Human cyritestin genes (*CYRN*1 and *CYRN*2) are non-functional

Pawel GRZMIL*, Youngmin KIM*, Rahman SHAMSADIN*, Jürgen NEESEN*, Ibrahim M. ADHAM*, Uwe A. O. HEINLEIN†, Ullrich J. SCHWARZER‡ and Wolfgang ENGEL*[1]

*Institut für Humangenetik, Georg-August-Universität Göttingen, Heinrich-Düker Weg 12, 37073 Göttingen, Germany, †Institut für Genetik, Heinrich-Heine-Universität Düsseldorf, Universitätsstraße 1, 40225 Düsseldorf, Germany, and ‡Urologische Praxis, Biberstraße 2b, 85354 Freising, Germany

The mouse cyritestin gene is a member of the ADAM (a disintegrin and metalloprotease) gene family and codes for a membrane-anchored sperm protein. Recently, it was shown that cyritestin is critical for male fertility in the mouse. Spermatozoa of cyritestin-deficient mice are not able to bind to the zona pellucida of the oocyte and therefore unable to fertilize the egg. However, zona-free oocytes can be fertilized and the resulting embryos show normal development. In contrast to the mouse, where only one gene for cyritestin (*Cyrn*) is reported, two cyritestin genes (*CYRN*1 and *CYRN*2) are known in humans. The human *CYRN*1 and *CYRN*2 genes are located on chromosomes 8 and 16, respectively. We report that 27% of fertile men are deficient for the *CYRN*1 gene but that all have a *CYRN*2 gene, suggesting that the *CYRN*2 gene is the orthologous mouse cyritestin gene in humans and might be involved in sperm–egg interactions. However, the characterization of *CYRN*2 transcripts from testicular RNA of *CYRN*1-deficient men demonstrated many termination codons in the synthesized cyritestin cDNA. Furthermore, Western-blot analysis with human testicular protein extracts using an anti-cyritestin antibody failed to detect any cyritestin protein. These results demonstrate clearly that both cyritestin genes are non-functional in humans.

Key words: deletion inheritance, male infertility, sperm–egg interaction, testicular expression.

INTRODUCTION

Genes belonging to the ADAM (a disintegrin and metalloprotease) family were suggested to be involved in sperm–egg binding and fusion [1,2]. All members of this protein family contain characteristic features like a signal peptide, prodomain, metalloprotease-like domain, disintegrin domain, cysteine-rich domain, epidermal-growth-factor-like repeat, and transmembrane and cytoplasmatic domains in their prepropeptide [3–5]. The presence of the disintegrin domain similar to that of soluble snake venom toxins led to the suggestion that members of the ADAM family are involved in cell–cell adhesion through binding to integrins. Fertilin, a member of the ADAM family, is known to play an important role in binding and/or fusion between sperm and the egg plasma membrane [3,6–10].

Cyritestin, another member of the ADAM family, also known as ADAM 3 or tMDC1, is the product of the *Cyrn* gene, which is localized on mouse chromosome 8 [11]. The gene is specifically expressed in male germ cells [4]. Whereas the transcription of the mouse *Cyrn* gene is detectable in the testis at postnatal day 14, the protein is first demonstrated at postnatal day 18 [12]. Experiments using anti-cyritestin antibodies have demonstrated that cyritestin is localized in the acrosomal region of spermatids and spermatozoa and undergoes post-translational modification during sperm passage through the epididymis [12]. The results of *in vitro*-fertilization experiments, using a peptide corresponding to the disintegrin domain of cyritestin, suggested that cyritestin is one of the major components of sperm–egg fusion events at the level of the egg membrane [13,14]. Cyritestin-deficient male mice were found to be infertile. Experiments *in vitro* revealed that the infertility is due to the inability of the cyritestin-deficient sperm to bind to the zona pellucida, whereas the binding and fertilization of these spermatozoa to zona-free oocytes were normal [15].

In contrast to the mouse, the human genome contains two cyritestin genes, *CYRN*1 and *CYRN*2, which are localized on chromosome 8p12–21 and 16q12, respectively [16,17]. Both genes were found to be expressed only in the testis [16]. A Western-blot analysis using rabbit antisera against human and macaque *CYRN*1 peptide revealed no immunoreactivity with human testis and sperm extracts. The presence of a variety of deletions, insertions and in-frame termination codons led the authors to suggest that the *CYRN*1 gene is non-functional in humans [18]. Our Southern-blot analysis revealed that in some individuals the *CYRN*1 gene is deleted. This finding led us to screen for this deletion in men and to repeat the Western-blot analysis, because we could not exclude the possibility that the protein extracts used by Frayne and Hall [18] were taken from a man with a *CYRN*1 deletion. We have analysed both fertile and infertile men. The *CYRN*1 deletion was found in both groups with the same frequency, which suggests that this mutation has no relevance for fertility. Furthermore, the deletion of the *CYRN*1 gene in fertile men suggests that the *CYRN*1 gene is non-functional in humans. However, all probands have been found to be endowed with the *CYRN*2 gene in their genome, suggesting that the *CYRN*2 gene is functional and might replace the non-functional *CYRN*1 gene. However, our Western-blot investigations revealed that the *CYRN*2 gene is also non-functional in humans. These results indicate that, in humans, another yet unknown gene could replace the function of cyritestin in sperm–egg interactions.

MATERIALS AND METHODS

Probands

Blood samples from 120 men with proven fertility (co-workers and probands) were used for DNA isolation. Testis material was obtained from 50 postmortem men, for which histological

Abbreviations used: ADAM, a disintegrin and metalloprotease; RT-PCR, reverse transcriptase PCR; THEG, testicular haploid expressed gene.

[1] To whom correspondence should be addressed (e-mail wengel@gwdg.de).

The nucleotide sequence data reported in this paper appear in the GenBank and EMBL Nucleotide Sequence Databases under the accession number AF 334867.

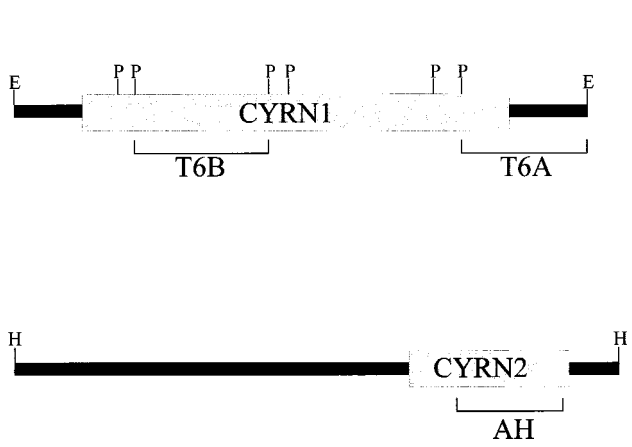

Figure 1 Localization of DNA probes used for Southern-blot analyses

(**A**) A schematic representation of the *CYRN*1 cDNA clone T6. The grey box indicates the putative translated region, and black boxes indicate untranslated regions. Positions of the 3′-specific *CYRN*1 probe T6A and the 5′ probe T6B are shown. (**B**) The 4 kb *Hin*dIII genomic fragment 4kb-Hd. The identified *CYRN*2 exon is indicated by the grey box. The fragment AH was used as a *CYRN*2-specific probe in Southern-blot analyses. P, E and H are restriction sites for enzymes *Pst*I, *Eco*RI and *Hin*dIII, respectively.

analyses showed normal spermatogenesis. The testis tissue was used for DNA, RNA and protein isolation. Blood samples from 12 infertile men whose spermatozoa showed an inability to bind and to fuse with oocytes in *in vitro*-fertilization assays were used for DNA isolation. Blood samples from eight females (co-workers) were used for DNA isolation. Kidney tissue was obtained from a postmortem man and used for protein isolation. Macaque testis tissue was obtained from the Deutsches Primaten Zentrum (DPZ) in Göttingen, Germany, and used for protein isolation.

Generation of DNA probes for Southern-blot analysis

Human cyritestin 1 cDNA clone T6 (accession number X89657) was digested with *Eco*RI and *Pst*I enzymes. Two fragments were isolated as *CYRN*1-specific probes: a 574 bp-long fragment from the 5′ region of the cDNA sequence (positions 312–886) and a 884 bp fragment from the 3′ end (positions 1751–2635), called T6B and T6A, respectively (Figure 1A). These fragments were subcloned, sequenced and used for Southern-blot experiments. A *CYRN*2-specific probe, AH, was generated by PCR using cosF (5′-CACTGGACACATTCAGAAACAG-3′) and cosR (5′-TC-ATTGTTAGATCAGGCCAGTT-3′) primers and *CYRN*2 genomic clone (4kb-Hd, accession number Y10615) as a template (Figure 1B). PCR products were subcloned into the pGEM-T vector (Promega, Southampton, U.K.) and sequenced.

Southern-blot analysis

High-molecular-mass human genomic DNA was isolated using standard methods. DNA (20 μg) was digested with *Hin*dIII restriction enzyme, fractionated on 1% agarose gel and transferred to Hybond C membrane. Probes were labelled with ^{32}P using the Rediprime II labelling system (Amersham, Braunschweig, Germany) according to the manufacturer's instructions. The membrane was hybridized at 65 °C in Membrane Hybridization Buffer (KPL) and washed with the following stringency: 20 min in 2 × SSC buffer at room temperature, 10 min in 2 × SSC/0.1% SDS at 65 °C and 10 min in 0.2 × SSC at 65 °C (where 1 × SSC is 0.15 M NaCl/0.015 M sodium citrate).

Reverse transcriptase PCR (RT-PCR)

Total RNA from human testes was isolated using the Rneasy mini kit (Qiagen, Hilden, Germany) according to the manual supplied. For RT-PCR, RNA (5 μg) was annealed with oligo-dT_{17} primer. The cDNA was synthesized using 200 units of Superscript reverse transcriptase (Life Technologies, Karlsruhe, Germany) at 42 °C for 50 min. PCR was carried out with 5 μl of synthesized cDNA, 10 pmol of reverse and forward primers and 3 units of *Taq* polymerase (Life Technologies). *CYRN*1 expression was analysed by RT-PCR using IS1 (5′-GTTACTGT-CCAGGAACTACATGC-3′) and IS6 (5′-ACTGTGAACACT-GTCTATCCC-3′), *CYRN*1-specific primers, and the cycling conditions were 2 min at 94 °C followed by 30 cycles of 1 min at 94 °C, 1 min at 60 °C and 2 min at 72 °C.

For the study of the *CYRN*2 expression, cosF- and cosR-specific primers were used. Cycling conditions were 4 min at 94 °C, then 35 cycles of 1 min at 94 °C, 1 min at 65 °C and 2 min at 72 °C, and ending with 5 min at 72 °C. The PCR products were electrophoresed in 1.5% agarose gel. To see whether the obtained PCR products in fact belong to the *CYRN*2 gene, the amplification products were extracted from the gel, subcloned into pGEM-T vector (Promega) and sequenced on both strands. The integrity of the RNA samples was checked by RT-PCR for 5 S rRNA using primers and conditions as described in [19].

Western-blot analysis of human cyritestin

Anti-*CYRN* antisera were produced by Eurogentec (Seraing, Belgium) using the synthetic peptide CLSAHARNGSKQ derived from the putative cysteine-rich domain of human *CYRN*1 and *CYRN*2, conjugated to the keyhole-limpet haemocyanin protein. The polyclonal antiserum was affinity purified using HiTrap *N*-hydroxysuccinimide-activated Sepharose columns according to the manufacturer's instructions (Amersham). Proteins were extracted from human testis, human kidney and macaque testis by homogenization in lysis buffer (150 mM NaCl, 10 mM EDTA, 50 mM Tris/HCl, pH 7.6, 1% Triton X-100 and 1% sodium deoxycholate) containing protease inhibitors (1 μg/μl leupeptin/3 μg/μl aprotinin). Lysates were sonicated and centrifuged at 7000 ***g*** for 10 min at room temperature, and supernatants were collected. Proteins (100 μg/lane) were separated by electrophoresis in a 10% polyacrylamide gel and electroblotted on PVDF membranes (Macherey and Nagel, Düren, Germany). Blots were incubated overnight with anti-CYRN antibodies at 4 °C. After extensive washing, blots were incubated for 1 h at room temperature with anti-rabbit IgG conjugated with alkaline phosphatase (Sigma, Duisenhofen, Germany). The detection of bound antibodies was achieved by colour reaction with 18.8 mg/ml Nitro Blue Tetrazolium chloride (NBT) and 9.4 mg/ml 5-bromo-4-chloro-3-indolylphosphate toluidine salt (BCPIP) in 67% DMSO. The protein quality was checked by incubation with a monoclonal anti-α-tubulin antibody (Sigma) [20].

Database searches and sequence analysis

The GenBank database was searched for genomic sequences of human cyritestin using the advanced BLAST program [21] at the

National Center for Biotechnology Information home page (http://www.ncbi.nlm.nih.gov/blast). One human clone was identified, RP11-189E14, localized on chromosome 16 (working drafting sequence, locus AC007861). The *CYRN*2 gene corresponds to nucleotides 155128–156067 in this clone.

RESULTS

Deletion of the *CYRN*1 gene is not associated with male infertility

In *Hin*dIII-digested genomic DNA, the *CYRN*1 5′ probe (T6B, Figure 1A) detected a 10 kb fragment (Figure 2A), whereas a 4 kb fragment was observed (Figure 2C) using the *CYRN*2 probe (AH, Figure 1B). Southern-blot analyses of DNA from 12 infertile men, whose spermatozoa showed an inability to bind and to fuse with oocytes in *in vitro*-fertilization assays, revealed that the 5′ probe of *CYRN*1 did not detect the 10 kb *Hin*dIII fragment in the genomes of three of these men. To investigate whether only the 5′ region or the complete *CYRN*1 gene was deleted, the T6A probe (Figure 1A) corresponding to the 3′ end of the cDNA was used to rehybridize the Southern blots. The 3′ probe of *CYRN*1 cDNA detected a 7.5 kb *Hin*dIII fragment (Figure 2B) only in the genomes of those with a *CYRN*1-positive genotype. These results demonstrate that the complete *CYRN*1 locus was deleted in the three infertile men. To address the question of whether the deletion of *CYRN*1 is associated with male infertility, we have genotyped fertile men and women for the deletion of the *CYRN*1 gene. Of 120 men, 27% were classified as *CYRN*1-deficient, and 73% as *CYRN*1-positive. The *CYRN*1 deletion was also found in two of eight females. To determine the presence of *CYRN*2 in the genomes of the studied cases, Southern blots were rehybridized with the *CYRN*2-specific probe. The 4 kb *Hin*dIII fragment of the *CYRN*2 gene was detected in all individuals analysed (Figure 2C). These data indicate that deletion of the *CYRN*1 gene is neither associated with male infertility nor functional in humans.

To investigate the inheritance of the *CYRN*1 deletion, the genomic DNA of a family with six children was analysed (Figure 3A). Southern-blot analysis demonstrated that the mother and

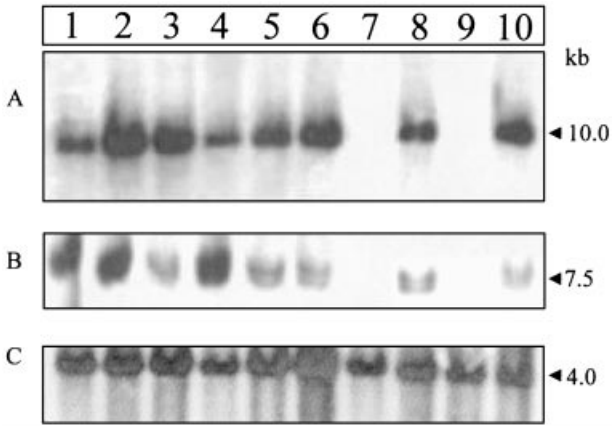

Figure 2 Southern-blot analyses of human cyritestin genes

Genomic DNA was isolated from 10 infertile men and digested with *Hin*dIII restriction enzyme. (**A**) Southern-blot analysis using the 5′ probe (T6B) of the *CYRN*1 cDNA. The 10 kb *Hin*dIII fragment was deleted in the genomes of individuals 7 and 9. (**B**) Probe T6A was used to demonstrate that the 3′ region of the *CYRN*1 gene was also deleted in the genomes of individuals 7 and 9. In individuals with the *CYRN*1-positive genotype a 7.5 kb fragment was detected by the T6A probe. (**C**) Southern-blot analysis using the *CYRN*2-specific probe AH. A 4 kb fragment was detected in all individuals analysed.

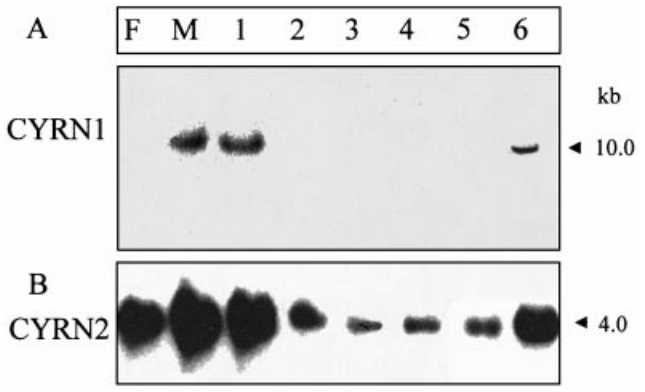

Figure 3 Mendelian inheritance of the *CYRN*1 deletion

DNA from the father (F), the mother (M) and six children (1–6) from one family was restricted with *Hin*dIII and hybridized with the *CYRN*1 probe T6B (**A**) or the *CYRN*2 probe AH (**B**). Deletion of the *CYRN*1 gene was found in the father and four children (2–5). The presence of the 10 kb fragment of the *CYRN*1 gene in the DNA of the mother and children 1 and 6 indicates that they are heterozygous for the *CYRN*1 deletion. (**B**) The genomes of all family members contained the 4 kb *Hin*dIII fragment of the *CYRN*2 gene.

two children were *CYRN*1-positive, whereas the father and four children were *CYRN*1-deficient. These results indicate that the mother is heterozygous and the father is homozygous for the *CYRN*1 deletion. According to the hybridization pattern with the DNA of the six children, four were homozygous and two were heterozygous for the *CYRN*1 deletion. To prove the DNA's quality and also the presence of the *CYRN*2 gene, the membrane was rehybridized with the *CYRN*2-specific probe. All family members were found to contain the *CYRN*2 gene in their genome (Figure 3B).

Cyritestin gene expression in men with the *CYRN*1 deletion

To confirm that the *CYRN*1 gene is completely deleted, we performed RT-PCR using testicular RNA of fertile men and *CYRN*1-specific primers IS1 and IS6 (Figure 4). No *CYRN*1 transcripts were found in the *CYRN*1-deficient cases, whereas two splice variants were amplified in the testicular RNA of those with a *CYRN*1-positive genotype. Integrity of the RNA was analysed by RT-PCR for 5 S rRNA using primers and conditions as described in [19] (Figure 4). The fact that the *CYRN*1 gene can be deleted in fertile men indicates that it is not a critical gene for male fertility in humans. To address the question of whether *CYRN*2 is able to replace *CYRN*1, testicular RNA from *CYRN*1-deficient men was used for amplification of *CYRN*2 cDNA. The expected 650 bp *CYRN*2 cDNA fragment was obtained (Figure 5A). Because both *CYRN*2-specific primers are located in the same exon, genomic DNA contamination of the testicular RNA was excluded. Using THEG (testicular haploid expressed gene)-specific primers, the typical two splice products (280 bp and 352 bp) for THEG cDNA were amplified, but not the PCR product of 3 kb, which is obtained with genomic DNA (Figure 5B) [22].

Western-blot analysis for cyritestin

To investigate the presence of human cyritestin proteins in the testes and spermatozoa, Western-blot analysis was performed. Antibodies were generated in rabbits against a synthetic peptide containing the conserved amino acid sequence located in the

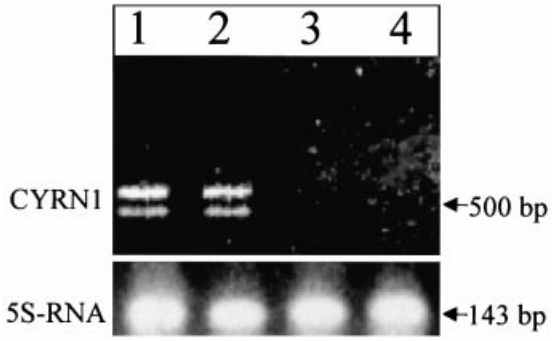

Figure 4 RT-PCR analysis of the expression of *CYRN*1

Testicular RNA was extracted from men with *CYRN*1-positive (lanes 1 and 2) and *CYRN*1-deficient (lanes 3 and 4) genotypes. Specific primers IS1 and IS6 were used to amplify *CYRN*1 fragments. No amplification products were detectable in men with the *CYRN*1-deficient genotype. The integrity of the RNA was proven by amplification of a fragment of the 5 S rRNA.

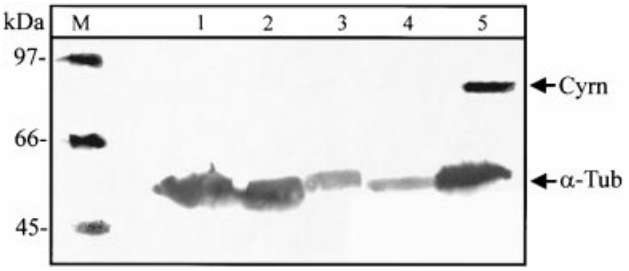

Figure 6 Western-blot analysis

Protein extracts from the testis of a man with the *CYRN*1-positive genotype (lane 1), a man with a *CYRN*1-deficient genotype (lane 2), human spermatozoa (lane 3), human kidney (lane 4) and macaque testis (lane 5) were probed with anti-CYRN antibodies. CYRN protein bands could not be detected in human protein extracts; however, an 86 kDa protein band was observed in protein extract from macaque testis tissue. Loaded protein amounts were determined using monoclonal anti-α-tubulin antibodies (α-Tub). M, molecular-mass standard.

cysteine-rich domains of both human *CYRN*1 and *CYRN*2. Testicular and spermatozoan protein extracts from *CYRN*1-positive and *CYRN*1-deficient men were probed with anti-CYRN antibodies. Macaque testis and human kidney protein extracts were used as positive and negative controls, respectively. An 86 kDa protein was detected in macaque testicular extract but not in extracts of human testis, spermatozoa or kidney (Figure 6). Protein quality and integrity was analysed by incubation of the blot with anti-α-tubulin antibody.

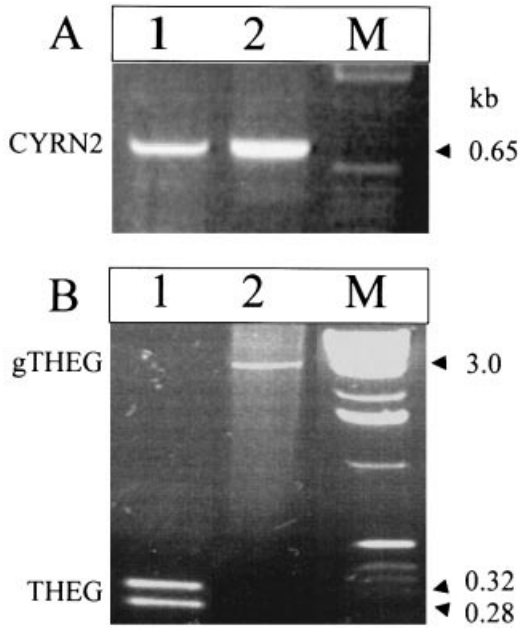

Figure 5 Expression analysis of the *CYRN*2 gene

(**A**) RT-PCR was carried out with testicular RNA from a man with a *CYRN*1-null genotype and *CYRN*2-specific primers cosF and cosR. A 650 bp product was obtained (lane 1). A fragment of equal size was amplified using genomic DNA (lane 2). (**B**) To exclude possible contamination of the RNA with genomic DNA, an RT-PCR experiment with THEG-specific primers was performed. Only the two expected THEG cDNA fragments of 320 and 280 bp were obtained (lane 1). The amplified 3 kb THEG genomic fragment (gTHEG) in the PCR analysis using genomic DNA as a template (lane 2) was not amplified in the RT-PCR assay with the testicular RNA (lane 1). M, molecular standard.

Sequence analysis of the *CYRN*2 gene

The amplified cDNA fragment (AH) of the *CYRN*2 gene as well as the genomic clone 4kb-Hd (Figure 1B) were sequenced. To get more sequence information about the *CYRN*2 gene, the 700 bp cDNA sequence (accession number Y10615) was aligned with the human genome database using the search tool BLAST. This comparison revealed 100% identity with one genomic clone (accession number AC007861) located on chromosome 16, corresponding to the chromosomal localization of the *CYRN*2 gene [16]. Analysis of both the *CYRN*2 cDNA and the corresponding genomic sequences revealed many stop codons in all possible reading frames. Although the amino acid sequences between the stop codons showed high similarity with the macaque cyritestin protein [23], it is very unlikely that this gene could produce a functional protein in humans.

DISCUSSION

The mouse is endowed with one cyritestin gene on chromosome 8 [11]. Mice that are deficient for cyritestin were found to be infertile due to the inability of their spermatozoa to bind to the zona pellucida of the oocyte [15]. Therefore it was suggested that a human orthologous gene could play a role in sperm–egg interaction. In the human genome two cyritestin genes are present, *CYRN*1 and *CYRN*2. Frayne and Hall [18] reported the results of Western-blot analysis with human testicular protein and an anti-CYRN1 antibody. They found that no *CYRN*1 protein is demonstrable in human testis, although a positive result was obtained with proteins from macaque testis. From these results the authors concluded that the *CYRN*1 gene (also called tMDC1) is non-functional in humans [18]. Our data support this conclusion.

The results of our screening study for the *CYRN*1 gene in fertile individuals demonstrate a large deletion in the locus for *CYRN*1 on human chromosome 8. Of the 120 individuals tested, 32 (27%) had a deletion in *CYRN*1 gene. A study of the inheritance of the *CYRN*1 deletion in a family with six children clearly demonstrated Mendelian inheritance. At the transcrip-

tional level it was found that in testicular RNA of fertile men with the *CYRN*1 gene deletion no *CYRN*1 transcript could be detected by RT-PCR. Thus, it was shown that the *CYRN*1 gene has no function in human male fertility.

As stated, in the human genome a second cyritestin gene (*CYRN*2) is present on chromosome 16. Therefore, we suggested that this gene could be the orthologous human gene to the mouse cyritestin gene, whose inactivation results in male infertility in mice [15]. This hypothesis was supported by the observation that the DNA of all individuals lacking the *CYRN*1 gene showed hybridization signals in Southern-blot analysis with a *CYRN*2-specific probe. Testicular RNA from men with the *CYRN*1 deletion was used for amplification with *CYRN*2-specific primers, and RT-PCR products were obtained. This result led us to suggest that the *CYRN*2 gene is the orthologous human gene to the mouse cyritestin gene and therefore of importance for male fertility. Therefore, the expression of the *CYRN*2 gene was studied in human testis. The RNA from individuals with the *CYRN*1 gene deletion was taken for amplification with *CYRN*2-specific primers and the transcript was found in all individuals tested. This result supported the hypothesis that *CYRN*2 can replace the *CYRN*1 gene in its function.

The sequencing of the *CYRN*2 RT-PCR product resulted in some single-base-pair differences when compared with the published sequence of a subcloned genomic fragment (4kb-Hd) [16] but was found to be identical to a genomic sequence of the *CYRN*2 gene on chromosome 16 in the human genome database. To obtain more information about the cDNA and the genomic structure of the *CYRN*2 gene, the cDNA sequence of the *CYRN*1 gene was compared with the sequence of *CYRN*2 in the human genome database. An additional 233 bp was found that shared 87% identity with the *CYRN*1 cDNA in the 5′ direction. The deduced amino acid sequence was found to contain the cysteine-rich, epidermal-growth-factor-like and transmembrane domains of a typical cyritestin protein, but not the disintegrin and metalloprotease domains. Since the working draft sequence clone for *CYRN*2 is not yet completed and the known sequence of the genomic clone 5′ from our cDNA is 22 kb, we cannot exclude the possibility that the missing disintegrin and metalloprotease domains are localized in sequence 5′ of the 22 kb region, which might be an intron. Most importantly, the known *CYRN*2 cDNA was found to contain many stop codons in all three possible reading frames. This result indicates that the *CYRN*2 gene in humans does not code for a functional protein. This is supported by the results of a Western-blot analysis. Our antibody, which was generated against sequences identical in putative *CYRN*1 and *CYRN*2 proteins, gave no staining with testicular proteins of individuals lacking the *CYRN*1 gene but which were endowed with the *CYRN*2 gene. Because the peptide used for raising the antisera includes a potential N-glycosylation sequence, it cannot be excluded that the antisera did not recognize the respective epitope. However, this possibility is rather unlikely because the antisera detected the macaque cyritestin protein (Figure 6).

Taking together all results obtained for the cyritestin genes, it is clear that, in contrast to the mouse, in humans the cyritestin genes are non-functional. Concerning the members of the ADAM gene family, this observation with the cyritestin genes is not unique. In the mouse, fertilin-α is important for sperm–egg binding [23,24], whereas this gene in the human is non-functional and possibly replaced in terms of function by ADAM 20. In the case of cyritestin we have no indication as to which other ADAM gene could replace it. It is also possible that in the human genome a third cyritestin gene exists, but all searches in the human GenBank database were negative.

The results of Frayne and Hall [18] and those reported here are important for the relevance of results obtained in the mouse with respect to our understanding of fertilization in humans. Results obtained from mice in this field, even from knockout mice, can now hardly be assigned to the fertilization process in humans.

This work was supported by the Deutsche Forschungsgemeinschaft (grant no. SFB 271 to W.E.). We thank Susanne Renzing from the Deutsche Primaten Zentrum (DPZ, Göttingen, Germany) for the gift of macaque testis tissue and Angelika Winkler for her excellent secretarial help.

REFERENCES

1 Wolfsberg, T. G., Primakoff, P., Myles, D. G. and White, J. M. (1995) ADAM, a novel family of membrane proteins containing a disintegrin and metalloprotease domain: multipotential functions in cell-cell and cell-matrix interactions. J. Cell Biol. **131**, 275–278
2 Wolfsberg, T. G., Straight, P. D., Gerena, R. L., Huovila, A. P., Primakoff, P., Myles, D. G. and White, J. M. (1995) ADAM, a widely distributed and developmentally regulated gene family encoding membrane proteins with a disintegrin and metalloprotease domain. Dev. Biol. **169**, 378–383
3 Wolfsberg, T. G., Bazan, J. F., Blobel, C. P., Myles, D. G., Primakoff, P. and White, J. M. (1993) The precursor region of a protein active in sperm-egg fusion contains a metalloprotease and a disintegrin domain: structural, functional, and evolutionary implications. Proc. Natl. Acad. Sci. U.S.A. **90**, 10783–10787
4 Heinlein, U. A. O., Wallat, S., Senftleben, A. and Lemaire, L. (1994) Male germ cell expressed mouse gene TAZ83 encodes a putative, cysteine-rich transmembrane protein (cyritestin) sharing homologies with snake toxins and sperm-egg fusion proteins. Dev. Growth Differ. **36**, 49–58
5 Huovila, A. P. J., Almeida, E. A. and White, J. M. (1996) ADAMs and cell fusion. Curr. Opin. Cell Biol. **8**, 692–699
6 Blobel, C. P., Myles, D. G., Primakoff, P. and White, J. M. (1990) Proteolytic processing of a protein involved in sperm-egg fusion correlates with acquisition of fertilization competence. J. Cell Biol. **111**, 69–78
7 Blobel, C. P., Wolfsberg, T. G., Turck, C. W., Myles, D. G., Primakoff, P. and White, J. M. (1992) A potential fusion peptide and an integrin ligand domain in a protein active in sperm-egg fusion. Nature (London) **356**, 248–252
8 Myles, D. G., Kimmel, L. H., Blobel, C. P., White, J. M. and Primakoff, P. (1994) Identification of a binding site in the disintegrin domain of fertilin required for sperm-egg fusion. Proc. Natl. Acad. Sci. U.S.A. **91**, 4195–4198
9 Primakoff, P., Hyatt, H. and Tredick-Kline, J. (1987) Identification and purification of a sperm surface protein with a potential role in sperm-egg membrane fusion. J. Cell Biol. **104**, 141–149
10 Bigler, D., Chen, M., Waters, S. and White, J. M. (1997) A model for sperm-egg binding and fusion based on ADAMs and integrins. Trends Cell Biol. **7**, 220–225
11 Lemaire, L., Johnson, K. R., Bammer, S., Petry, P., Ruddle, F. H. and Heinlein, U. A. (1994) Chromosomal assignment of three novel mouse genes expressed in testicular cells. Genomics **21**, 409–414
12 Linder, B., Bammer, S. and Heinlein, U. A. (1995) Delayed translation and posttranslational processing of cyritestin, an integral transmembrane protein of the mouse acrosome. Exp. Cell Res. **221**, 66–72
13 Linder, B. and Heinlein, U. A. (1997) Decreased *in vitro* fertilization efficiencies in the presence of specific cyritestin peptides. Dev. Growth Differ. **39**, 243–247
14 Yuan, R., Primakoff, P. and Myles, D. G. (1997) A role for the disintegrin domain of cyritestin, a sperm surface protein belonging to the ADAM family, in mouse sperm-egg plasma membrane adhesion and fusion. J. Cell Biol. **137**, 105–112
15 Shamsadin, R., Adham, I. M., Nayernia, K., Heinlein, U. A., Oberwinkler, H. and Engel, W. (1999) Male mice deficient for germ-cell cyritestin are infertile. Biol. Reprod. **61**, 1445–1451
16 Adham, I. M., Kim, Y., Shamsadin, R., Heinlein, U. A., Von Beust, G., Mattei, M. G. and Engel, W. (1998) Molecular cloning, chromosomal localization, and expression analysis of CYRN1 and CYRN2, two human genes coding for cyritestin, a sperm protein involved in gamete interaction. DNA Cell Biol. **17**, 161–168
17 Koehler, M. R., von Beust, G., Engel, W. and Schmid, M. (1997) Assignment of the germ cell cyritestin gene 2 (CYRN2) to human chromosome 16q12.1 by fluorescence in situ hybridization to 5-azacytidine induced, partially decondensed chromosomes. Cytogenet. Cell Genet. **77**, 178–179
18 Frayne, J. and Hall, L. (1998) The gene for the human tMDC I sperm surface protein is non-functional: implications for its proposed role in mammalian sperm-egg recognition. Biochem. J. **334**, 171–176
19 Foley, K. P., Leonard, M. W. and Engel, J. D. (1993) Quantitation of RNA using the polymerase chain reaction. Trends Genet. **9**, 380–385
20 Piperno, G., LeDizet, M. and Chang, X. J. (1987) Microtubules containing acetylated alpha-tubulin in mammalian cells in culture. J. Cell Biol. **104**, 289–302

21 Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D. J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. **25**, 3389–3402
22 Mannan, A., Lücke, K., Dixkens, C., Neesen, J., Kämper, M., Engel, W. and Burfeind, P. (2000) Alternative splicing, chromosomal assigment and subcellular localization of the testicular haploid expressed gene. Cytogenet. Cell Genet. **91**, 171–179
23 Evans, J. P., Schultz, R. M. and Kopf, G. S. (1998) Roles of the disintegrin domains of mouse fertilins alpha and beta in fertilization. Biol. Reprod. **59**, 145–152
24 Evans, J. P., Schultz, R. M. and Kopf, G. S. (1997) Characterization of the binding of recombinant mouse sperm fertilin alpha subunit to mouse eggs: evidence for function as a cell adhesion molecule in sperm-egg binding. Dev. Biol. **187**, 94–106

Received 21 February 2001/28 March 2001; accepted 4 May 2001