

results suggest that intracellular  $Ca^{2+}$  may play an important role in induction of *mPer1* transcription in the mouse embryonic fibroblast NIH-3T3 cells.

#### **D106** Organ Induction by Combined dose of bFGF and HGF on *Xenopus* Early Embryo

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Fibroblast growth factors (FGFs) are known to have multiple functions in early development of vertebrates including mesodermal formation, gastrulation and anteroposterior patterning. Hepatocyte growth factor (HGF) also functions in development and hepatic repair, but have no effect on organ induction from animal cap assay. In this study, the pattern of organ induction from *Xenopus* presumptive ectoderm by combined dose of bFGF and HGF were investigated. Explants were cultured in combined solutions for 3 days, examined histologically, immunohistochemically, and were analysed with RT-PCR. As a result, the synergistic effect was seen in the combined dose of bFGF and HGF rather than in the single dose of bFGF. Eyes were developed at high rates in each concentration, 1-10 ng/ml of bFGF and HGF respectively. RPE65 was detected both in induced eye and normal embryonic eye with monoclonal antibodies, 40A11 and 25F5. The expression of retinal specific opsin and muscle actin were detected by RT-PCR in explants.

#### **D107** Modulation of Thr-Phosphorylation of Integrin 1 during Muscle Differentiation

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We have previously demonstrated that the engagement of the integrin  $\alpha 7$  in E63 skeletal muscle cells by anti-7 antibodies and laminin triggered transient elevations in the intracellular free  $Ca^{2+}$  concentration ( $[Ca^{2+}]_i$ ) (Kwon et al., 2000). In this study, we extend these observations in an attempt to determine both the function of integrin phosphorylation and how phosphorylation levels are regulated. Okadaic acid treatment of E63 muscle cells induced increased phosphorylation of integrin  $\beta 1A$ , inhibition of integrin activation, loss of focal adhesions and disruption of the stress fibers. In the presence of okadaic acid, PP2A association with integrin 1A was reduced while 1D remained bound with PP2A. Both coimmunoprecipitation and in vitro phosphatase assays revealed that dephosphorylation of residues TT788-789 in the 1A cytoplasmic domain was dependent upon PP2A activity. These results suggest that PP2A may be a primary regulator of integrin 1A Thr-phosphorylation and subsequent integrin activation. Taken together, we propose that dephosphorylation or deletion of residues TT788-789 in the cytoplasmic domain of integrin 1A may contribute to the linkage of integrins to focal adhesion proteins and induce integrin activation.

#### **D108** The effects of melatonin on the in vitro immature mouse oocytes maturation and preimplantation embryos development

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Melatonin, secreted by the pineal gland, is known to regulate the ovary function and reproduction in mammals. Several reports have demonstrated that melatonin is an efficient free radical scavenger and general antioxidant. The present study was done to clarify the effects of melatonin on mouse oocytes maturation and embryos development in vitro. Melatonin ( $10^{-10}$ ,  $10^{-8}$ ,

$10^{-5}$  M) increased GVBD (Germinal Vesicle Breakdown) and first polar body formation in mouse oocytes. Furthermore, melatonin added to the medium even in the presence of dbcAMP (0.1mM) showed rather higher percentage of the GVBD and the first polar body formation compared to the dbcAMP treatment group. However, melatonin did not show any effect in the immature mouse oocytes that was arrested by hypoxanthine (3mM). Melatonin has not showed any effect in the 2-cell embryos. The present study suggests that melatonin supports immature mouse oocytes maturation but has no effect in the mouse embryos development in vitro.

**D109** Distribution of Ca<sup>2+</sup>/calmodulin-dependent protein kinase II during the mouse oocyte meiotic maturation

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During the meiosis resumption, Ca<sup>2+</sup>-transient or Ca<sup>2+</sup>-oscillation is taking place in the ooplasm. It has not been known that what is the initial trigger for transient Ca<sup>2+</sup> increase. Recently Ca<sup>2+</sup>/calmodulin dependent protein kinase II (CaM KII) was found to be a Calcium-oscillation decoder in in vitro experiment. CaM KII is a multifunctional serine/threonine kinase in various cells. Present studies were performed to investigate the distribution of CaM KII during the mouse oocytes maturation. In immunocytochemical study using monoclonal antibody of CaM KII (α-subunit), CaM KII was found to be colocalized with tubulin near the chromosome. In 6hr in vitro cultured GBVD oocyte, CaM KII was localized closer to the spindle pole than tubulin. In 3 and 4hr cultured GBVD oocytes, CaM KII was colocalized with microtubule-associated protein (MAPs). MAPs have shown to regulate the microtubule stability by binding to the

microtubule surface. In this study, α-subunit of CaM KII has shown to be expressed in brain, heart, kidney, testes and ovary. CaM KII has been expressed in the follicle, including granulosa cell and oocyte. From these results, CaM KII seems to regulate the microtubule stabilization through MAPs phosphorylation in the process of oocyte maturation.

**D110** Molecular cloning of Xenopus trithorax group brahma gene (XBrm)

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During embryogenesis, many different cells are generated in varieties of tissues and organs. They are required to maintain differential expression patterns of many important developmental regulatory genes. This maintenance system is known to be mediated through the trithorax gene (trxG) of transcriptional activator and the polycomb group (PcG) of transcriptional. Both group of genes are regulated the action of ATP-dependent chromatin remodeling complexes. Chromatin remodeling complexes mediate a change in chromatin structure, assisted by sequence-specific DNA binding trxG proteins such as BRM and GAGA, and perhaps by recruiting histone deacetylases to stably induce a hyperacetylated active state. We have isolated partial cDNA clone encoding putative Xenopus homologues of trxG related genes, XBrm. We examined that the patterns of expressions in Xenopus embryos, and putative Xenopus homologues of trxG related genes were detectable in tissues by in situ hybridization, We were revealed RT-PCR in Xenopus embryos of each stage and observed the phenotype in embryos by microinjection. RT-PCR exhibited XBrm in Xenopus embryos between egg and stage 40. The transcript was found to show strong maternal signals in the early stage, but became reduced after