

Division of Animal Sciences

Research Background

Animal products, which are nutritionally better balanced and abundant in essential amino acids, are important for human diets. In Japan, low cost and high quality livestock production must be achieved, despite the severe limitation of land resources and high labour cost in comparison with countries as U.S.A. or Australia. In order to overcome these difficulties, it is important to improve livestock production technology, especially in animal breeding, reproduction, and management. In addition to the pursuit of economic efficiency, the concept of animal welfare has recently encouraged American and European countries to provide new standards for domestic animal management. Moreover, in new industries other than livestock production, there is a global competitive environment for research on transgenic domestic animals. On the other hand, gene sequencing in domestic animals is conducted in collaboration with international consortiums. The Animal Science Division accumulated genome information and has systematically used the information to improve domestic animals and create transgenic domestic animals for other fields such as medicine. To counteract the recent decrease in the conception rate in cattle and to improve somatic cell cloning technology, we conducted basic research to solve major problems in reproductive biology. Our research on the central regulatory mechanisms in instinctive behaviour would contribute significantly to the development of animal production and husbandry. We have contributed internationally by participating in the international consortium on swine genome sequencing.

Animal Genome Research Unit

The unit aims to improve the genome resources of domestic animals and to develop systems that promote the use of genome information to contribute to “the high quality and safe production of livestock” and “the new use of livestock as physiological models of humans.” The unit also identifies genome regions related to economically important traits and develops DNA markers that can be applied to improvement of livestock and to animal identification and traceability.

Reproductive Biology Research Unit

The unit aims to improve the conception rate directly concerned with the animal industry and to establish new reproductive technology involving oogenesis and spermatogenesis. The unit investigates the proliferation and differentiation of germ cells and stem cells, the molecular mechanism of meiosis, and the process of implantation and placental formation.

Neurobiology Research Unit

The unit aims to elucidate the central mechanisms of learning, stress response, growth and reproduction in domestic animals to improve livestock management systems. The unit investigates neural functions of hypothalamic-pituitary axis and limbic-cortex system and its control mechanisms by environmental factors.

The major research topics in the fiscal year 2008 are described as follows:

Animal Genome Research Unit

Numbers of loci of swine leukocyte antigen classical class I genes differ depending on haplotypes

The major histocompatibility complex (MHC) is one of the most complicated regions in the whole mammalian genome. It encodes many proteins that are closely involved in the immune response through the presentation of peptidic antigens to T lymphocytes. In the MHC region, many paralogous genes have been generated in the process of evolution from particular ancestral genes. The process of evolution of MHC class I genes in mammals is extremely complicated and there are many types of species-specific expansions of class I genes. Consequently, the structure of the gene family of MHC class I varies among species, and it is often difficult to find counterparts of a particular class I gene in closely related species.

The porcine MHC class I genes are divided into two groups – classical and nonclassical – on the basis of their locations on the genome and their similarity to human or other mammalian class I genes. There are six classical class I genes: *SLA-1*, *SLA-2*, *SLA-3*, *SLA-4*, *SLA-5*, and *SLA-9*. Among the proteins encoded by these loci, *SLA-1*, *SLA-2*, and *SLA-3* are functional and play a role in the presentation of intracellular peptidic antigens to cytotoxic T cells. *SLA-4* and *SLA-9* are pseudogenes, and there is no evidence for the expression of *SLA-5*. An additional class I pseudogene, *SLA-11*, which has characteristics of both the classical and nonclassical class I genes, is located adjacent to the region containing the classical class I genes. The entire sequence of the porcine MHC (swine leukocyte antigen; SLA) region was completely sequenced recently by using a particular haplotype named Hp-1.1 found in a Large White pig. The sequencing of this region has demonstrated that the classical class I genes are arranged in a single contiguous region, and are ordered as *SLA-1*, *SLA-5*, *SLA-9*, *SLA-3*, *SLA-2*, *SLA-4*, and *SLA-11* from the p-terminus of the chromosome. On the other hand, there are three nonclassical class I genes, *SLA-6*, *SLA-7* and *SLA-8*, in a cluster.

Studies to date have indicated that there is copy number variance of classical class I genes in the porcine MHC region. In particular, several studies have implied that the typical classical class I gene, named *SLA-1*, which is clearly defined in the Hp-1.0 haplotype (the portion of the region containing class I genes of the Hp-1.1 haplotype), has duplications or deletions on the chromosome. On the other hand, another type of classical class I gene, named *SLA-3*, has deletions in particular haplotypes. However, genotyping of *SLA-1* and *SLA-3* in individuals whose haplotypes are not already known has often been performed by using cDNA, because the complexity and polymorphism of the sequences flanking the classical class I genes hinder the design of appropriate primers for polymerase chain reaction (PCR) amplification in genotyping. Therefore, the actual number of *SLA-1* or *SLA-3* loci on the genome has not been clearly demonstrated. We previously designed 40 microsatellite (MS) markers for the genotyping of haplotypes of *SLA*. The MS markers within the genomic region carrying MHC classical class I genes frequently produced more than two fragments from diploid genomic DNA. This implies that in these individuals there was duplication of the genomic region containing the classical class I genes, unlike in the Hp-1.0 haplotype. Previous studies have also implied that such copy number variation between haplotypes exists in MHC class I genes of other artiodactyls, i.e., cattle and sheep. In these animals, as in pigs, different haplotypes express varying numbers of class I genes. The variation in the number of class I genes expressed in cattle and sheep makes it difficult to designate locus names.

We constructed two contigs using a bacterial artificial chromosome (BAC) library, which was generated with a Landrace breed pig, covering the region carrying MHC classical class I genes. The two contigs were derived from haplotypes different from Hp-1.0. We determined the whole sequences of the BAC clones contained in the two contigs. The contig corresponding to the first

haplotype, which was designated Hp-28.0, was 541,313 bp long and ranged from the genomic region carrying *TRIM26* and other members of the *TRIM* gene family (this region is located on the side of the p-terminal flanked by *SLA-1* on swine chromosome [SSC] 7 of the Hp-1.0 sequence), to *SLA-11*, which is the class I gene most distal to the p-terminal of SSC7 in the region containing classical class I genes. The contig was extended to the middle of *TRIM39*, adjacent to *SLA-11*. The second contig, which was derived from another haplotype, which was designated Hp-62.0, was 254,346 bp long. It ranged from *TRIM10* to *SLA-4*. Southern blotting with genomic DNA used for construction of the BAC library showed that one additional locus was inferred besides the loci contained in the contig; it might have corresponded to *SLA-11* on the Hp-62.0 haplotype, although we could not isolate the BAC clones containing *SLA-11* of Hp-62.0 from the library. Both of the contigs contained the genomic region containing loci corresponding to the typical *SLA* classical class I genes *SLA-1*, *SLA-2*, and *SLA-3*.

The sequences derived from the Hp-28.0 and Hp-62.0 haplotypes contained 13 and 8 *SLA* classical class I genes, respectively (Fig 1). This was strikingly different from the case in the Hp-1.0 haplotype, which possesses seven *SLA* classical class I genes. We performed sequence comparisons of the observed loci with those on the Hp-1.0 haplotype. We detected five loci homologous to *SLA-1/SLA-3* on the Hp-28.0 haplotype (*A01*, *A03*, *A05*, *A08*, and *A10*). One of the loci corresponding to *SLA-1/SLA-3* had a 2-bp deletion in exon 5 that generated a stop codon in exon 6 (*A05*). Another locus corresponding to *SLA-1/SLA-3* had a 4-bp elongation in exon 6, resulting in a stop codon in exon 7 (*A03*). On the other hand, the Hp-62.0 haplotype had three loci (*B01*, *B04*, and *B06*) homologous to *SLA-1/SLA-3*, one of which possessed an elongation similar to *A03*, resulting in a stop codon in exon 7 (*B04*). Phylogenetic analysis using entire coding sequences or using sequences of exons 2 and 3, as in a previous report of *SLA* class I nomenclature and the database for MHC polymorphisms, IPD-MHC (<http://www.ebi.ac.uk/ipd/mhc/>), demonstrated that *A10* and *B06* were included in the clade of known *SLA-3* sequences; we therefore classified these loci into

SLA-3. Among the remaining loci similar to *SLA-1/SLA-3*, *A01*, *A08* and *B01* were grouped into a large clade containing many *SLA-1* alleles. In this study, we designated *SLA-1* for these loci similar to authentic *SLA-1*. On the other hand, *A03*, *A05*, and *B04* were grouped by themselves, away from the other *SLA-1* and *SLA-3* loci, although these loci shared two characteristic bases – C at position 51 and G at position 118 in the coding sequence (CDS) – that clearly discriminate known *SLA-1* alleles from *SLA-3*. These loci were designated *SLA-12* (Fig 1).

We assigned the blocks to make the duplication and deletion process clear. The region encompassing two characteristic repetitive sequences, 5S rRNA and *Tigger1a*, was designated block A. The regions encompassing *SLA-1*-like (*SLA-1* and *SLA-12*), *SLA-5*, *SLA-9* and *SLA-3* loci and their flanking characteristic sequences were designated B, C, D and E, respectively. There were four repetitive units in the Hp-28.0 haplotype for the sequence corresponding to blocks B, C, D, and A in the Hp-1.0 haplotype (Fig 2), although several regions were deleted in the repetitive units. The deletion of block D in the first repetitive unit (designated Hp-28.0-I) corresponded to the region carrying an *SLA-9* locus and its upstream sequence. The second repetitive unit (Hp-28.0-II) lacked a large sequence, block C and the p-terminal side of block D, ranging from the downstream of *A03* (*SLA-12*) to the (GGGGA)_n MS repeat located downstream of the absent *SLA-5* locus. The second unit also lacked the SCRE upstream of *A03* in block B. The most proximal unit to the centromere (Hp-28.0-IV) had a small deletion including a locus corresponding to *SLA-9* (block D). In the Hp-62.0 haplotype, a large duplication of the sequence covering blocks B to A was observed; it also lacked block C, including a locus corresponding to *SLA-5* and its upstream region in the unit proximal to the centromere (Hp-62.0-II). These observations show that active duplication and deletion processes have occurred in the region upstream of *SLA-3* in the respective *SLA* haplotypes (Fig 2).

The pig is considered a useful model animal for biomedical research – especially for transplantation research – because of the similarity of its organ size and cardiovascular system structure to

those in humans. In such studies or transplantation applications using pigs, precise knowledge of the expression of antigen-presenting *SLA* molecules is indispensable. Determination of copy number variance of loci of MHC genes as presented in this study contributes to the analysis of how *SLA* haplotypes affect porcine immunological responses and disease resistance, and it will also help in the experimental design of transplantation research in pigs.

Detection of QTL for average daily gain in a Large White population (a commercial pig breed)

Growth rate is one of the important traits in pork production. Many researchers have investigated quantitative trait loci (QTL) affecting growth rates (or average daily gain), and some significant QTL effects were reported. Most QTLs were detected by using F2 populations between pigs from Asian local breeds and European commercial breeds. It is well known that European

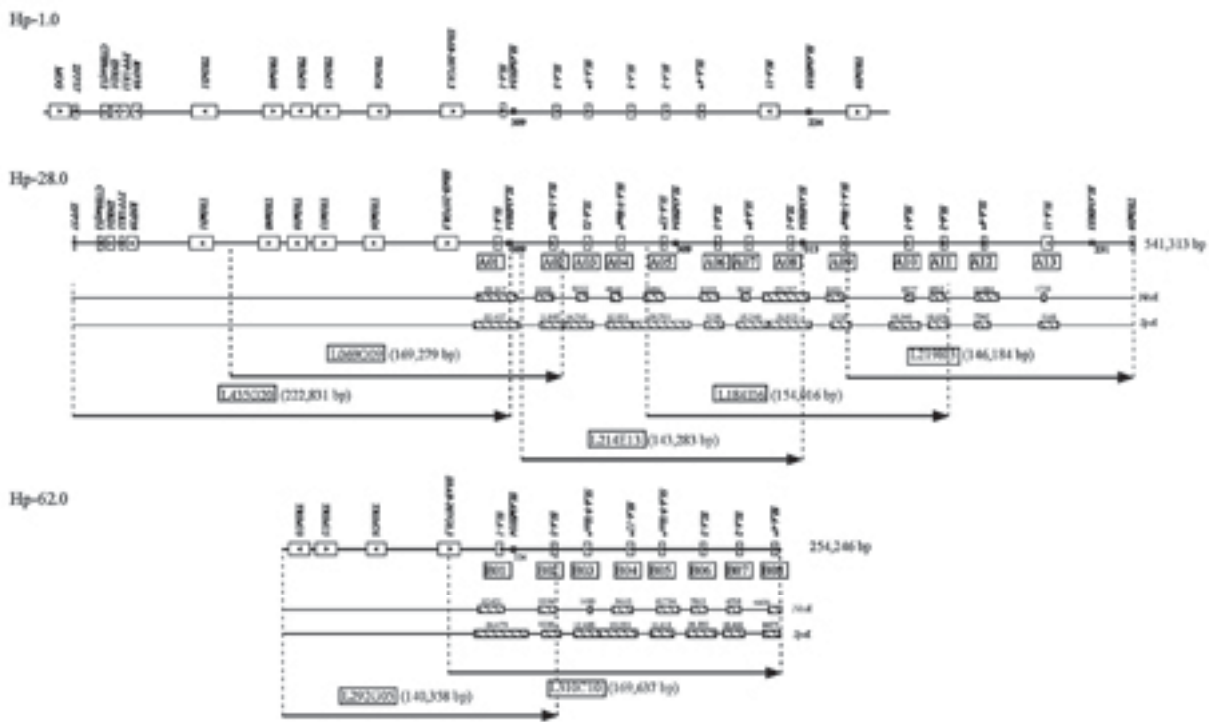


Fig 1. BAC contigs derived from two haplotypes possessed by a Landrace individual.

The haplotypes of the individual differed from haplotype Hp-1.0, which had been sequenced completely in previous studies. Genes on the genomic sequences are indicated by rectangles with arrows showing their orientation, according to a previous study. Loci with stop codon(s) in the region corresponding to CDSs or loci regarded as pseudogenes are asterisked. Detected genes corresponding to *SLA* classical class I genes are demonstrated as *A01* to *A13* on the Hp-28.0 haplotype and *B01* to *B08* on the Hp-62.0 haplotype, with the corresponding class I genes named on the Hp-1.0 haplotype. *SLA-1*-like loci with different characteristic bases, newly found in this study, were designated as *SLA-12*. The duplicated loci were followed by sequential letters (a, b and c) from the most centromeric locus. MS markers (*SLAMS034* and *SLAMS035*) from our previous reports are indicated by gray rectangles on the genomic sequences, along with the lengths of the PCR amplicons for the respective loci. BAC clones consisting of the contigs for the respective haplotypes are indicated by the large arrows with lengths in base pairs in parentheses. Locations of the fragments detected by Southern hybridization with the universal *SLA* classical class I probe are shown under the respective genomic sequences by hatched rectangles, with lengths in base pairs.

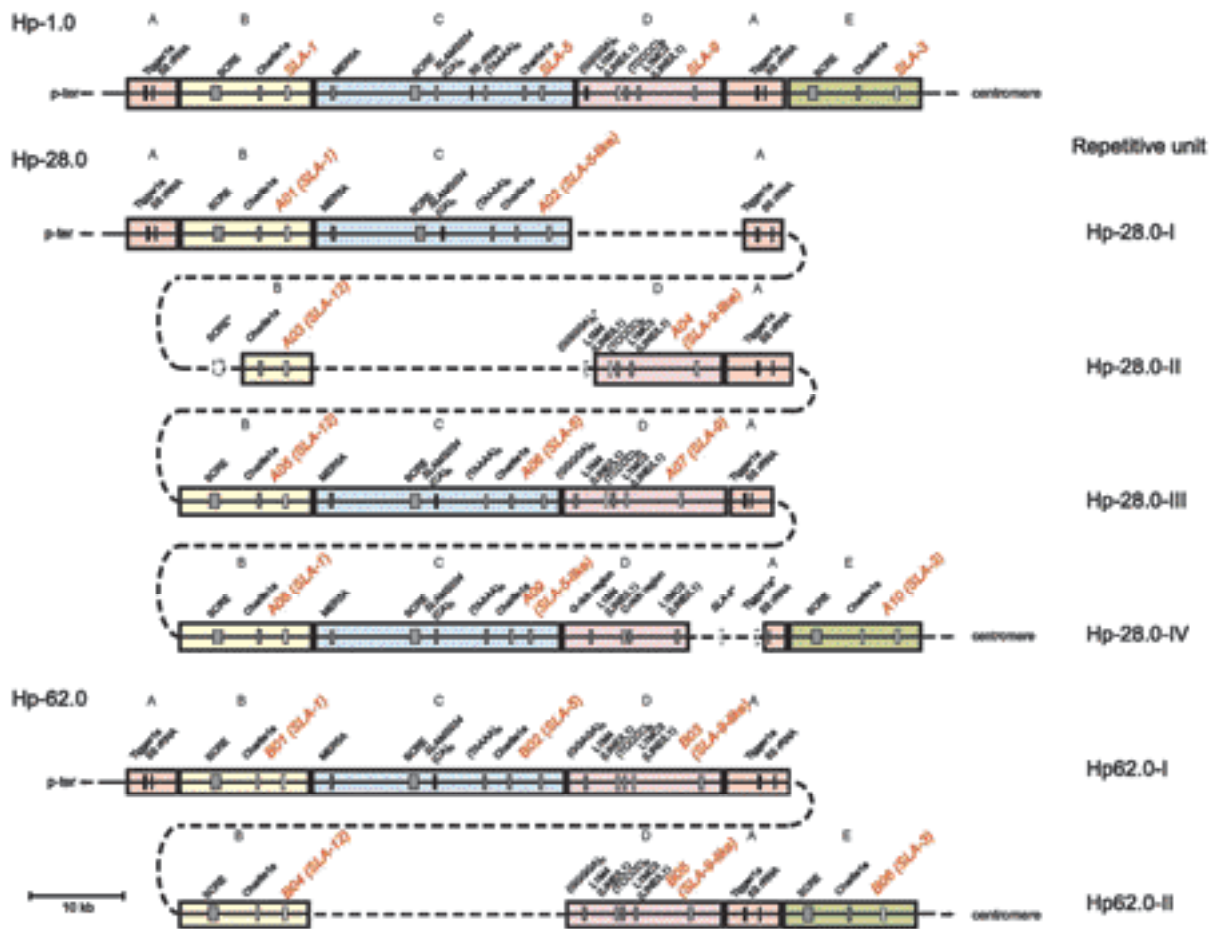


Fig 2. Structural comparison around the duplicated regions containing loci corresponding to *SLA-1*, *SLA-5*, and *SLA-9* among haplotypes.

SLA classical class I loci, as well as characteristic repetitive sequences and MS repeats adjacent to the class I loci, are indicated in the three haplotypes. The regions encompassing two characteristic repetitive sequences, 5S rRNA and Tigger1a, were designated block A. The other regions encompassing loci and their characteristic flanking sequences were designated as follows: B (*SLA-1/SLA-12*), C (*SLA-5*), D (*SLA-9*), and E (*SLA-3*). Loci that might have been deleted from the putative ancestral genomic sequence are asterisked. Repetitive units in the Hp-28.0 and Hp-62.0 haplotypes are designated Hp-28.0-I to -IV and Hp-62.0-I to -II, respectively, at the right side of the figure.

commercial breeds have been improved for many years and that they grow faster than Asian local breed pigs and wild boars. For improvement of commercial breed pigs currently, it is necessary to identify QTLs which have not been fixed in commercial breed pigs.

We have investigated QTL affecting economical traits by using F2 populations and detected three QTL for growth rates on SSC (*Sus scrofa* chromosome) 4, 7 and 8 in an F2 population between a Japanese wild boar and Large White breed pigs (Fig 3). If diversity of these QTL were detected in commercial breed pigs, their application for

pig breeding would be possible. In this study, we tried to detect the segregation of these QTL in a commercial Large White population bred in Tokushima prefecture.

We used the data from 1,063 animals derived from five sires (Table 1, 2), which were produced in Livestock Research Institute of Tokushima prefecture from 1996 to 2004. Traits for growth rate are ADG1 (average daily gain 1): from weaning (28 day) to slaughter, and ADG2: from beginning of fattening (80.3 ± 9.0 day, 38.0 ± 8.5 kg) to slaughter. Among the groups classified by the sires, average weights at slaughter are almost

equal; from 112.2 ± 6.0 kg to 113.5 ± 7.6 kg, but average rearing periods varied significantly from 177.7 ± 13.8 days to 187.2 ± 10.4 days. For growth rates, ADG1 were from 668.8 ± 59.1 g/day to 708.2 ± 71.1 g/day and ADG2 were from 712.2 ± 81.6 g/day to 762.9 ± 99.6 g/day.

Five microsatellite markers, which were heterozygous in the sires, were arranged in each of the QTL regions on SSC4, 7 and 8, and were genotyped for meat animals. The QTL regions transmitted from the sires were determined by the haplotype of microsatellite markers, and half-sib analyses for ADG1 and ADG2 were carried out with the five sires. The result revealed that segregation of QTL effects for ADG2 were

detected on SSC4 with two sires, No.404 and No.4407 (Table 3). The differences of allele effects detected for No.404 and No.4407 were 37.6 g/day and 32.8 g/day (Fig 4), respectively, while the additive effect of the QTL on SSC4 in the F2 family between Japanese wild boar and Large White was 38.3 g/day. The proportion of phenotypic variance of ADG2 explained by the QTL on SSC4 was 6.4% and 5.2% in the cases of No.404 and No.4407, respectively. These effects of the QTL for ADG2 are corresponding to approximately four days difference of the fattening period. We think that this QTL will be useful to the breeding of Large White population and now the fine mapping of this QTL is in progress.

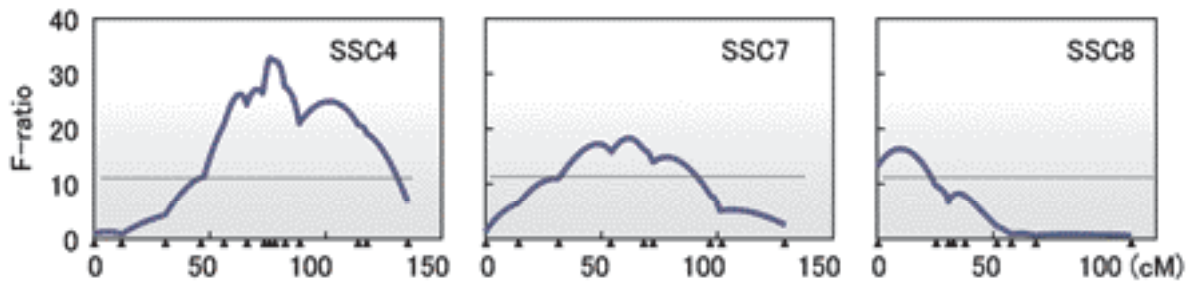


Fig 3. QTL for average daily gain detected in F2 population between Japanese wild boar and Large White breed pigs.

Table 1. Summary of the data for ADG1

Sire	Weaning		Slaughter		n	ADG1
	day	weight	day	weight		
404	28.0 ± 0.6	7.7 ± 1.6	185.4 ± 13.5	112.2 ± 6.0	205	668.8 ± 59.1
503	28.1 ± 1.0	7.3 ± 1.8	187.2 ± 10.4	113.4 ± 6.9	212	668.8 ± 47.9
2201	27.8 ± 0.8	7.9 ± 1.7	178.0 ± 14.9	112.7 ± 7.6	356	704.5 ± 71.3
4407	28.0 ± 1.0	7.9 ± 1.6	177.7 ± 13.8	113.1 ± 7.1	189	708.2 ± 71.1
8305	28.3 ± 0.7	8.0 ± 1.7	186.4 ± 14.1	113.5 ± 7.6	101	671.1 ± 62.2

± SD

Table 2. Summary of the data for ADG2

Sire	Beginning of fattening		Slaughter		n	ADG2
	day	weight	day	weight		
404	84.8 ± 6.9	38.2 ± 5.4	181.9 ± 12.8	111.8 ± 5.6	124	747.5 ± 92.9
503	81.2 ± 9.6	36.6 ± 8.7	185.1 ± 10.6	112.4 ± 6.8	119	716.5 ± 67.2
2201	80.9 ± 7.9	40.6 ± 8.6	177.4 ± 15.0	113.2 ± 7.0	298	750.3 ± 102.7
4407	74.7 ± 8.8	35.6 ± 9.0	178.3 ± 14.1	113.0 ± 7.2	166	762.9 ± 99.6
8305	78.5 ± 9.2	36.3 ± 8.3	186.9 ± 13.7	113.5 ± 7.8	94	712.2 ± 81.6

± SD

Table 3. Result of half-sib analysis of ADG1 and ADG2

	Sire	F-ratio / % variance explained			
		SSC4		SSC7	SSC8
ADG1	404	5.77		0.62	0.61
	503	0.23		0.55	0.34
	2201	0.02		0.10	0.93
	4407	3.18		0.14	0.72
	8305	1.68		1.58	0.00
ADG2	404	8.12*	6.4%	0.10	0.29
	503	0.04		0.01	0.00
	2201	0.14		0.63	0.18
	4407	7.10*	5.2%	0.05	1.05
	8305	0.28		6.38	0.12

* $p < 0.05$, multiple comparison test

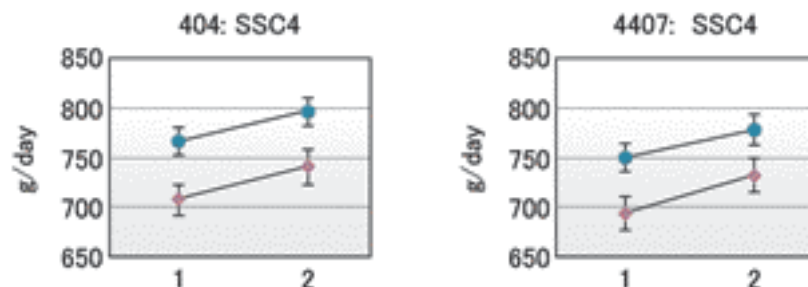


Fig 4. QTL effects detected in half-sib analyses of ADG2.

Circles and diamonds indicate the data from castrated animals and female animals, respectively. "1" and "2" means two groups of offspring classified by the alleles transmitted from the sires. Vertical bars indicate ± 1 SE.

Reproductive Biology Research Unit

Reproductive Biology Research Unit is seeking to clarify the molecular mechanisms regulating development/differentiation of germ and placental cells, and stem cells such as embryonic stem (ES) cells in mammals. The major research topics of this year are as follows:

Production of blastocysts from reconstituted oocytes derived from porcine primordial oocytes grafted into nude mice

Primordial follicles are a store for ovarian follicles and a potential resource of oocytes for medical, agricultural, and zoological purposes. We have been able to endow porcine oocytes in primordial follicles (primordial oocytes) with fertilizing ability by a combination of ovarian xenografting and subsequent in vitro culture of the collected oocytes (Kaneko et al. 2006). However, only a few of the fertilized oocytes (1%) attained the blastocyst stage probably due to a poor cytoplasmic maturation of the porcine oocytes grown in nude mice. It is important to improve the developmental competence of the recovered oocytes in order to utilize the primordial oocytes for agricultural purposes. Here we introduce a new technology that is expected to enable us to rescue the recovered oocytes with low developmental ability. Ovarian tissues from 20-day-old piglets, in which most of the follicles were primordial, were transplanted under the capsules of kidneys of ovariectomized nude mice. The mice then received continuous treatment of follicle stimulating hormone in order to enhance the growth of antral follicles in the xenografts. After in vitro maturation, porcine oocytes recovered from the xenografts were subjected to a serial centrifugation, and then, from the resulting cytoplasmic fragments, fragments containing metaphase II chromosomes (defined as karyoplasts) were selected. On the other hand, matured oocytes obtained from the prepubertal pig ovaries, which proved to have full developmental competence (Kikuchi et al. 2002), were also subjected to the same centrifugation procedures

and cytoplasmic fragments without metaphase II chromosomes (defined as cytoplasts) were selected. One karyoplast was electro-fused with 2 cytoplasts to produce reconstituted oocytes (Fig 5). These reconstituted oocytes were then transferred to in-vitro fertilization and cultured for 7 days. Out of the 56 reconstituted oocytes, 4 oocytes developed to the blastocyst stage (Fig 6): the blastocyst rate was 7%. The number of cells of the blastocysts was 13.6 ± 1.8 (mean \pm SE). These results demonstrate successful production of the reconstituted oocytes by fusion of one karyoplast, prepared from the xenogeneic oocytes, with 2 cytoplasts from the normal in vitro matured oocytes. Reconstruction of oocytes enhanced the developmental ability of the porcine oocytes grown in nude mice.

Establishment of pluripotent stem cells in domestic animals

Pluripotent stem cells would be beneficial for generating precise genetically-modified animals based on genomic information. In mice, several types of pluripotent stem cells, such as embryonic stem (ES) cells, embryonic germ (EG) cells, and induced pluripotent stem (iPS) cells, that have the ability to generate germline chimera have been established, but not in domestic animals. We have attempted to establish pluripotent stem cells in rabbit, bovine and pig, and already have isolated some candidate cell lines. Rabbit ES cells formed slightly flattened colonies in contrast to mouse ES cells. They have alkaline phosphatase activity and express several undifferentiated cells markers, such as Pou5f1 (Oct3/4). Flow cytometry analysis also showed that SSEA-1 positive cells were contained in the cells. Bovine ES-like cells showed a property almost the same as rabbit. In pig, we isolated cells that formed tight round-shape colonies like those of mouse ES cells. Although alkaline phosphatase activity and Pou5f1 (Oct3/4), *NANOG* expression were detected in the cells, they were not included the SSEA-1 positive cells. The differentiation ability

of these cells to all three germ layers and germ cells is being estimated by in vitro and in vivo assays. We have also focused on the germ-line stem (GS) cells in bovine. Using flow cytometry, we established a useful system to enrich the cells expressing germ cell marker genes from whole calf testis (Fig 7). Studies are underway to establish GS cell lines from enriched spermatogonial cells. The knowledge gained from our studies will be useful for establishing protocols for multipotent stem cell lines of domestic animals, elucidation of the mechanisms of early development, and regenerative medicine using stem cell technology.

Functions of prolactin-related proteins in ruminants

The prolactin-related proteins (PRPs) are non-classical members of the prolactin/growth hormone family. Among ruminants, they are expressed in the cotyledonary villi of cattle and goat. We investigated placental PRP in sheep in order to gain a comprehensive understanding of the function and evolution of these molecules. We also examined the sequence properties, expression and lactogenic activation of the cloned genes. We cloned two novel ovine PRPs, named oPRP1 and oPRP2. oPRP2 had a typical PRP sequence similar to bovine PRP1 (bPRP1). oPRP1 had a short sequence identical with bovine or caprine

type PRP but the reading frame was shifted. Both oPRPs were expressed in trophoblast giant binucleate cells (BNC) as in cattle and goat (Fig 8). oPRP1 expression declined from the early to the middle stage of gestation. In contrast, oPRP2 expression remained constant throughout the gestation period (Fig 9). oPRP2 was translated to form a mature protein in a mammalian cell expression system. Western blotting showed a molecular mass of 35 kDa for the FLAG-tag fusion oPRP2 protein. This recombinant protein and bPRP1 were bioassayed using Nb2 lymphoma cells; it was confirmed that neither ruminant PRP had lactogenic activity because the Nb2 lymphoma cells did not proliferate. We have identified two novel PRPs, oPRP1 and oPRP2, in ovine placenta. Both these ovine PRPs were localized and quantitatively expressed in BNC. Absence of lactogenic activity was confirmed for the oPRP2 molecule. The deduced molecular weight of oPRP1 was comparable with 16K fragment of pituitary prolactin. The 16K fragment of pituitary PRL is generated by post-translational enzymatic digestion and inhibits angiogenesis. It is anticipated that novel and known ruminant PRPs have common functions, except for lactogenic activity.

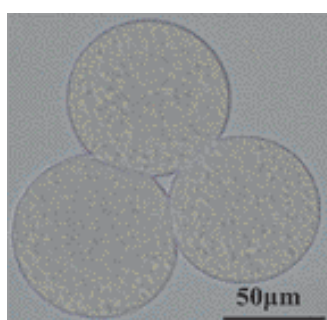


Fig 5. Three cytoplasmic fragments just after electro-fusion.

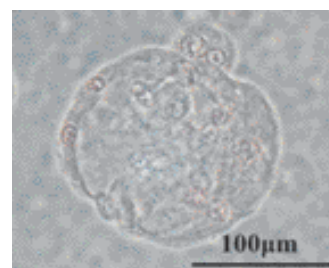


Fig 6. A blastocyst having 23 cells developed from a fusion oocyte.

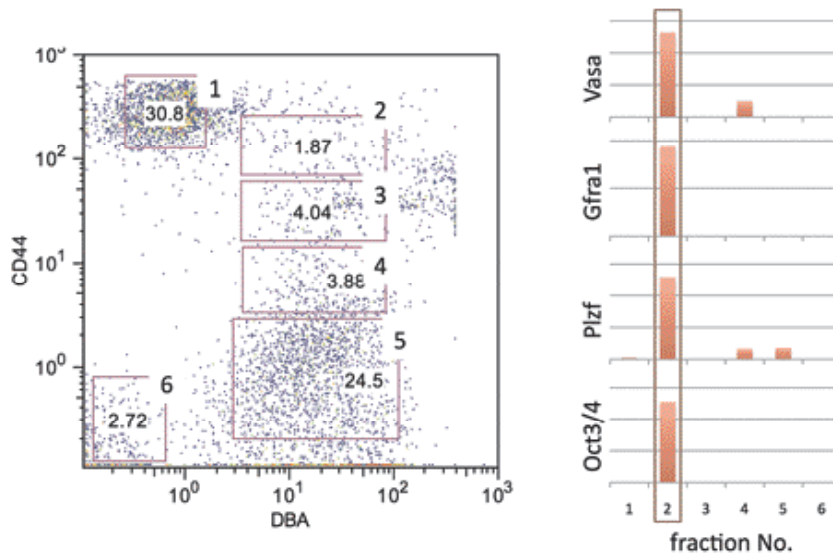


Fig 7. Enrichment of spermatogonial cells from calf testicular cells by flow cytometry.

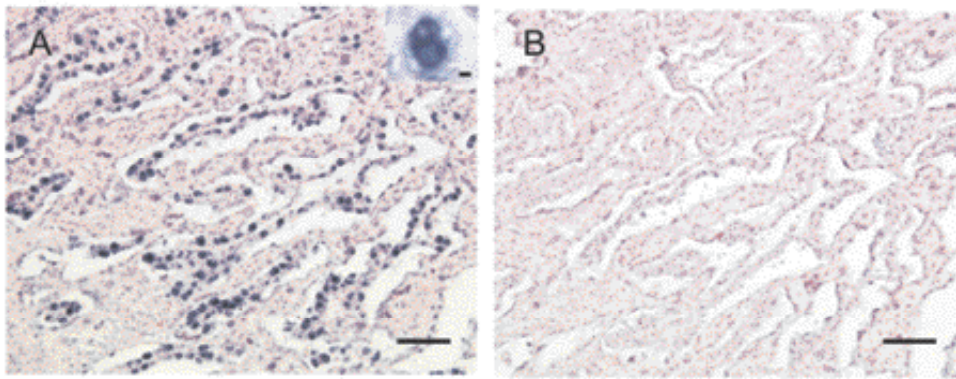


Fig 8. *In situ* hybridization of oPRP1 in ovine placenta on Day 45 of gestation. (A) anti-sense cRNA probe. (B) sense cRNA probe. Scale bars = 100 μm (main areas in A and B) and 4 μm (right upper area in A).

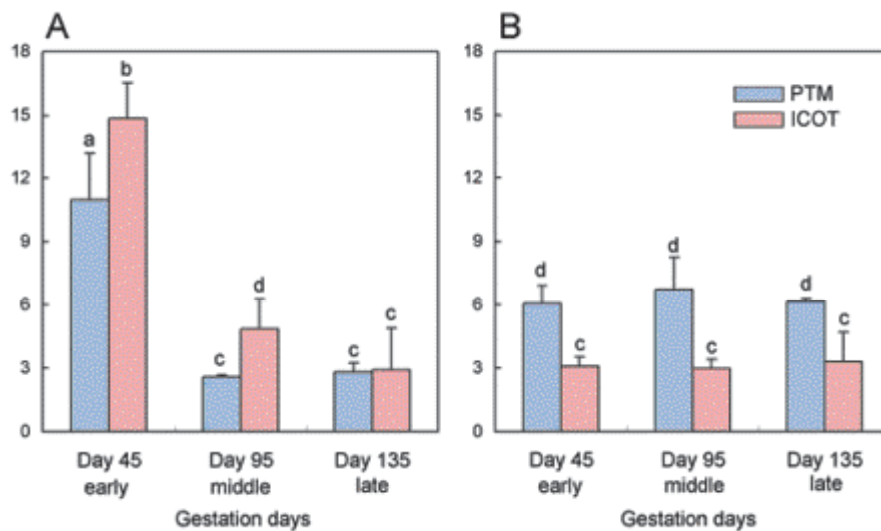


Fig 9. Real-time PCR analysis of (A) oPRP1 and (B) oPRP2 mRNA in ovine placenta. Expression of these mRNAs was normalized to the expression of GAPDH measured in the corresponding RNA preparation. Values are means ± SEM. Values with different letters (a, b, c and d) are significantly different (P < 0.05).

Neurobiology Research Unit

The role of neuromedin U and neuromedin S in modulating stress responses in cattle

The neuropeptides neuromedin U (NMU) and neuromedin S (NMS) were identified as endogenous ligands for two orphan G protein-coupled receptors, FM-3/GPR66 and FM-4/TGR-1, currently identified as NMU type-1 and type-2 (NMUR2) receptors, respectively. Since the NMUR2 is highly expressed in the central nervous system, the physiological roles of these neuropeptides in the central nervous system have been investigated mainly in rodents. Although NMU and NMS are reported to modulate stress responses mainly through corticotropin-releasing hormone (CRH) system in rodents, the *in vivo* effects of centrally administered NMU or NMS on stress regulation have not been fully elucidated in cattle. We examined adrenocorticotrophic hormone levels, body temperature, and behavioral responses to intracerebroventricularly (ICV) administered rat NMU or rat NMS in steers.

ICV NMU and NMS (0.2, 2, and 20 nmol/200 μ l) evoked a dose-related increase in plasma cortisol concentrations (CORT) (Fig 10). There was a significant time-treatment interaction for the time course of CORT ($p < 0.001$). ICV NMU evoked a

dose-related increase in rectal temperature (RT) (Fig 11). There was a significant time-treatment interaction for the change in RT from the pre-injection value ($p < 0.05$). There was a significant difference among treatments in the percentage of time spent lying (Friedman's test, $v_2 = 15.6$, $p < 0.01$) and in the total number of head shaking (Friedman's test, $v_2 = 14.49$, $p < 0.01$) (Table 1). A high dose of NMS tended to shorten the duration of lying and increase the number of head shaking.

These results together with our previous findings on the central roles of CRH and arginine vasopressin in cattle suggest that central NMU and NMS might take part in the regulation of HPA axis, body temperature, and behavioral activation via different pathways from those of rodents, at least partly. (Adapted from Yayou et al. (2009))

Reference

Yayou, Kitagawa S, Ito S, Kasuya E, Sutoh M (2009). Effects of intracerebroventricular administration of neuromedin U or neuromedin S in steers. *General and Comparative Endocrinology* **163**: 324–328)

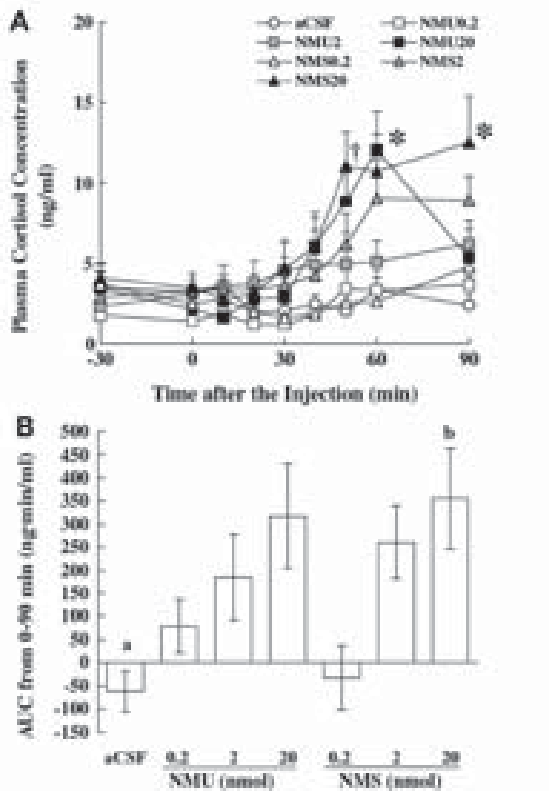


Fig 10. Effects of intracerebroventricular injections of 200µl aCSF and 0.2, 2, and 20 nmol neuromedin U(NMU) or neuromedin S(NMS) on temporal changes in mean (+SE) plasma cortisol concentrations (CORT) in streets (A) and the mean (±SE) area under the CORT curve(AUC) from 0 to 90 min after the injection (B).

* Significant difference from the pre-injection value (-30 and 0 min) ($p < 0.05$) † Tendency to differ from the pre-injection value ($p < 0.1$). Different superscript letters indicate statistical differences ($p < 0.1$ between a and b).

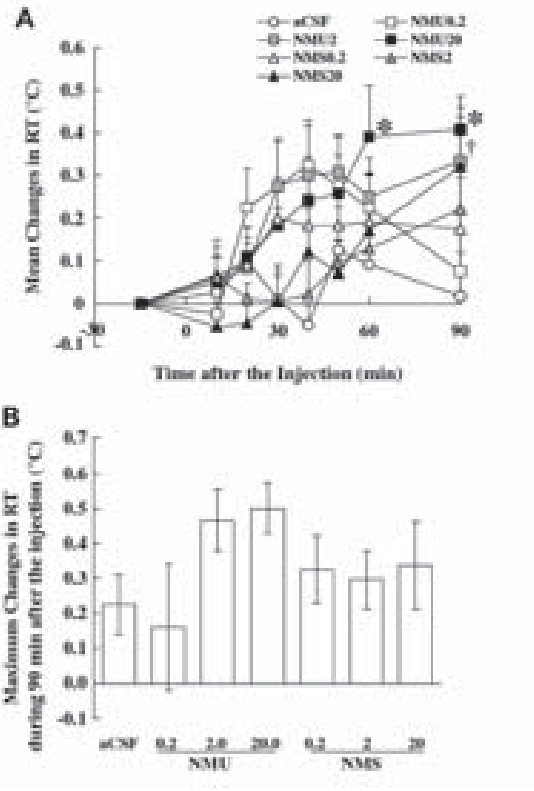


Fig 11. Effects of intracerebroventricular injections of 200µl aCSF and 0.2, 2, and 20 nmol neuromedin U(NMU) or neuromedin S(NMS) on temporal changes in mean (+SEM) change in rectal temperature (RT) from the pre-injection value (mean of values at -30 and 0 min) in streets (A) and the mean (±SE) of the maximum change in RT from the pre-injection value during 90 min after the injection (0-90min) (B).

* Significant difference from the pre-injection value ($p < 0.05$). † Tendency to differ from the pre-injection value ($p < 0.1$). There was a significant main effects of treatment ($p < 0.05$) on the maximum changes in RT.

Table 1. Effects of intracerebroventricular injections of 200µl aCSF and 0.2, 2 and 20 nmol of neuromedin U(NMU) or neuromedinsS(NMS) on behavior during 90 min after the injection in streets

	aCSF	NMU			NMS			χ^2	P
		0.2 nmol	2 nmol	20 nmol	0.2 nmol	2 nmol	20 nmol		
Lying (X)									
Mean	57.4	78.5	64.2	64.3	77	75.1*	39.4 ^{cd}	15.6	0.004
SD	28.7	16.1	11.5	11.4	9	20.7	38.4		
Resting (X)									
Mean	30.6	41.4	32.6	45.1	46.3	40	43.5	4.71	0.619
SD	8	11.6	19.6	13.6	17.9	17.4	21.2		
Self-grooming (No.)									
Mean	11	4.4	5.8	8.3	6	8.3	12	5.01	0.577
SD	10.4	4.2	7.6	5.1	5.4	10.1	18.2		
Head shaking (No.)									
Mean	0	0	0.7	0.2	0	0.2	1.7	14.49	0.009
SD	0	0	1.6	0.4	0	0.4	2		
Head rubbing (No.)									
Mean	3.3	0.8	2.7	4.3	3	3.3	4.2	7.27	0.304
SD	3.5	0.8	2.7	1.8	3	2.3	2.2		
Water across (No.)									
Mean	6.2	3	3.2	4	2.2	4	6.8	6.29	0.412
SD	4.4	3.1	3.9	2.4	2.2	3.4	7.8		

p: p-values obtained by Friedman's test.

Different superscript letters indicate statistical differences (Nemenyi multiple comparison: $p < 0.05$: between a and b, and $p < 0.1$: between c and d).