Arch. Tierz., Dummerstorf 44 (2001) 6, 677-686

[1], Research Institute of Molecular and Cell Biology, National Institute of Advanced Industrial Science & Technology, Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8566, Japan;
[2], Itoham Central Research Institute, 1-2 Kubogaoka, Moriya, Kitasoma, Ibaraki 302-0104, Japan;
[3], Kanagawa Cancer Research Institute, Nakao 1-1-2, Asahi-ku, Yokohama 241-0815, Japan;
[4], National Institute of Livestock and Glassland Science, Ikenodai 2, Kukizaki, Inashiki, Ibaraki 305-0901, Japan

SHIZUYO SUTOU[1], MASAAKI KONDO[2], MIYUKI MATSUDA[3], KAZUHIKO KAWAKURA[4], YASUHIDE OHINATA[1], YOUJI MITSUI[1], and SHOICHI MATSUKUMA[3]

# H-Y antigens as Y chromosome-encoded gene products and serologically detectable male antigens (SDM) as testis- or spermatogenesis-linked autosomal gene products

## Summary

Male-specific transplantation antigen H-Y was proposed to be the testis-determining factor (TDF) in 1975, while SRY was found to be the TDF gene in 1990. What then of H-Y antigen? H-Y antigen was categorized into two entities, viz., T-cell mediated H-Y antigens (H-Y) and serologically detectable male antigens (SDM). Several H-Y genes such as *Smcy*, *Uty* and *Dty* have been identified and these are all Y-linked. H-Y is male-specific and clinically important in cell, tissue, or organ transplantations. Male-enhanced antigen 1 (*Mea1*) was isolated from an expression library using polyclonal anti-H-Y antibody. We cloned and characterized mouse and bovine *Mea1/MEA1*, the gene product of which was mainly localized in elongated spermatids. *Mea2* was identified using monoclonal anti-H-Y antibody. MEA2 protein is localized in the Golgi apparatus of spermatocytes and spermatids. Colocalization of MEA2 protein with γ-adaptin in clathrin-coated vesicles was demonstrated. Disruption of *Mea2* resulted in spermatogenic failure. These findings suggest that *Mea2* is involved in transportation of materials needed for acrosome components in spermatogenesis. Its human homologue is Golgin-160 which was detected in an SLE autoimmune disease patient. Müllerian inhibiting substance (MIS) was reported to have SDM activity. Taken together, SDM is a collective name for protein antigens associated with testis activity (MIS) or spermatogenesis (MEA1, MEA2) and may be antigenic when expressed in females. From the viewpoint of autoimmune diseases, the identification and characterization of SDMs will be clinically important.

Key Words: H-Y antigen, spermatogenesis, serologically detectable male antigens, SDM, *Mea1*, *Mea2*, MIS

## Zusammenfassung

Titel der Arbeit: **Serologische nachweisbare männliche Antigene (SDM): HY-Antigene, die codiert sind auf dem Y-Chromosom, charakterisiert als Testis- oder Spermatogenesis-linked autosomale Genprodukte**

HY-Antigene werden in zwei Kategorien eingeteilt: T-zellen-vermittelte HY-Antigene und serologisch nachweisbare männliche Antigene (SDM). SDM umfassen Protein Antigene, die mit der Testis-Aktivität (MIS) oder Spermatogenese (MEA1, MEA2) im Zusammenhang stehen und antigenetisch im weiblichen Organismus fungieren können. Im Hinblick auf Autoimmunkrankheiten ist die Identifizierung und Charakterisierung der SDM von klinischem Interesse.

Da HY-Antigen männlich spezifisch ist, sind sie von klinischer Bedeutung bei Zell-, Gewebe- und Organtransplantationen. In der vorliegenden Arbeit wurde das MEA1-Protein bei Maus und Rind geklont und charakterisiert. Das Genprodukt war hauptsächlich in verlängerten Spermatiden lokalisiert, währenddessen das MEA2-Protein im Golgiapparat der Spermatozyten und Spermatiden nachgewiesen wurde. Weiterhin wurde neben MEA2-Protein das γ-Adaptin in Vesikeln gefunden. MEA 2-Protein ist erforderlich für die Akrosomenreaktion und es ist homolog zum Golgin 160, das bei SLE-autoimmunerkrankten Patienten nachgewiesen wurde. Es wurde berichtet, dass die Müllersche Hemmsubstanz (MIS) SDM-Aktivität besitzt.

Schlüsselwörter: HY-Antigen, Spermatogenese, serologisch nachweisbares männliches Antigen, SDM, Mea1, Mea2, MIS

## Introduction

Originally, H-Y antigen (histocompatibility-Y) was postulated as a male-specific transplantation antigen in skin-graft experiments using genetically identical mice in which male grafts were rejected by females, but female grafts not by males (EICHWALD and SILMSER, 1955). So far as the method to detect H-Y antigen was limited to skin-grafting, tests were also limited to mice and rats by availability of isogenic animals. Antibody against male cells, however, was found in females which had rejected male skin-grafts or had been immunized with male cells (GOLGBERG et al., 1971). The antibody expanded the H-Y world from mammals to birds, amphibians, and arthropods in which heterogametic cells (males (XY) in mammals, females (ZW) in chicks, females (ZW) in *Xenopus* frogs, males (XY) in leopard frogs, and not female but male cells in lobsters) were shown to have H-Y antigen (SHAREV et al., 1980; WACHTEL et al., 1974, 1975a). On the basis of these findings and others, the late Dr. S. Ohno and his colleagues put forward the H-Y antigen hypothesis of sex-determination where transplantation antigen H-Y works as a tissue-inducing factor to entice the testes in the X-Y sex-determination system or ovaries in the Z-W system (WACHTEL et al., 1975b).

Upon the understanding that one and the same H-Y antigen determines the sex of a wide variety of living organisms, many attempts to identify the H-Y antigen-coding gene and to isolate the antigen itself were made. In these attempts, some controversial data were generated. For example, a male mouse whose skin was not rejected by females but whose cells were reactive to anti-H-Y antibody was produced after X-irradiation (MELVOLD et al., 1977). Scrutinizing accumulated data, SILVERS et al. (1982) argued that H-Y antigen detected by cell-mediated reaction (referred to as H-Y hereinafter) and serologically detectable antigens (referred to as SDM hereinafter) must be different entities.

Before a gene encoding H-Y and the chemical nature of H-Y was identified, H-Y was negated as TDF/Tdy. Sex-reversal mice (Sxr, now called $Sxr^a$) with 40, $XXsxr^a$, in which most of the Y chromosome short arm was translocated to its long arm and in turn to the long arm of the X chromosome at meiotic recombination, became males and were H-Y antigen-positive, supporting the theory of testis-induction by H-Y. Another sex-reversal mouse (now called $Sxr^b$) with 40, $XXsxr^b$, a deletion mutant derived from $Sxr^a$, however, was Tdy-positive and H-Y-negative, indicating that the testis is not induced by H-Y (MC LAREN et al., 1984). $Sxr^a$ mice with 39, $Xsxr^a$ could produce sperm even if abnormal, but entire spermatogenesis was lacking in $Sxr^b$ mice with 39, $Xsxr^b$, suggesting that H-Y is involved in spermatogenesis (BURGOYNE et al., 1986). In humans too, H-Y is not TDF; TDF is located on the short arm of the Y chromosome, and the H-Y gene on the long arm (SIMPSON et al., 1987). *Sry* was unequivocally identified as Tdy using *Sry*-transgenic mice in which mice with 40, XX chromosomes developed the testes (SINCLAIR et al., 1990).

The first H-Y was identified in 1995 (SCOTT et al.). H-Y was not one and the same; several H-Ys have been clarified as Y-linked genes (Table). SDM was more elusive. What is the molecule that commonly appears on the heterogametic cells of mammals, birds, frogs, and arthropods? The acrosomal region of mouse sperm was shown to harbor SDM, but its chemical nature was not identified (KOO et al., 1973). Müllerian inhibiting substance (MIS) secreted from Sertoli cells was reported to have SDM activity (MÜLLER et al., 1993). Does MIS appear on the heterogametic cell surface of

mammals, birds, frogs, and arthropods? No. Through research into *Mea1*/*MEA1* which was isolated using polyclonal anti-H-Y antibody and *Mea2* which was isolated using monoclonal anti-H-Y antibody, we reached the conclusion that SDM is not one and the same, but consists of several or many protein antigens that are involved in testicular activities or spermatogenesis. We emphasize that research into SDM is of importance from the clinical view point. Here we present a total image of H-Y antigen consisting of H-Y and SDM.

Table
The wide variety of H-Y antigens

| Animals | Gene | Location[a] | H-Y species | Peptide/product | Reference |
|---|---|---|---|---|---|
| **Transplantation/T cell-mediated H-Y antigens** | | | | | |
| Mouse | *Smcy* | Y | H-YK$^k$ | TENSGKDI | Scott et al., 1995 |
| | *Smcy* | Y | H-YD$^k$ | | Simpson et al., 1997 |
| | *Smcy* | Y | H-YD$^b$(B6.1.16) | KCSRNRQYL | Markiewicz et al., 1998 |
| | *Uty* | Y | H-YD$^b$(CTL10) | WMHHNMDLI | Greenfield, 1996 |
| | *Dby* | Y | H-YA$^b$ | NAGFNSNRANSSRSS | Scott et al., 2000 |
| | *Dby* | Y | H-YE$^k$ | REEALHQFRSGRKPI | ibid. |
| Mouse and rat | ? | ? | | KQYQKSTER | Simmons et al, 1997 |
| | ? | ? | | AVLNKSNREVR | ibid. |
| Human | *SMCY* | Y | HLA-B7 | SPSVDKARAEL | Wang, 1995 |
| | *SMCY* | Y | HLA-A2 | FIDSYICQV | Meadows et al., 1997 |
| | *UTY* | Y | HLA-B8 | LPHNHYDL | Warren et al., 2000 |
| | *DFFRY* | Y | HLA-A1 | | Vogt et al., 2000 |
| **Serologically detectable male (SDM) antigens** | | | | | |
| Mouse | ? | ? | | (Acrosome cap) | Koo et al., 1973 |
| | *MIS* | A (10) | | Müllerian inhibiting substance | Müller et al., 1993 |
| | *Mea1* | A (17) | | Male-enhanced antigen-1 | Lau et al., 1989, Kondo et al., 1996 |
| | *Mea2* (*Golga3*) | A (5) | | Male-enhanced antigen-2 | Su et al., 1992; Kondo & Sutou, 1997 |
| Human | *GOLGA3* | A (12) | | Golgin-160 | Flitzler et al., 1993 |

a. Y: Y Chromosome; A (): autosome (chromosome number).

## Materials and Methods

Mouse *Mea1* cDNA was originally isolated by screening a mouse testis cDNA library with a radio-labeled PCR product (LAU et al., 1989). We cloned bovine *MEA1* cDNA

by screening a bovine testis cDNA library with a radio-labeled mouse *Mea1* cDNA probe. Bovine *MEA1* genomic DNA was isolated by screening a bovine genomic DNA library with a radio-labeled bovine *MEA1* cDNA. Characterization of *Mea1/MEA1* by Southern and Northern blottings was reported previously (KONDO et al., 1996a). Bovine *MEA1* cDNA was put into an expression vector pET and 6-histidine-tagged MEA1 protein was expressed in *E. coli* strain BL21 (DL3). The cell lysate was mixed with ProBond (Invitrogen) and eluted with imidazole. This 6-histidine-fused MEA1 protein was used to make monoclonal antibody as described previously (KONDO et al., 1996b). This antibody (IgG2a-Lκ type) detected a single band with expected size in Western blotting of *E. coli* lysate. In a mammalian expression system using COS1 cells, transfected cells with the *MEA1*-expression vector were reactive to this antibody, while mock-infected cells showed no signals. This antibody against bovine MEA1 was reactive to those of mice, pigs, and goats so far tested. The ascites fluid from a mouse inoculated with MEA1-producing hybridoma was used for MEA1-localization study.

Mouse *Mea2* cDNA was isolated by screening a mouse testis cDNA library with a radio-labeled PCR product (KONDO and SUTOU, 1997) on the basis of information presented by SU et al. (1992). Characterization of mouse *Mea2* has been described in part previously (MATSUKUMA et al., 1999). The synthetic peptide consisting of C-terminal 30 amino acid residues was used to immunize a rabbit and antibody against MEA2 was raised. The affinity-purified antibody detected a 160 kDa band in Western blotting of the mouse testis.

To examine localization of MEA1 and MEA2 proteins in the testis, paraffin-embedded and imprinting specimens were prepared. Pieces of caprine and swine testes were fixed in Bouin's fluid and, after dehydration in ethanol series, embedded in paraffin according to the usual method. Thin sections were dewaxed and blocked with 5% rabbit serum. The culture medium of the hybridoma producing anti-MEA1 antibody was diluted twice with 5% fetal calf serum and used to stain specimens for over 1 hr. After washing specimens with phosphate-buffered saline (PBS) for 15 min 3 times, they were treated with biotinylated anti-mouse IgG rabbit antibody (DAKO A/S, Glostrup, Denmark) for over 1 hr. After three washings with PBS, specimens were treated with streptavidin-conjugated alkaline phosphatase (Roche Diagnostics Corp., Mannheim, Germany; 1:100 dilution with 2% fetal calf serum) for 1 hr. Specimens were washed with PBS and distilled water briefly, then treated with new fuchsin (DAKO A/S). Counterstain was made with hematoxylin.

Imprinting specimens of the mouse testis were also prepared. A testis was cut into halves and the cut-edge was pressed onto the surface of a glass slide. Specimens were fixed with ice-cold 4% paraformamide for 15 min and blocked with 1% bovine serum albumin/5% skim milk (blocker) for 1 hr. Specimens were stained with antibody (1:1000 dilution) for 1 h, washed for 5 min 3 times with the blocker, and stained for 30 min with Cy2-conjugated donkey anti-mouse IgG (Jackson ImmunoResearch Laboratories, Inc., PA, USA, 1:1000 dilution) for MEA1 staining and with Cy3-conjugated goat anti-rabbit IgG (Amersham Pharmacia Biotech UK Limited, Buckinghamshire, UK, 1:1000 dilution) for MEA2. They were washed 3 times with the blocker and stained for 15 min with 4', 6-diamidino-2-phenylindole dihydrochloride hydrate (DAPI, 50 pg/ml) for MEA1 or with a mixture of DAPI (50 pg/ml) and FITC-labeled lectin PNA (5 ng/ml) for MEA2. Slides were covered with a glass slip using Cell/Mount (Biomeda Corp., Foster City, CA, USA). Specimens were

examined under a fluorescent microscope (Leica Mikroskopie und Systeme, GmbH, Wetzlar, Germany).

## Results and Discussion

### *Transplantation or T cell-mediated H-Y antigen*

The first H-Y was identified as a gene product of *Smcy* 40 years after the discovery of H-Y (SCOTT et al., 1995). *Smcy* was later found to encode three H-Y peptide epitopes H-YK$^k$, H-YD$^k$, and H-YD$^b$ which are presented on the cell surface with the H-2 K$^k$, H-2 D$^k$, and H-2 D$^b$ major histocompatibility complex (MHC) class I molecules, respectively (Table). Other H-Y antigens have been identified and are also listed in the Table. H-Y peptide epitopes are presented on the cell surface together with not only class I but also class II MHC molecules (SCOTT et al., 2000). H-Y peptide epitopes so far ascertained are encoded by genes on the Y chromosome, but genes for some H-Ys remain to be identified. Either presently unidentified Y chromosomal genes or autosomal genes under the control of genes on the Y chromosome may be responsible for these orphan H-Y peptides. As minor histocompatibility antigens, H-Y may be the targets of host versus graft and graft versus leukemia responses following cell, tissue, or organ grafting from males to females. In this sense, research into H-Y is clinically important.

### *Serologically detectable male antigen*

Inbred female animals which had rejected male skin-grafts or had been immunized with male cells were found to raise antibody against H-Y (H-Y antibody, GOLDBERG et al., 1971). Serological detection of SDM with H-Y antibody made it possible to examine which sex has SDM in various animals (SHALEV et al., 1980; WACHTEL et al., 1974, 1975a). SDM is not species-specific, but heterogamete-specific in not only vertebrates but also non-vertebrates. Sperm was first found to be reactive to this antibody (GOLDBERG et al., 1971). This SDM is localized on the acrosomal region of sperm (KOO et al., 1973), but its chemical nature remains to be clarified. Since SDM is expressed on embryos of more than 8-cell stages (KROC et al., 1976), SDM could be utilized to sex embryos (WHITE et al., 1983). SDM has been thus found on cell surfaces of a wide variety of animals and developmental stages. At first, these were accepted as one and the same molecular species. Now that they are known to be heterogeneous (Table), SDMs presented on the surfaces of various cell types are likely to be different entities.

Retrospectively, MIS (Müllerian inhibiting substance or anti-Müllerian hormone (AMH)) was the first molecularly defined SDM (MÜLLER et al., 1993). MIS is secreted by Sertoli cells of the testis during male organogenesis so that the Müllerian ducts, precursor of the uterus and major parts of the vagina, are degenerated and instead the Wolffian ducts develop into the epididymal, deferential, and ejaculatory ducts and seminal vesicles. Since MIS is a product of testis activities, it is reasonable that MIS is recognized as non-self by the immune surveillance of females. At that time, however, MIS could not explain all characteristics assigned to SDM.

### *MEA1*

Retrospectively again, the second defined SDM is *Mea1* (male-enhanced antigen-1). LAU et al. (1989) isolated *Mea1* with polyclonal H-Y antibody from an expression

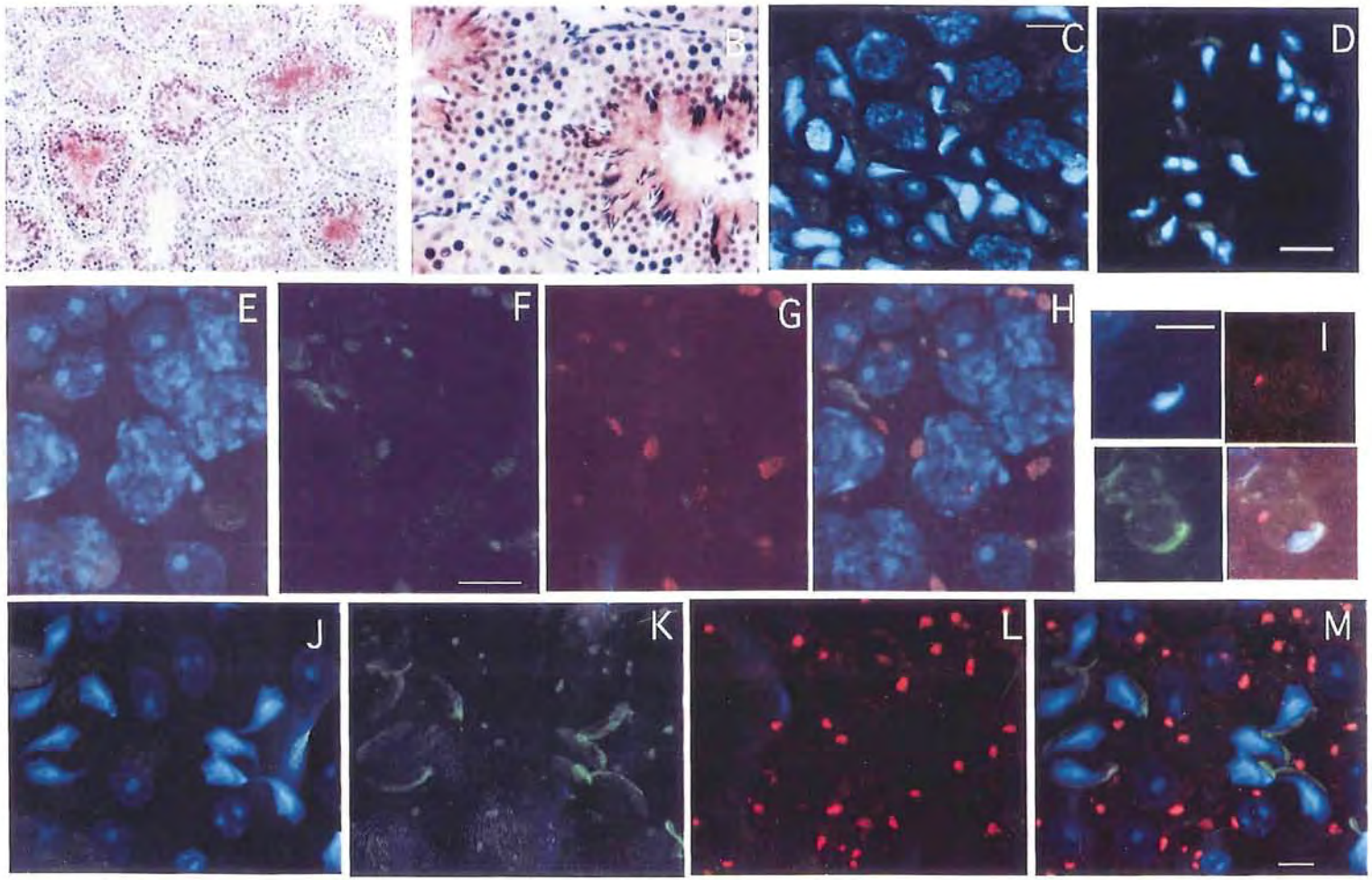
library of the mouse testis. We isolated bovine cDNA and genomic DNA in addition to mouse cDNA and characterized them (KONDO et al., 1994, 1996a). *In situ* hybridization using the antisense strand of *MEA1* cDNA indicated that the message of *MEA1/Mea1* is expressed in spermatocytes and spermatids in bovines and mice. Monoclonal antibody against bovine recombinant MEA1 protein produced in an *E. coli* system was raised. Immunohistochemical staining of the murine and bovine testes using cryosections was reported previously (KONDO et al., 1996b). The result indicated that MEA1 is strongly expressed in the cytoplasm of elongated spermatids in both species. These single-stained cryosections, however, have proved rather difficult for pinpointing protein locations (KONDO et al., 1996b). Since the anti-MEA1 antibody was reactive to caprine and swine in addition to murine and bovine MEA1, immunohistochemical staining of the caprine and swine testes using paraffin-embedded sections was performed (Fig. 1A and B, respectively). Differential expression of MEA1 in the different seminiferous tubules (Fig. A) showed that MEA1 is expressed stage-specifically. Analyses of the stages suggested that the most prominent expression occurs in spermatids at mouse-equivalent steps 13-15. MEA1 is also expressed in the late maturation steps of swine elongated spermatids (Fig. 1B).

Imprinting specimens were useful to obtain single germ cell layers without cutting or injuring the cells. Weak expression of MEA1 in caprine and swine round spermatids was seen in thin sections (Fig. 1AB). Imprinting specimens of the mouse testis indicated that MEA1 occurs in the cytoplasm of elongated spermatids, but is negligible or scanty in that of pachytene spermatocytes or round spermatids (Fig. 1C). MEA1 in elongated spermatids is clearly shown in Fig. 1D.

Even though *MEA1* is expressed beyond the 8-cell stage of bovine embryos as revealed by RT-PCR (KONDO et al., 1994), immunostaining of bovine blastocysts with our monoclonal antibody was negative, indicating that MEA1 is not translated or present on the cell surfaces. Thus, MEA1 seems to be a different SDM to the one expressed on the 8-cell stage embryos (KROC et al., 1976). Since transcription of genes in later stages of spermatogenesis is difficult, mRNAs are produced earlier and preserved for a protracted period. Transcripts of *Mea1* appear in pachytene spermatocytes and MEA1 protein occurs mainly in elongated spermatids (Fig. 1A-D; KONDO et al., 1996b). Since the time span between pachytene spermatocytes and elongated spermatids at steps 13-15 is one to two weeks, mechanisms to keep *Mea1* mRNA stable must be functioning.

Figure 1 (see next page)

Fig. 1: Localization of MEA1 (A-D) and MEA2 (E-M) in male germ cells. Scale bars indicate 10 μm. A, paraffin-embedded section of the caprine testis stained with alkaline phosphatase and new fuchsin. Counterstain was made with hematoxylin. B, paraffin-embedded section of the swine testis stained as for A. C and D, imprinting specimens of the mouse testis stained with DAPI and Cy2. Spermatocytes, round spermatids, and elongated spermatids (C) and elongated spermatids (D) are shown. E-H, imprinting specimen of the mouse testis stained with DAPI (E), PNA (F), Cy3 (G), and merge of the three (H). Spermatocytes and round spermatids are shown. I, an elongated spermatid stained with Cy3 (red), DAPI (blue), PNA (green), and merge of the three (three-colored). J-M, imprinting specimen of the mouse testis stained with DAPI (J), PNA (K), Cy3 (L), and merge of the three (M). Round spermatids and elongated spermatids are shown.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*MEA2*

The third defined SDM is *Mea2* (male-enhanced antigen-2). SU et al. (1991) indicated, using monoclonal H-Y antibody, the existence of *Mea2* on mouse chromosome 5. We subsequently cloned mouse *Mea2* cDNA (KONDO and SUTOU, 1997). On the other hand, one of us (Matsukuma) has raised transgenic mice (line T604), chromosomes 5 and 19 of which were reciprocally translocated (MATSUKUMA et al., 1989). Only male homozygotes showed conspicuous characteristics, viz., sterility because of testicular atrophy and aspermia. Southern blot analysis using *Mea2* cDNA indicated that the translocation breakage site was located in the *Mea2* region. Comparison of sequences between cDNA and genomic DNA indicated that translocation occurred at intron 7 in a total of 24 exons. Affinity-purified antibody against the C-terminal peptide of MEA2 detected a 160 kDa protein from wild type mice, but not from homozygotic *Mea2*- males. MEA2 protein has a long coiled-coil structure. MEA2 is co-localized with γ-adaptin, a component of clasrin-coated vesicles in the trans-Golgi network. Yeast two-hybrid experiments identified three major sequences, filamin 24th domain-like, TRAX-like, and EAP-300-like sequences (MATSUKUMA et al., 1999 and unpublished data). MEA2 is localized in the Golgi apparatus of spermatocytes at the pachytene stage (Fig. 1E-H) and spermatids (Fig. 1E-H and J-M) in the normal testis; no signals were found in the testis of homozygotic T604 males which lack spermatogenesis. At the beginning of spermiogenesis, the proacrosomal vesicles containing the proacrosomal granules coalesce to form a single acrosomal vesicle under the Golgi apparatus. As the acrosome matures, the Golgi apparatus gradually moves to the cytoplasm. MEA2 is localized in the Golgi apparatus after separation from the acrosome (Fig. 1I). Although the contribution of the Golgi apparatus to acrosome formation is accepted, the role of MEA2 in the Golgi apparatus remains to be clarified. Findings discussed above, however, suggest the involvement of MEA2 in transportation of materials to the acrosome or trafficking of clasrin-coated vesicles. The third defined SDM is surely involved in spermatogenesis.

*SDM is not one and the same*

All three SDMs molecularly defined so far are associated with activities of the testis (MIS) or spermatogenesis (MEA1 and MEA2). SDM on the acrosomal region of sperm (KOO et al., 1973) is also associated with spermatogenesis. These findings lead to a generalized conclusion that SDMs are products of testis- or sperm-associated genes that are usually repressed in females. When these genes are derepressed erroneously or females are immunized artificially with SDMs, SDMs must function as antigens.

*SDM and autoimmune deseases*

That most patients of autoimmune diseases are women is well documented. FLITZLER et al. (1993) isolated an antigen Golgin-160 with antibody from a woman suffering from systemic lupus erythematosus (SLE). Comparison of amino acid sequences of human Golgin-160 and mouse MEA2 revealed that these two are homologues, because amino acid identity of the two is 83% (KONDO and SUTOU, 1997) and Golgin-160 is a component of the Golgi apparatus (FLITZLER et al., 1993). This is the first and so far only example of SDM functioning as an autoantigen in an

autoimmune disease. Since many genes are associated with spermatogenesis and functions of the testis, there must be many SDMs yet to be identified. From the viewpoint of autoimmune diseases, the identification and characterization of SDMs will be clinically important.

*SDM and avian sex-determination*

Let's return to the original enigma. Given that SDMs of mammals are testis- or sperm-associated, what are SDMs on the cell surfaces of organisms with the Z/W sex-determining system? Simply, these might be proteins associated with oogenesis or functions of the ovaries. However, when phylogenetic conservation of genes associated with spermatogenesis or testicular functions is taken into consideration, it is incredible that antibodies against mammalian testis- or sperm-associated proteins are reactive to, e.g., avian proteins associated with oogenesis or ovarian functions. A possible explanation may reside in A/X or A/Z chromosome-counting molecules. Sex of *Drosophila melanogaster*, whose Y chromosome is a dummy, and sex of *Caenorhabditis elegans*, which does not have a Y chromosome, is determined on the ratio of autosomes to X chromosomes. Avian sex determination is not well understood, but the ratio of autosomes to Z chromosomes is deeply involved (ELLENGREN, 2000). Although mammalian sex is determined by presence or absence of the Y chromosome, the ratio of autosomes to X chromosomes is counted in the mammalian dosage compensation system. Counting molecules encoded by autosomes are neutralized by those on the X chromosomes in mammalian females, but extra-molecules not neutralized in males may provide SDM to females. These autosomal molecules may be neutralized by gene products on the Z chromosomes in avian males, but remain unneutralized in avian females, thus providing SDM reactive to mammalian H-Y antibody if mammalian and avian counting molecules have the same or similar epitopes. It is of interest to learn that when genetic male chick embryos were treated with estradiol, SDM was detected in the ovotestis, indicating that avian SDM is autosome- or Z-linked (MÜLLER et al., 1979).

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Received: 2001-07-12

Accepted: 2001-08-27

Corresponding author
SHIZUYO SUTOU, Ph.D.
Research Institute of Molecular and Cell Biology, National Institute of Advanced Industrial Science & Technology, Central 6, 1-1-1 Higashi,
Tsukuba, Ibaraki 305-8566, Japan

E-Mail: s-sutou@aist.go.jp