Introduction

In mammals, the testicular sperm are neither motile nor fertile under the physiological conditions. They acquire motility and fertility through the post-testicular maturating processes such as epididymal maturation, ejaculation and capacitation, which induce sperm acrosome reaction and subsequent fertilization. These processes have been suggested to be attained by the direct and indirect interaction of sperm with somatic cells (Dacheux *et al.*, 1998 ; Jin *et al.*, 1997 ; Nagdas *et al.*, 1992 ; Okamura *et al.*, 1988 ; Tulsiani *et al.*, 1993a ; Skudlarek *et al.*, 1993 ; Eccleston *et al.*, 1994 ; Hamilton *et al.*, 1986 ; Moore *et al.*, 1989 ; Olson and Hamilton, 1978 ; Poirier, 1975 ; Brown *et al.*, 1983 ; Garberi *et al.*, 1982 ; Jones *et al.*, 1981 ; Jones and Brown, 1982 ; Jones *et al.*, 1983 ; Jones *et al.*, 1985 ; Jones and Brown, 1987).

A number of secretory proteins in the reproductive tracts were reported to bind to or to interact with the surface of the maturating sperm and were indicated to have specific roles in the development of fertilizing ability. Sperm surface proteins which have affinity for the zona pellucida or egg plasma membranes have also been reported (Abou-Haila and Tulsiani, 2000 ; Benoff *et al.*, 1993b ; Benoff *et al.*, 1993a ; Benoff,

1997 ; Cornwall *et al.*, 1991 ; Tulsiani *et al.*, 1990a ; Tulsiani *et al.*, 1995a ; Tulsiani *et al.*, 1995b ; Tulsiani *et al.*, 1996 ; Yoshida-Komiya *et al.*, 1999 ; Bookbinder *et al.*, 1995 ; Eccleston *et al.*, 1994 ; Foster *et al.*, 1997 ; Goluboff *et al.*, 1995 ; Johnston *et al.*, 1995 ; Boettger-Tong *et al.*, 1993 ; Rivkin *et al.*, 2000 ; Saling, 1981 ; Shur and Hall, 1982 ; Tanphaichitr *et al.*, 1993 ; Ram *et al.*, 1989 ; Tanphaichitr *et al.*, 1992 ; Shur *et al.*, 1998 ; Cheng *et al.*, 1994 ; Mertz *et al.*, 1995). Furthermore, it is suggested that some sperm surface proteins with enzymatic activities function as receptors in the recognition of and binding to the complementary carbohydrate moieties present on the zona pellucida at the initial step of fertilization (Saling, 1981 ; Cornwall *et al.*, 1991 ; Tulsiani *et al.*, 1995a ; Tulsiani *et al.*, 1995b ; Tulsiani *et al.*, 1990a ; Tulsiani *et al.*, 1997 ; Shalgi and Raz, 1997 ; Sinowatz *et al.*, 1998 ; Shalgi *et al.*, 1986). In rat, mouse, hamster, bull and human, α-D-mannosidase in the sperm plasma membranes has been indicated to be one of such enzymes to bind to mannose residues on the zona pellucida (Benoff *et al.*, 1993b ; Benoff *et al.*, 1993c ; Benoff *et al.*, 1993a ; Benoff *et al.*, 1996 ; Benoff *et al.*, 1997b ; Benoff *et al.*, 1997a ; Chayko *et al.*, 2000 ; Cornwall *et al.*, 1991 ; Jin *et al.*, 1999 ; Loeser *et al.*, 1999 ; Loeser and Tulsiani, 1999 ; Pereira *et al.*, 1998 ; Revah *et al.*, 2000 ; Tulsiani *et al.*, 1989 ; Tulsiani *et al.*, 1990b ; Tulsiani *et al.*, 1990a ; Tulsiani *et al.*, 1992 ; Tulsiani *et al.*, 1993b ; Tulsiani *et al.*, 1995a ; Tulsiani *et al.*, 1995b ; Tulsiani *et al.*, 1996 ; Yoshida-Komiya *et al.*,

1999 ; Flanagan *et al.*, 1982 ; Jayakumar *et al.*, 1992).

Recently, we have found a novel 135-kDa α-D-mannosidase (MAN2B2) which has little similarity in the amino acid sequences and the substrate specificities with other types of the mammalian α-D-mannosidases such as Golgi-mannosidase Ⅱ, ER-mannosidase, Man9-mannosidase and lysosomal mannosidase (Okamura *et al.*, 1992 ; Okamura *et al.*, 1995). The porcine MAN2B2 is specifically secreted from the border region between the caput and corpus epididymis, and then binds to the equatorial segment of immature sperm head as a 27-kDa fragment. During the epididymal maturation, porcine MAN2B2 migrates to the sperm surface, localizing itself to a crescent-shaped area just behind the acrosome and to the apical rim of the sperm head where sperm interact with oocyte. In addition, α-D-mannosidase in the rat sperm plasma membrane has been reported to play an important role in fertilization (Cornwall *et al.*, 1991 ; Loeser *et al.*, 1999 ; Pereira *et al.*, 1998 ; Tulsiani *et al.*, 1989 ; Tulsiani *et al.*, 1990b ; Tulsiani *et al.*, 1990a ; Tulsiani *et al.*, 1992 ; Tulsiani *et al.*, 1993b ; Tulsiani *et al.*, 1995a ; Tulsiani *et al.*, 1996 ; Yoshida-Komiya *et al.*, 1999). Although its structural characterization has not been done yet, the enzyme is very similar to the MAN2B2 in such properties as molecular mass, optimum pH and substrate specificity, which strongly indicates that the rat sperm plasma membrane α-D-mannosidase is a homologue of porcine MAN2B2. These results

strongly suggest that MAN2B2 is involved in the sperm-egg interaction.

In the present study, we cloned both cDNA and gene of mouse MAN2B2 homologue (mMAN2B2) from the testis cDNA library and the 129SVJ mouse genomic library, respectively. We found that the mMAN2B2 mRNA is expressed in the germ cells bordering on the basement membrane of the seminiferous tubule, mainly in spermatogonia, at particular stages of spermatogenesis (Appendix I). On the other hand, it was found that mMAN2B2 protein itself was first detected in the acrosomal vesicle in round spermatid and present in the acrosome during the spermiogenesis and the epididymal maturation. We also found that the anti mMAN2B2 antibody reduced the fertilization rate.