after GSS treatment, the content of 1-MeAde in the ovaries reaches a level sufficient to induce oocyte maturation (Mita & Nakamura, 1994). It has also been shown that 1-MeAde released in response to GSS is newly synthesised, rather than originating from intracellular stores or as a breakdown product of some 1-MeAde-containing substance such as ribonucleic acid (Shirai, 1972; Mita, 1991). This suggested that 1-MeAde would be produced from intracellular adenine-related compounds. Therefore, acid extracts from ovarian follicle cells of *A. pectinifera* were analysed by high-performance liquid chromatography (HPLC). This revealed that GSS caused a reduction in the intracellular level of ATP without any change in the levels of ADP and AMP (Mita *et al.*, 1996*a*). Interestingly, the decrease in the amount of ATP following treatment with GSS was similar to the amount of 1-MeAde produced, suggesting that ATP is a good candidate for the

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precursor substance in 1-MeAde biosynthesis. Furthermore, when isolated follicle cells were preloaded with [U-^{14}C]adenine or [U-^{14}C]adenosine to accumulate radiolabelled ATP within the cells, and then further incubated with GSS, the intracellular level of radiolabelled ATP was found to decrease, concomitant with a marked increase in the level of [^{14}C]1-MeAde (Mita *et al.*, 1999*a*). However, there was no change in the levels of ADP and AMP regardless of the presence or absence of GSS. These findings strongly suggest that the adenine molecule of 1-MeAde is derived from ATP.

GSS-dependent 1-MeAde production has been shown to involve a methylation process in which S-adenosylmethionine (SAM) is used as a methyl donor (Shirai, 1972; Mita *et al.*, 1996*b*). It is noteworthy that cyclic AMP plays an important role in mediating the action of GSS on 1-MeAde production (Mita *et al.*, 1987). The methylation step in 1-MeAde production has been shown to be associated with an increase in the level of cyclic AMP (Mita, 1992). It has also been shown that 3-isobutyl-1-methylxanthine (IBMX), a potent inhibitor of cyclic AMP phosphodiesterase, induces not only 1-MeAde production but also SAM reduction in follicle cells (Mita *et al.*, 1996*b*), suggesting that cyclic AMP is the agent responsible for mediating the transfer of the methyl group from SAM to produce 1-MeAde.

In a cell-free system using follicle cells homogenised with a buffer solution, 1-MeAde was also produced from ATP and SAM in the presence of cyclic AMP in a concentration-dependent manner. Thus it was confirmed that 1-MeAde is synthesised from ATP via a methylation process involving SAM in starfish ovarian follicle cells. On the other hand, starfish ovarian follicle cells have been shown to be capable of producing 1-MeAde without GSS when they are incubated with 1-methyladenosine and 1-methylAMP (Kanatani & Shirai, 1971; Shirai & Kanatani, 1973). These substances may be intermediates of 1-MeAde biosynthesis. Recently, it was reported that a cyclic AMP-dependent protein kinase is present in follicle cells (Mita *et al.*, 1996*b*). It is possible that a unique methyltransferase is activated by protein kinase upon stimulation with cyclic AMP to produce an intermediate of 1-MeAde, although it is unclear whether ATP is methylated directly or indirectly by SAM.

Among the various adenine derivatives, only 1-MeAde has biologically effective maturation-inducing activity. In general, ATP plays a very important role in biological reactions. The chemical energy obtained from the high-energy phosphate bond can be likened to currency. ATP is also known to be used as a substrate in the production of the second messenger, cyclic AMP. This study is the first to show that ATP is a substrate for the biosynthesis of 1-MeAde, which is a kind of hormone.

It has been demonstrated that 1-MeAde controls meiosis by interacting with stereospecific receptors localised exclusively on the oocyte plasma membrane (Kanatani & Hiramoto, 1970; Yoshikuni *et al.*, 1988; Tadenuma *et al.*, 1992). Previous studies have shown that in the molecule structure, a lipophilic substituent on N-1, an amino group at the 6-position, and a cationic charge are prerequisites for maturation-inducing activity (Kanatani & Shirai, 1971; Doree *et al.*, 1976). All the active compounds are sufficiently strong bases to exist in appreciably cationic form at pH 7, the positive charges being distributed mainly in the pyrimidine ring. It has also been demonstrated that 1-benzyladenine is as active as 1-MeAde (Doree *et al.*, 1976), although the degree of biological activity decreases as the side-chain at N_1 becomes longer (Kanatani & Shirai, 1971). Recently, it was shown that among the 1-benzyladenine analogues, 1-(*p*-nitrobenzyl)adenine induced oocyte maturation in the starfish *A. pectinifera*, although its activity was about 30 times less than that of 1-benzyladenine (Saneyoshi *et al.*, 1998). In contrast, 1-(*o*-nitrobenzyl)adenine and 1-(*m*-nitrobenzyl)adenine had no maturation-inducing activity. This result suggests that the biological activity is associated with the regional specific structure of 1-nitrobenzyladenine. It is also noteworthy that 1-(*o*-nitrobenzyl)adenine and 1-(*m*-nitrobenzyl)adenine inhibit 1-MeAde-induced oocyte maturation. The inhibitory effect of 1-(*m*-nitrobenzyl)adenine is stronger than that of 1-(*o*-nitrobenzyl)adenine. It seems likely that the nitro group of the benzene ring interacts with the 1-MeAde receptor, suggesting that the regional isospecific structure of the N_1 site plays an important role in receptor binding. Furthermore, the active compound 1-nitrobenzyladenines would be useful for the design of photoaffinity labelling reagents for the 1-MeAde receptor.

With respect to hormonal action of 1-MeAde, binding to its receptor triggers transfer of its signal to the cytoplasm, followed by activation of maturation or M-phase promoting factor (MPF), which is the direct trigger of germinal vesicle breakdown (Kishimoto & Kanatani, 1976). MPF, first discovered in frog maturing oocytes, has been identified as a complex of cdc2 kinase with cyclin B (Masui & Markert, 1971). Recently, it was shown that injection of the $\beta\gamma$ subunits of G-proteins into the cytoplasm of starfish oocytes induces reinitiation of meiosis (Chiba *et al.*, 1993). Also, pertussis toxin inhibits 1-MeAde-induced oocyte maturation (Shilling *et al.*, 1989). These data suggest that a signal transduction pathway from the receptor to MPF is involved in the action of pertussis-toxin-sensitive G-proteins. Since MPF activity is similar among invertebrates and vertebrates, it is concluded that the physiological action of 1-MeAde in inducing oocyte maturation in starfish can be ascribed to the production of MPF in the oocyte cytoplasm.

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References

Chiba, K., Kotani, K., Tadenuma, H., Katada, T. & Hoshi, M. (1993). *Mol. Biol. Cell* **4**, 1027–34.

Doree, M., Guerrier, P. & Leonard, N.J. (1976). *Proc. Natl. Acad. Sci. USA* **73**, 1669–73.

Kanatani, H. (1985). *Biol. Fertilization* **1**, 119–40.

Kanatani, H. & Hiramoto, H. (1970). *Exp. Cell Res.* **61**, 280–4.

Kanatani, H. & Shirai, H. (1971). *Dev. Growth Differ.* **13**, 53–64.

Kanatani, H., Shirai, H., Nakanishi, K. & Kurokawa, T. (1969). *Nature* **221**, 273–4.

Kishimoto, T. & Kanatani, H. (1976). *Nature* **260**, 321–2.

Kishimoto, T., Usui, N. & Kanatani, H. (1984). *Dev. Biol.* **101**, 28–34.

Masui, Y. & Markert, C.L. (1971). *J. Exp. Zool.* **177**, 129–46.

Masui, Y. & Clarke, H.J. (1979). *Int. Rev. Cytol.* **57**, 185–282.

Mita, M. (1991). *Comp. Biochem. Physiol.* **99B**, 459–62.

Mita, M. (1992). *Gen. Comp. Endocrinol.* **86**, 54–62.

Mita, M. & Nagahama, Y. (1991). *Dev. Biol.* **144**, 262–8.

Mita, M. & Nakamura, M. (1994). *J. Exp. Zool.* **269**, 140–5.

Mita, M., Ueta, N. & Nagahama, Y. (1987). *Biochem. Biophys. Res. Commun.* **147**, 8–12.

Mita, M., Yasumasu, I., Nagahama, Y. & Saneyoshi, M. (1996*a*). *Dev. Growth Differ.* **38**, 413–18.

Mita, M., Yasumasu, I. & Nagahama, Y. (1996*b*). *Comp. Biochem. Physiol.* **115**, 111–16.

Mita, M., Yoshikuni, M. & Nagahama, Y. (1999*a*). *Biochim. Biophys. Acta* **1428**, 13–20.

Mita, M., Saneyoshi, M., Yoshikuni, M. & Nagahama, Y. (1999*b*). *Mol. Reprod. Dev.* **54**, 63–8.

Nagahama, Y. & Adachi, S. (1985). *Dev. Biol.* **109**, 428–35.

Saneyoshi, M., Saito, M. & Mita, M. (1998). *Zygote* **6**, S147.

Shilling, F., Chiba, K., Hoshi, M., Kishimoto, T. & Jaffe, L.A. (1989). *Dev. Biol.* **133**, 605–8.

Shirai, H. (1972). *Exp. Cell Res.* **74**, 124–30.

Shirai, H. & Kanatani, H. (1973). *Dev. Growth Differ.* **15**, 217–24.

Tadenuma, H., Takahashi, K., Chiba, K., Hoshi, M. & Katada, T. (1992). *Biochem. Biophys. Res. Commun.* **186**, 114–21.

Yoshikuni, M., Ishikawa, K., Isobe, M., Goto, T. & Nagahama, Y. (1988). *Proc. Natl. Acad. Sci. USA* **85**, 1874–7.

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