

deficient in these *Gfr* mutants. We also found that *Gfr* is involved in the fucosylation of *N*-linked glycans on Notch and its *O*-fucosylation, as well as those of bulk proteins. However, despite the essential role of Notch *O*-fucosylation, the *Gfr* homozygote was viable. Thus, our results also indicate that the *Drosophila* genome encodes at least one other GDP-fucose transporter that is involved in *O*-fucosylation of Notch. Finally, our results implicate the reduction of Notch signaling in the pathology of CDG IIc.

PHOSPHOINOSITIDE PHOSPHATASE ACTIVITY COUPLED TO AN INTRINSIC VOLTAGE SENSOR

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Changes in membrane potential affect ion channels and transporters, which then alter intracellular chemical conditions. Other signalling pathways coupled to membrane potential have been suggested but their underlying mechanisms are unknown. Here we describe a novel protein from the ascidian *Ciona intestinalis* that has a transmembrane voltage-sensing domain homologous to the S1-S4 segments of voltage-gated channels and a cytoplasmic domain similar to phosphatase and tensin homologue (PTEN). This protein, named *C. intestinalis* voltage-sensor-containing phosphatase (Ci-VSP), displays channel-like 'gating' currents and directly translates changes in membrane potential into the turnover of phosphoinositides. The activity of the phosphoinositide phosphatase in Ci-VSP is tuned within a physiological range of membrane potential. Immunocytochemical studies show that Ci-VSP is expressed in *Ciona* sperm tail membranes, indicating a possible role in sperm function or morphology. Our data demonstrate that voltage sensing can function beyond channel proteins and thus more ubiquitously than previously realized

CHANGING OF PHOSPHORYLATION STATUS OF MAPKS DURING THE INDUCTION OF ANHYDROBIOSIS IN *POLYPEDILUM VANDERPLANKI*

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Anhydrobiosis is a totally ametabolic state used by for example yeast, tardigrades and nematodes. The larva of the African chironomid *Polypedilum vanderplanki* can enter anhydrobiosis, and withstand complete desiccation. In *P. vanderplanki*, specific gene expression is observed during induction of anhydrobiosis. To understand the molecular mechanisms of anhydrobiosis, understanding signal transduction should be essential. However, no study has been reported about this field except for yeast. An osmosensing mechanism in the budding yeast (*Saccharomyces cerevisiae*) involves a MAP kinase cascade. Mitogen-activated protein kinases (MAPK) are a family of serine/threonine protein kinases widely conserved among eukaryotes and are involved in many cellular programs such as cell proliferation, cell differentiation, cell movement and cell death. Here, we report the changing of activation status of MAPKs during the induction of anhydrobiosis, and discuss the role of MAPKs on the anhydrobiosis in *P. vanderplanki*.

ULTRASTRUCTURAL STUDY OF THE GRANULES OF THE EPIDERMAL MUCOUS CELLS OF THE LAND PLANARIAN, *BIPALIUM NOBILE*, FOLLOWING PEPSIN TREATMENT

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We reported that the granules of epidermal mucous cells in *Bipalium nobile* can be classified into 6 types based on observation by light and electron microscopy at the 73rd Annual Meeting. These were the lattice (L) granules, the rhabdites (R) and other four types of granules. Of the four, two of them were metachromatic but the others were not metachromatically stained with toluidine blue. Both types uniformly had high or low electron density. In the present study, we examined the disappearance of granules following treatment using the protease pepsin. After 30 minutes, we found a decrease of the four types of granules other than L and R. At 60 minutes, there remained L and R granules, but the others had almost disappeared and metachromasia was not demonstrated. These observations suggest that the four types of granules contain more protein than the L and the R granules and play different roles from the L and the R.

ANALYSIS OF THROMBOCYTES WITH A MONOCLONAL ANTIBODY IN *XENOPUS LAEVIS*

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Xenopus thrombocytes are nucleated and larger than mammalian platelets derived from megakaryocytes. Murine monoclonal antibodies to *Xenopus* peripheral blood cells were generated, and screened three independent hybridomas that produce immunoglobulin G (IgG). Immunostaining, chemical staining and *in situ* hybridization of *Xenopus* blood cells were comparatively performed to determine the specificity of the antibodies. Among them, T12 antibody was revealed to recognize intact thrombocytes, and was not compete with anti-human GpIIb/IIIa IgG, indicating that the antigen of T12 was not GpIIb/IIIa homologue in *Xenopus*. A thrombocytopenic model was furthermore developed by consecutive intracardiac injections of T12. In this model, the number of peripheral thrombocytes decreased with the nadir occurring at 3 days after injection, and methyl-green pyronine-Y and/or toluidine blue positive blood cells appeared thereafter. T12 antibody should be useful to investigate the cellular origin of thrombocytes and the mechanism(s) of thrombocytopoiesis in *Xenopus*.

IDENTIFICATION OF HEMATOPOIETIC PROGENITORS AND ERYTHROID STIMULATING ACTIVITY IN ADULT *XENOPUS LAEVIS*

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An *in vitro* semi-solid cell culture system was established to enable identification of hematopoietic progenitors in adult *Xenopus laevis*. Cells from putative erythropoietic tissues were cultured in semi-solid methylcellulose medium to form hematopoietic colonies. Serum of adult *Xenopus* with severe anemia induced by phenylhydrazine (PHZ) injections was added to stimulate the erythroid colony formation. CFU-e (colony forming unit-erythroid) was detected in normal liver, and remarkably in circulating blood of anemic *Xenopus*. In addition, red-white mixed colonies were developed from bone marrow and spleen, demonstrating the existence of bi-potential hematopoietic progenitors. As many of the major hematopoietic factors are still unknown in non-mammalian, we examined the erythropoietic activity detected in the anemic serum by this assay to analyze its characteristics. The highest activity was detected in the day4 PHZ-treated serum. Further application of this system should give us important information about *Xenopus* erythropoietin.

MORPHOLOGY AND CHARACTERISTICS OF *XENOPUS* THROMBOCYTES

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To reveal hematopoietic development in *Xenopus laevis*, morphology and cytological characteristics of thrombocytes were studied in comparison to mammalian megakaryocytes/platelets. *Xenopus* thrombocytes are known as large, nucleated, oblong shaped cells. In activated state, they change to round, tightened shape and gather through their function relating to hemostasis and thrombosis. Morphological characteristics such as lobulated nuclear chromatin, granules, microparticles and open canalicular system-like-structures were observed through transmission electron microscopy, resembling key structures of mammalian megakaryocytes and platelets. *Xenopus* thrombocytes were acetylcholinesterase positive, as megakaryocytes in rodents. *Xenopus* thrombocytes were also positive to antibodies against human fibrinogen receptor (GpIIb/IIIa) and thrombopoietin receptor (c-Mpl), indicating that major glycoproteins associated with aggregation, proliferation and differentiation in megakaryocyte/platelet lineage are conserved through phylogeny.

MAGNETIC TESTS AND FERROMAGNETIC RESONANCE ON *DAPHNIA* RESTING EGGS

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Daphnia normally reproduce by parthenogenesis. In unfavourable environments, however, robust resting eggs are induced after they switch to sexual reproduction. Resting eggs can remain viable for decades and survive in the harsh environment of a predator's digestive system: However, there is a little physico-chemical information about the resting eggs itself. A previous study reported the existence of magnetic material in *Daphnia* resting eggs (Kawasaki *et al.*, *Zool Sci.*, 2004). To further study of a magnetic characteristic of *Daphnia* eggs, field-cool (FC) and zero-field-cool (ZFC) measurements were investigated by superconducting quantum interference device (SQUID). FC and ZFC measurements showed that both of (1) superparamagnetic particles and (2) magnetic particles which size is bigger than superparamagnetic particles, exist in the eggs. A detection

of FMR of the eggs was also investigated by electron spin resonance (ESR) spectroscopy. Compared with FMR line patterns of previously reports in which FMR patterns of magnetite in ants and magnetotactic bacteria were shown, some characteristics of FMR were also detected from the spectrum of *Daphnia* eggs.

CELL BEHAVIOR DATA BASE (1) : AN ANALYSIS ON ELEMENTS OF CELL BEHAVIOR

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Cell behavior instances are described in many different books and scientific journals spread all over the world. In order to facilitate knowledge sharing and to gain more general understanding of cells as a living and acting unit, we have been accumulating cell behavioral instances in a database on a basic format of "cells do something under such and such circumstances". We report here an attempt to classify cell behavior cases using 500 of our real data. In spite that the instances are described in a variety of ways, we have concluded that these behaviors are built by 10 elements at most, namely "take in", "secrete", "migrate", "divide", "adhere", "change shape", "display", "commit suicide" and "exert tension".

CELL BEHAVIOR DATA BASE (2) : PREPARATION FOR BUILDING A CELL BEHAVIOR "ONTOLOGY"

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A database compiling complex phenomena such as cell behavior is apt to contain a wide variety of descriptions using different words of resembling meanings. For instance, "macrophage" as representative cells displaying active phagocytosis is variously named as "phagocytes", "immune cells", "granulocytes", "phago-amoebocytes" and so on according to animal species. In order to neutralize such variation of description and make the database more useful to general users, it is necessary to plant an ontology (correlation between words) into the database. We report here on an attempt to extract examples of such vocabulary-descriptive variations from some 1000 real data and to neutralize these variations.

CELL BEHAVIOR DATA BASE (3) : AN ATTEMPT TO INTRODUCE MOTION PICTURES

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One of the characteristics of our "Cell Behavior Data Base" is that it contains one or more figures for each cell behavioral instances to provide information of a high quality of help the readers understand precisely the seemingly complex phenomena of cell behavior on a common bases. The figures porvoke vivid interests not only in scientific investigators, but also in students of wide variety of fields and grades. In order to enlarge the effect of the figures of our database, we have started to take in motion pictures showing actual cell behavior cases into the database. In this report, we will show some of the examples of these motion pictures along with the technical problems we have encountered while developing a program to implant these motion pictures. We will also discuss the way to clear the copy right issues.

A NEW MODEL FOR ION ABSORPTION BY MITOCHONDRION-RICH CELLS IN MOZAMBIQUE TILAPIA: INVOLVEMENT OF Na/K/2Cl COTRANSPORTER AND Na/Cl COTRANSPORTER

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A currently accepted model for ion secretion by teleost mitochondrion-rich cells (MRCs) consists of Na/K-ATPase, Na/K/2Cl cotransporter (NKCC) and Cl channel (CFTR). However, a mechanism for ion absorption by MRCs is still controversial. We have cloned two homologues of NKCC1 (basolateral type), one homologue of NKCC2 (apical type) and one homologue of thiazide-sensitive Na/Cl cotransporter (TSC) from the gills of Mozambique tilapia. A new model for ion absorption is proposed based on localization of NKCCs and TSC.

THE MITOTIC APPARATUS OF THE SILKWORM SPERMATOCYTES CULTIVATED *IN VITRO*

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Bombyx spermatogonia are bipotential, producing two kinds of sperm, eupyrene and apyrene. Differentiation into these two kinds of sperm has remained unknown. Only eupyrene sperm bundles appeared in our long term cultivation system. In the absence of *Bombyx* hemolymph (BH), however, some spermatocytes developed into chimera sperm bundles, that disclosed the characteristics of both eupyrene and apyrene. In this study, we examined the influence of the cultivation without BH. Spermatocytes of chimera sperm bundles were as small as normal apyrene and synchronicity of meiotic division in a cyst was lost. The mitotic apparatus (MA) of eupyrene spermatocytes is barrel shaped and asters are separated from poles. On the other hand, chimera MA was feeble to induce abnormal chromosome alignment and segregation. Small asters were not separated from the poles. Chimera MA appeared much like apyrene meiotic spindle. It is suggested that chimera sperm are deviated from the course of eupyrene spermatogenesis because of some defects in the MA induced by the absence of BH. Some components of MA maybe correlated with differentiation of apyrene sperm.

THE GERMINAL PROLIFERATION CENTER OF TESTIS IN THE SILKWORM, *BOMBYX MORI*

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Silkworm males produce dimorphic sperm, eupyrene and apyrene. Eupyrene meiosis occurs after the 4th ecdysis, and apyrene meiosis starts around the spinning stage. It has been nuclear, however, that the germinal proliferation center (GPC) at the apical tip of the testicular follicle does or does not change with along the individual development. In this study, we morphologically investigated GPC from the 2nd larval instar to late pupal stages. Many spermatogonia-like cells concentrically surrounded the apical cell (AC), forming a cell cluster. The spermatogonia-like cells were divided into 3 types. (1) Cells were closely gathered around the AC. They may be germinal stem cells (GSCs). (2) Cells were scattered around the AC, but kept connection by cytoplasmic projections. They may be the primary spermatogonia. (3) The multiplying spermatogonia were wrapped by some cyst cells. The spermatocysts containing spermatogonia were separated from the AC. The structure of the GPC was unchanged from the 2nd larval stage to the proeclosion stage. These results indicate that spermatogonia are produced continuously from the GSCs up to the stage shortly before adult eclosion.

MITOTIC APPARATUS AND DISTRIBUTION OF γ -TUBULIN IN EUPYRENE AND APYRENE SPERMATOCYTES OF THE SILKWORM, *BOMBYX MORI* (LEPIDOPTERA)

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Lepidoptera males produce dimorphic sperm, eupyrene (Eup) and apyrene (Apy). To make clear the morphological differences in the two-type spermatocytes, the microtubule (MT) cytoskeleton of the mitotic apparatus (MA) was immuno-stained using antibodies against alpha- and gamma-tubulin. In Apy meiosis, irregular chromosome arrangements in metaphase, asynchronous chromosome movements and formation of chromosome bridges in anaphase, were conspicuous, while these phenomena were never observed during Eup meiosis. It was difficult to identify the kinetochore-MTs and overlap-MTs of Apy spindle especially in early anaphase. In Eup meiosis, however, shortening of kinetochore-MTs in early anaphase and elongation of overlap-MTs in the later half of anaphase was observed. Both Eup and Apy spermatocytes disclosed gamma-tubulin in the centrosomes. Faint signals were shown in the spindle region in metaphase and anaphase of Eup spermatocytes, but not in Apy. Although it is unclear how gamma-tubulin is directly related to MA formation and chromosome behavior, the difference in MT of the MA between Apy and Eup suggests one of the signs of differentiation into spermatogenesis of two-types.

PRESERVATION OF ADULT HUMAN SPERMATOGENIC CELLS BY TESTICULAR TISSUE XENOGRAFTING TO CASTRATED IMMUNODEFICIENT MICE

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Many complex aspects of testicular function in human spermatogenesis are elusively remained because of a lack of suitable *in vitro* or *in vivo* models. Recently, grafting of testicular tissue was developed and it has been used to differentiate the neonatal testes from several different species up to the level of complete spermatogenesis. In order to