The Sea Urchin Sperm Receptor for Egg Jelly Is a Modular Protein with Extensive Homology to the Human Polycystic Kidney Disease Protein, PKD1

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Abstract. During fertilization, the sea urchin sperm acrosome reaction (AR), an ion channel–regulated event, is triggered by glycoproteins in egg jelly (EJ). A 210-kD sperm membrane glycoprotein is the receptor for EJ (REJ). This conclusion is based on the following data: purified REJ binds species specifically to EJ dotted onto nitrocellulose, an mAb to REJ induces the sperm AR, antibody induction is blocked by purified REJ, and purified REJ absorbs the AR-inducing activity of EJ. Overlapping fragments of REJ cDNA were cloned (total length, 5,596 bp). The sequence was confirmed by microsequencing six peptides of mature REJ and by Western blotting with antibody to a synthetic peptide designed from the sequence. Complete deglycosylation of REJ followed by Western blotting yielded a size estimate in agreement with that of the mature amino acid sequence. REJ is modular in design; it contains one EGF module and two C-type lectin carbohydrate-recognition modules. Most importantly, it contains a novel module, herein named the REJ module (700 residues), which shares extensive homology with the human polycystic kidney disease protein (PKD1). Mutations in PKD1 cause autosomal dominant polycystic kidney disease, one of the most frequent genetic diseases of humans. The lesion in cellular physiology resulting from mutations in the PKD1 protein remains unknown. The homology between REJ modules of the sea urchin REJ and human PKD1 suggests that PKD1 could be involved in ionic regulation.

SEA urchin eggs possess an extracellular matrix termed egg jelly (EJ)[1]. Glycoprotein ligands in EJ induce the sperm acrosome reaction (AR) (Keller and Vacquier, 1994*a*; Suzuki, 1995). The AR is required for fertilization; it consists of the exocytosis of the acrosome granule and the polymerization of acrosomal actin to form the bindin-coated acrosomal process used by the sperm to attach to and fuse with the egg (Vacquier et al., 1995). Underlying the EJ-induced AR, increased sperm respiration, motility, and chemotaxis are signal transduction events that involve Ca^{2+} and Na^+ influx, H^+ and K^+ efflux (Darszon et al., 1989; Schackmann, 1989; Vacquier, 1986*a*), cyclic nucleotide changes, and protein kinase activation (Garbers, 1989).

Trypsin treatment of sea urchin sperm blocks AR induction by EJ, suggesting that receptor proteins regulate the ion channel events mediating the AR (Moy, G.W., and V.D. Vacquier, unpublished observations). To identify receptor proteins, mice were immunized with sea urchin sperm, and hybridomas were screened for secretion of mAbs reacting with extracellular epitopes. The majority of mAbs (85 out of 96) reacted with a sperm membrane glycoprotein of 210–260 kD, which was termed the "210-kD protein" (Trimmer, 1987). Previous work had shown that the isolated 210-kD protein bound to live eggs and to EJ dotted onto nitrocellulose (Podell and Vacquier, 1985).

Immunofluorescence (Trimmer et al., 1985) and immunogold (Longo et al., 1989) localization showed the 210-kD protein was present on the surface of the sperm flagellum and also on a thin belt (0.2-μm diam) of membrane directly over the acrosomal granule at the anterior apex of the sperm head. The eightfold increase in immunogold localization over the acrosome, as compared with the remainder of the sperm head, suggested this protein functioned in

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1. *Abbreviations used in this paper*: ADPKD, autosomal dominant polycystic kidney disease; AR, acrosome reaction; CRD, carbohydrate-recognition module; EJ, egg jelly; Fn3, fibronectin type 3; GA/SW, gluteraldehyde in seawater; HF, hydroflouric acid; HSW, filtered seawater containing 10 mM Hepes; ORF, open reading frame; PKD1, human polycystic kidney disease protein; REJ, receptor for egg jelly; SMV, sperm plasma membrane vesicle; WGA, wheat germ agglutinin.

AR induction. Loading sperm with Ca^{2+}-indicator dyes showed that mAbs to the 210-kD protein opened Ca^{2+} channels required for AR induction (Trimmer et al., 1986; Vacquier et al., 1988).

Here we demonstrate that the 210-kD protein is the receptor for egg jelly (REJ) ligands inducing the AR. Molecular cloning showed that REJ is modular in design, containing EGF and carbohydrate-recognition modules. In addition, it possesses a new module, the REJ module, which is also found in the human polycystic kidney disease protein (PKD1).

Materials and Methods

Gametes and Miscellaneous Procedures

Sperm of *Strongylocentrotus purpuratus* were spawned and stored undiluted on ice. Acrosome reactions were scored, and egg jelly was obtained and quantitated (Keller and Vacquier, 1994*a*; Vacquier, 1986*b*). Plasma membrane vesicles (Podell et al., 1984) were commercially deglycosylated by hydrofluoric acid (HF) (Immuno-Dynamics, Inc., La Jolla, CA). Methods to produce mAbs and Fab fragments were previously described, and mAb characterization by immunoprecipitation and Western blotting was presented (Trimmer et al., 1985; Trimmer, 1987; Vacquier et al., 1988). Protein was quantitated by the bicinchoninic acid assay (Pierce Chemical Co., Rockford, IL). All Fab, purified REJ protein, and EJ used for treatment of live sperm were dialyzed into filtered seawater containing 10 mM Hepes (HSW) (pH 8.0, unless noted otherwise). For Fig. 1, *A* and *B*, 10 μl Fab J10/14, J17/30 (IgG), EJ, or HSW was placed in 1.5-ml microcentrifuge tubes. 10 μl of a 1:100 dilution of undiluted semen ("dry sperm") was added, and after 5 min the cells were fixed by addition of 40 μl 5% glutaraldehyde in seawater (GA/SW). Acrosome reactions were scored by preparing thumb-squashed slides and viewing in phase contrast at a magnification of ~1,200 (Vacquier, 1986*b*). Percentage of AR was rounded to the nearest whole number. In Fig. 1 *B*, the various pHs were obtained using 10 mM Hepes seawater adjusted to pHs 6.8–8.0, and the sperm were diluted 1:100 into the pH adjusted seawaters. In Fig. 1 *B* the final concentration of EJ was equivalent to 12 μg fucose per ml (yielding 95% AR), and that of Fab J10/14 was 250 μg/ml (yielding 95% AR). For Fig. 1 *C*, 10 μl of Fab J10/14 was diluted 50% per dilution in HSW, pH 7.5, in a series of tubes, and 10 μl of a 1:100 dilution of dry sperm (in HSW, pH 7.5) was added. After 5 min 20 μl of EJ was added; the final concentration of EJ was 12 μg fucose per ml. After 5 min 80 μl of GA/SW was added, and the cells were scored for AR. For Fig. 1 *D*, 5 μl HSW containing purified REJ protein was diluted into a series of tubes, with the protein varying from 0 to 719 ng. 5 μl of Fab J10/14 (1.8 ng protein) was added to each tube and incubated 2 h at 22°C. 10 μl 1:100 diluted sperm in HSW, pH 8.0, was added, and after 5 min the samples were fixed by addition of 40 μl GA/SW. In Fig. 1, each data point represents 200–600 cells.

For Table I, 5 μl EJ (final concentration after adding sperm was equivalent to 780 ng fucose per ml) was mixed with 5 μl of either HSW or HSW containing 12.5 μg purified REJ protein (Fig. 3, lane *B*) for 2 h at 22°C, and 10 μl of 1:100 dilution of sperm was added (final concentration of REJ was 625 μg/ml). EJ was used in a linear region of the concentration dependence curve, in this case the amount resulting in 25% acrosome-reacted sperm. After 15 s the cells were fixed by addition of 40 μl GA/SW. 4% AR was observed in the seawater-only control. This value was subtracted from the other samples to arrive at 86% inhibition of the EJ-induced AR.

A synthetic peptide (KGVSNEDPDTDA) was conjugated to ovalbumin (Doolittle, 1987), and rabbit antibody was commercially prepared (Cocalico Biologicals Inc., Reamstown, PA). The whole antiserum was used without purification of specific IgGs. SDS-PAGE gels were performed according to the method of Laemmli (1970), and Western blots were performed and developed using the directions in the ECL kit (Amersham Corp., Arlington Heights, IL).

Purification of 210-kD Protein from Sperm

Sperm plasma membrane vesicles (SMV) were prepared by the pH-9 method (Podell et al., 1984). The proteins found in SMVs are shown in Fig. 3 *A* (Podell et al., 1984; Vacquier, 1986*b*). The 200,000 *g* (60 min) supernatant of SMVs was applied to a wheat germ agglutinin (WGA) agarose column (5 ml; E-Y Laboratories, Inc., San Mateo, CA). The column was washed with a minimum of 10 vol: 500 mM NaCl/50 mM Hepes/5 mM EDTA/1% Triton X-100, followed by 10 vol 150 mM NaCl/50 mM Hepes, pH 8.0/5 mM EDTA. The REJ protein was eluted in a single step of the second wash buffer containing 100 mM GlcNAc. The peak fractions ($A_{280\ nm}$) were concentrated using Centricon-50 concentrators (Amicon Corp., Danvers, MA), and the buffer was exchanged for HSW using Centricons (three times, 2 ml). The purity of the isolated REJ protein is shown in Fig. 3, lane *B*.

Peptide sequences of fragments of REJ were obtained by two methods starting with the WGA-eluted peak. Peptide sequences p2, p3, and p5 were from sperm of the congeneric species, *Strongylocentrotus franciscanus*. This is because the REJ is more abundant in this species. The WGA eluate was carboxymethylated, dialyzed into distilled water, and REJ separated on 7% acrylamide SDS-PAGE. The REJ band was excised after visualization by negative staining in 2 M KCl. CNBr cleavage was done for 2 h at 22°C (Matsudaira, 1989). The gel slices were washed twice in excess 125 mM Tris, pH 6.8 (10 min). Peptides were separated on a 20% acrylamide gel by SDS-PAGE and transferred to a polyvinyldifluoride membrane in 10 mM CAPS buffer, pH 11/10 % methanol. The polyvinyldifluoride membrane was stained in Coomassie blue and destained, peptide bands were excised, and gas-phase amino acid sequencing was performed (Matsudaira, 1989). Peptides p1, p4, and p6 were obtained from *S. purpuratus* REJ by running lanes of 80 μg WGA eluate on 5% SDS-PAGE gels, visualizing REJ using the $CuCl_2$ staining kit (Bio Rad Laboratories, Richmond, CA) and excising the center of the REJ band from the gel. The cut-outs were exposed to trypsin, and the tryptic peptides were separated by reverse-phase HPLC (Ferrara et al., 1993). The peak fractions were gas-phase microsequenced by the University of California, San Diego Protein Sequencing Laboratory.

Molecular Cloning, Sequencing, and Expression

A Lambda Zap cDNA library of *S. purpuratus* testis was prepared following the manufacturer's directions (Stratagene, La Jolla, CA). The library was screened with a polyclonal antibody raised to the REJ protein band cut from SDS-PAGE gels. The antiserum had been absorbed with an acetone powder of sea urchin ovaries and reacted with the REJ protein on Western blots (Fig. 3, lane *C*). Primary screening yielded 22 positive plaques, 14 of which had unique restriction maps. One clone (2 kb) was sequenced; it contained the sequences of four REJ-derived peptides (Fig. 2 *B*; *p2–p5*). All 14 unique clones were screened with an exact 57-mer oligonucleotide to the sequence of peptide p2. Six positives were obtained, two of which contained the full-length 210-kD open reading frame (ORF) with an overlap of 1,200 bp. Both ends of the ORF were confirmed by sequencing independently picked phage plaques. The deduced sequence was further confirmed by obtaining two additional sequences of tryptic fragments of REJ isolated from *S. purpuratus* sperm (Fig 2 *B*; *p1* and *p6*). Nested deletions were made using the Erase-a-Base System (Promega, Madison, WI), and T3 and T7 primers were used for sequencing. Gaps in the sequence were filled in using custom primers. All sequence elements overlapped, and both strands of all clones were sequenced using Sequenase 2.0 (United States Biochemical Corp., Cleveland, OH).

Computer Analyses

Hydrophilicity profiles were generated by the method of Kyte and Doolittle (1982) using a window of 14 residues. Homology searches used the BLAST (Altschul et al., 1990) and FASTA (Pearson and Lipman, 1988) algorithms using a variety of scoring matrices. Statistical significance of pairwise comparisons (Tables II and III) used the RFD2 program (500 randomizations, Ktup = 2) (Pearson, 1990). Multiple alignments were made using the NEWAT progressive alignment program (Feng and Doolittle, 1990), except in the case of the putative Fn3 modules of the PKD1 protein, where the published alignments were used (Hughes et al., 1995). Profiles (Gribskov et al., 1990) were computed from the aligned sequences using the Wisconsin Genetics Computer Group's program PROFILEMAKE and searched against GenBank (release 88) using the program PROFILESEARCH (Genetics Computer Group, 1991). These programs were accessed using the suite of programs in DNASYSTEM (Smith, 1988).

Results

The 210-kD Protein Is the Sperm REJ

Fab of the 210-kD mAb J10/14 induce the sperm AR in a concentration-dependent manner (Fig. 1 *A*). Both IgG

(50% AR at 1 μg/ml) and Fab (50% AR at 31 μg/ml) are inductive. Another mAb, J17/30, reacting with a 63-kD GPI-anchored sperm membrane protein (Mendoza et al., 1993) is noninductive (Fig. 1 *A*). Induction of the AR by EJ and Fab J10/14 exhibits different pH dependencies, with EJ being maximally inductive at and above pH 7.5, whereas mAb J10/14 is noninductive at pH 7.5 (Fig. 1 *B*). Preincubation of the cells with Fab J10/14 at pH 7.5 blocks EJ from inducing the AR in a concentration-dependent manner (Fig. 1 *C*). This result shows that after the J10/14 binding site is occupied by Fab, EJ ligands cannot displace the antibody and induce the AR. Preincubation of Fab J10/14 with purified 210-kD protein (Fig. 3, lane *B*) blocks the induction of the AR by the antibody (Fig. 1 *D*). At pH 8.0, preincubation of EJ with purified 210-kD protein blocks EJ from inducing the AR (Table I); in this experiment the inhibition by the isolated 210-kD protein was ~86% (Table I). The earlier data (Podell and Vacquier, 1985) and the new data of Fig. 1 and Table I show that the 210-kD protein is a critical sperm surface receptor for EJ ligands inducing the AR. This sperm membrane protein was named "REJ," denoting it as the receptor for egg jelly.

Table I. Purified 210-kD Protein Blocks Egg Jelly from Inducing the Sperm Acrosome Reaction

Inducer	Condition	Yes AR	No AR	Total	Percentage of AR
Egg Jelly					
	A. 10mM Hepes SW, pH 8.0	32	868	900	4
	B. 210-kD protein	19	281	300	6
	C. Egg jelly + 210 kD	63	837	900	7
	D. Egg jelly only	375	1,125	1,500	25

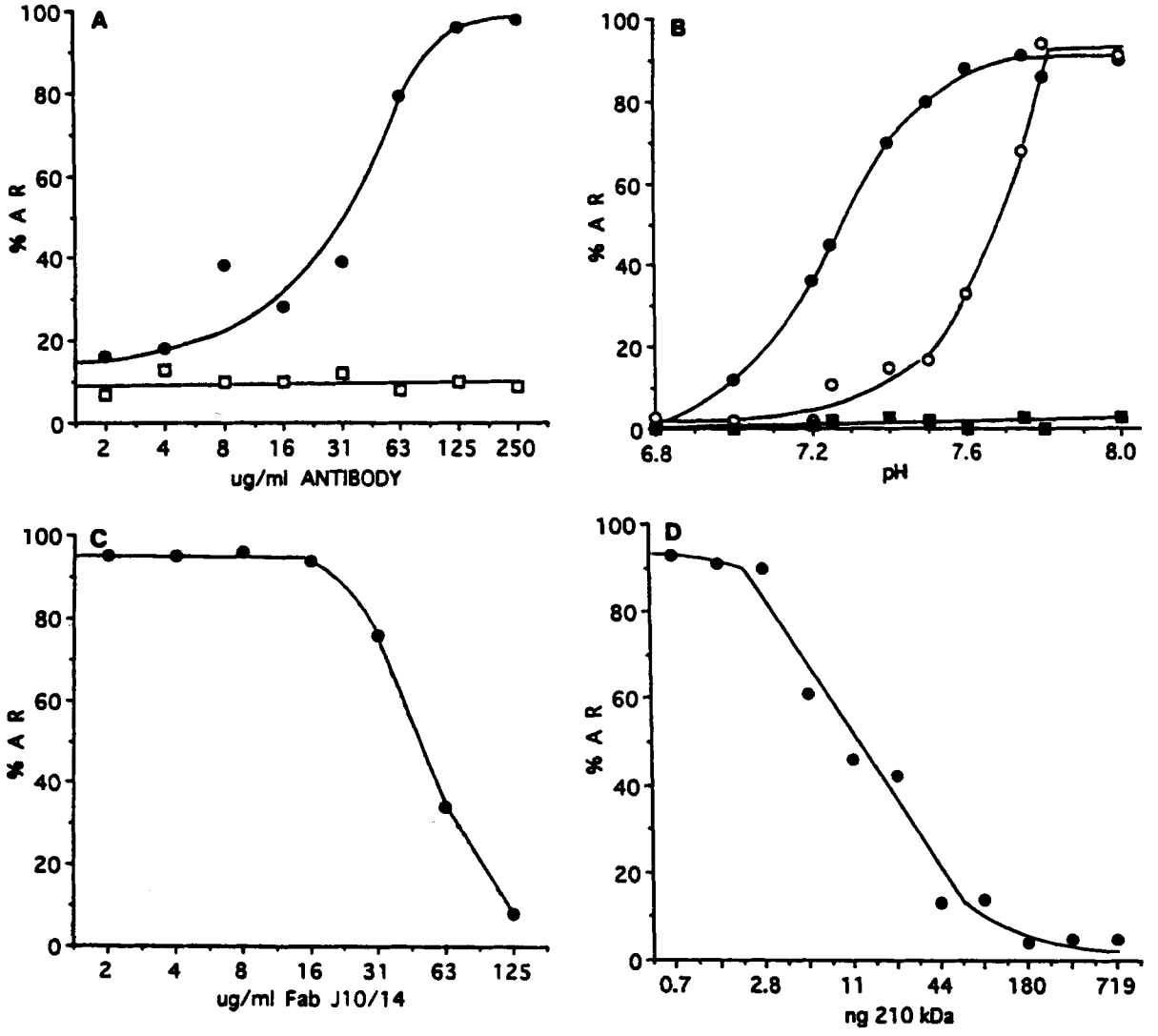

Figure 1. Effects of Fab J10/14, EJ, pH, and purified REJ on the *S. purpuratus* sperm acrosome reaction. See Materials and Methods for details. (*A*) Concentration dependence of Fab J10/14 induction of the AR at pH 8.0 (●). mAb J17/30 (IgG) reacting with a 63-kD sperm membrane protein (negative control, □). (*B*) pH dependence of the EJ-(●) and Fab J10/14–(○) induced AR; seawater control (■). (*C*) Concentration dependence of the inhibition of the EJ-induced AR by pretreatment of sperm with Fab J10/14 at pH 7.5. (*D*) Concentration dependence of the inhibition of the Fab J10/14–induced AR by purified REJ protein, pH 8.0. Half maximal inhibition occurred at a 2:1 molar ratio of REJ (210-kD protein) to Fab J10/14.

Sequence of REJ

The total nucleotide sequence obtained, 5,596 bp, consists of 201-bp 5′ untranslated region, 4,350-bp ORF, and 1,045-bp 3′ untranslated region. Northern blots of testis polyA$^+$ RNA yielded two closely spaced bands at ~7,200 bp (not shown). The arrangement of the modules contained in the 1,450 amino acid ORF is shown diagrammatically in Fig. 2 *A*. These consist of one EGF module (40 residues), two carbohydrate-recognition modules (CRDs) (119 and

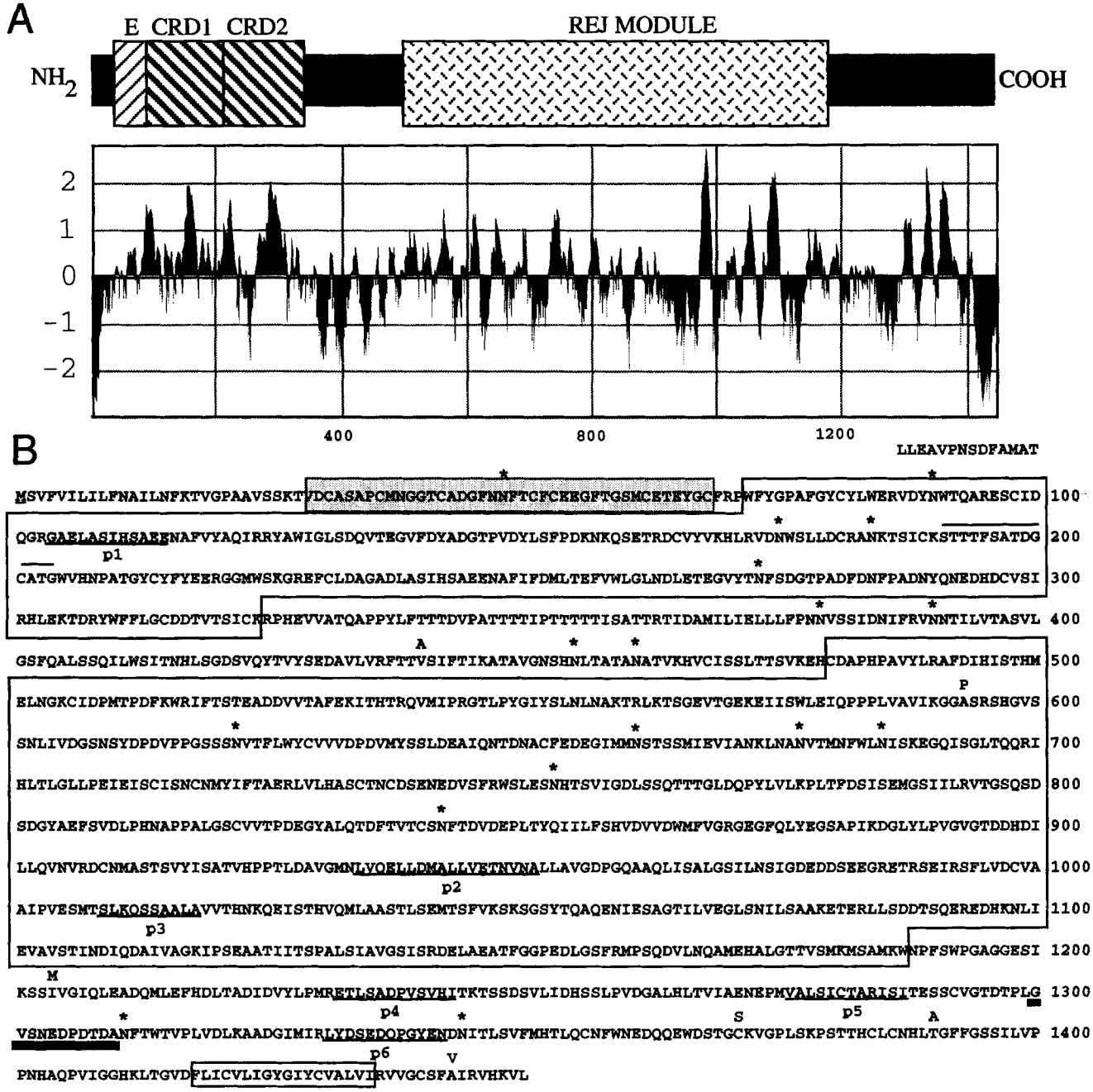

Figure 2. Deduced amino acid sequence of the REJ protein. (*A*) Diagrammatic scheme of the modular design of REJ presented over a hydrophilicity plot of the sequence. *E*, EGF module; *CRD*, carbohydrate-recognition domains 1 and 2. (*B*) The 1,450-residue ORF of REJ. Underlined Met at position 1 marks the putative start of the ORF; the putative NH_2 terminus of the mature protein is Lys^{27}. Shaded box marks the EGF module, followed by the two CRDs with a 14-residue spacer between them (*overline*). The REJ module is also boxed (positions 480–1,187), as is the 18-residue putative transmembrane segment in the COOH-terminal region. Asterisks mark 17 potential N-linked glycosylation sites. p1–p6 are peptide sequences obtained from fragments of mature REJ isolated from sperm. Thick underline (positions 1,300–1,310) denotes synthetic peptide used for generation of polyclonal antiserum. Six polymorphic sites are labeled by letter above their positions. These sequence data are available from EMBL/GenBank/DDBJ under accession number U40832.

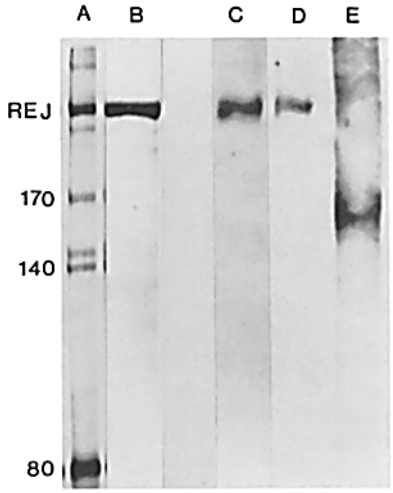

Figure 3. Western blots of REJ. Lanes *A* and *B* are silver-stained SDS-PAGE gels, and lanes *C–E* are Western blots of SMV developed with ECL. Lane *A*, 4 μg SMV protein; lane *B*, 1.2 μg WGA-purified REJ protein used in Table I and Fig. 1 *D*. (Lane *C*) Reaction of the absorbed rabbit antibody to REJ used for primary screening of the lambda Zap library. (Lane *D*) Reaction of antibody to the synthetic peptide reacting with SMV. (Lane *E*) Reaction of the antibody to the synthetic peptide reacted with SMV that had been deglycosylated by HF treatment. The reacting band shifts to ~160–170 kD. Numbers on left margin, M_r in kD.

120 residues), and a novel module, the REJ module (707 residues). The hydrophilicity plot of the sequence (Fig. 2 *A*) shows two hydrophobic regions. The probable signal sequence is at the NH_2 terminus, and the putative membrane-spanning sequence is close to the COOH terminus. The orientation of this putative membrane-spanning segment (*box*, residues 1,418–1,435) follows the "charge difference rule," having greater positive charge on the COOH-terminal side of the segment as compared with the NH_2-terminal side (Sipos and von Heijne, 1993). Detailed analysis of the short, internal, hydrophobic sequence elements throughout the sequence shows they could not span the membrane as alpha helices.

The full-length deduced amino acid sequence of REJ of 1,450 residues is shown in Fig. 2 *B* with the three types of modules boxed. The start and stop codons were confirmed by sequencing independently picked phage plaques. The putative initiation Met is underlined. This was chosen as the start of the ORF because its sequence of GCAACCAUGA conformed in 8 out of 10 sites to the preferred translation initiation sequence of $GCC^{A}/_{G}CCAUGG$ (Kozak, 1987).

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MOUSE NOTCH DECASTPCKNGAKCLDGPNTYTCVCTEGYTGTHCEVDIDEC
BRACHYDANIO DECASTPCKNGAKCTDGPNKYTCECTPGFSGIHCELDINEC
DROS DEVOPM DDCASFPCQNGGTCLDGIGDYSCLCVDGFDGKHCETDINEC
SEAURCH FIB NECASIPCLNGGICVDGINQFACTCLPGYTGILCETDINEC
XENOPUS XOT DDCQPNPCHNGGSCSDGINMFFCNCPAGFRGPKCEEDINEC
SEAURCH REJ VDCASAPCMNGGTCADGFNNFTCFCEEGFTGSMCETEY-GC
HUM FACT IX DQCESNPCLNGGSCKDDINSYECWCPFGFEGKNCELDV-TC
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Figure 4. Alignments of representative EGF modules compared to the EGF module of REJ. Dashes are included for alignment. These sequence data are available from EMBL/GenBank/DDBJ under the following accession numbers: mouse notch, 483581; *Brachydanio*, 433867; *Drosophila* developmental, 157993; *S. purpuratus* fibropellin, 310660; *Xenopus* xotch, 214925; and human factor IX, 182609.

In-frame sequence obtained 5′ to the putative Met^1 is also shown. The precise junction between the signal sequence and the mature protein is speculative. Given the "−3,−1 rule," the most probable junction is between Ser^{26} and Lys^{27}. This assignment would place Pro in the −6 position, which is optimal for marking the junction (von Heijne, 1990). The six polymorphic positions are noted with the alternative residue above the sequence; five are conservative replacements (the cDNA library was made from several individuals). The sequence was confirmed to be that of the REJ protein by amino acid sequencing of six fragments of mature REJ extracted from sperm (*underlines*, p1–p6). In addition, an antiserum raised to a synthetic peptide corresponding to residues 1,300–1,310 (*thick underline*) reacted with the REJ protein on Western blots of sperm plasma membrane vesicles (Fig. 3, lane *D*).

Although its mobility on SDS-PAGE is in the 210–260-kD range, the 1,424 amino acids of the putative mature REJ account for only 155,420 units of molecular mass. By chemical analysis, REJ protein is 49% carbohydrate (Podell and Vacquier, 1985). The 17 sites for potential N-linked glycosylation are marked with asterisks (Fig. 2 *B*). Digestion with peptide-N-glycosidase-F decreased the relative mass to ~190 kD. Complete deglycosylation by HF treatment of sperm plasma membrane vesicles (Podell et al., 1984), followed by SDS-PAGE and Western blotting with the synthetic peptide antibody to REJ, yielded a single reaction at ~160–170 kD (Fig. 3, lane *E*).

Sequence Modules of REJ

EGF Module. The EGF module (*shaded box*, positions 29–68) contains the diagnostic seven Cys residues (Fig. 4). This module is similar to the sea urchin fibropellins and various developmentally regulated proteins such as notch and xotch. Table II presents the quantitative pairwise

Table II. Quantitative Comparisons of EGF Modules

	NOTCH	BRACH	DDEVLP	FIBROP	XOTCH	REJ	FACT IX
NOTCH		78.0	58.5	58.5	48.8	52.5	47.5
BRACH	20.0		61.0	61.0	53.7	50.0	52.5
DDEVLP	18.7	15.0		58.5	58.4	55.0	55.0
FIBROP	16.3	18.5	16.8		56.1	55.0	50.0
XOTCH	14.9	14.7	14.3	17.1		50.0	57.5
REJ	12.4	11.7	13.1	14.7	12.1		45.0
FACT IX	12.8	13.4	13.5	13.4	14.0	13.0	

Values below the diagonal are pairwise alignment scores in SD units, and above the diagonal, percent identity in pairwise comparisons.

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REJCRD1 WFYGPAFGYCYLWERV-DYNWTQARESCIDQGRGAELASIHSAEENAFV-Y--AQI-RRYA--WIGLS
REJCRD2 WVHNPATGYCYFYEER-GGMWSKGREFCLDA--GADLASIHSAEENAFI-F--DML-TEFV--WLGLN
HUMCRD8 WI--PFHGHCYYIESSYTRNWGQASLECLRM--GSSLVSIESAAESSFLSYRVEPL-KSKTNFWIGLF
MBRA    W--QEYDGHCY WASTYQVRWNDAQLACQTVHPGAYLATIQSQLENAFI----SET-VSNNRLWIGLN
180PPLA WLFHQDAEYLFYPHSS---EWSSFEFVCGWL--RSDILTIHSAHEQEFILSKIKALSKYGANWWIGLQ
RHANS   EGSNAYSSYCYYFMED-HLSWAEADLFCQNMN-SGYLVSVLSQAEGNFLASLIKESGTTAANVWIGLH
PKD1    TEIFPGNGHCYRLVVE-KAAWLQAQEQCQAWA-GAALAMVDSPAVQRFL----VSRVTRSLDVWIGFS
INTRON+ WV      SC WF       W     Y       AHL  V S  EQ                 W  L

REJCRD1 DQVTEGVF-DYADGTPVDY---LSFPD--KNKQS-ETRDCVYVKHL-RVDN-WSLLD-CRANKTSICK
REJCRD2 DLETEGVYTTFSDGTPADF---DNFPA--DNYQN-EDHDCVSIRHLEKTDRYWFFLG-CDDTVTSICK
HUMCRD8 RNV-EGTW-LWINNSPVSF---VNWNT--GDPSG-ERNDCVALHA---SSGFWSNIH-CSSYKGYICK
MBRA    DIDLEGHY-VWSNGEATDF---TYWSS--NNPNNWENQDCGVVNY-DTVTGQWDDDD-CNKNKNFLCK
180PPLA EETANDEL-RWRDGTPVIY---QNWDKERDRSMNNQSQRCAFISS---ITGLWDREE-CSVSMPSICK
RHANS   DPKNNRRW-HWSSGSLFLY---KSWDT--GYPNNSNRGYCVSVTS-NSGYKKWRDNS-CDAQLSFVCK
PKD1    TVQGVEVG-PAPQGEAFSLESCQNWLP--GEPHPATAEHCVRL-----GPTGWCNTDLCSAPHSYVCE
INTRON+      G W KW  G    Y              G              D              RW
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Figure 5. Alignments of representative CRD modules compared to REJ. Dashes are included for alignment; the two REJ CRD modules are aligned to each other. Bold type denotes identities in five out of seven sequences. The intron $^+$ CRD consensus sequence (Taylor et al., 1990) is shown below the alignments. These sequence data are available from EMBL/GenBank/DDBJ under the following accession numbers: human mannose receptor repeat #8 (*HUM 8*), 187334; acorn barnacle *Megabalanus rosa* lectin BRA-3 (*MBRA*), 407227; 180-kD phospholipase A2 receptor precursor (*PPLA*), 456376; islet regenerating protein (*RHANS*), 206605; and PKD1, U24497.

comparisons of EGF modules; the numbers below the diagonal line are pairwise alignment scores in SD units. Values >7 SD units demonstrate homology (Doolittle, 1987). Above the diagonal line are percent sequence identities in the 40-residue modules. The sequences presented are representative of those retrieved by BLAST and FASTA searches of GenBank. The EGF module of REJ does not match the consensus sequence of a Ca^{2+}-binding EGF module (Rao et al., 1995).

Carbohydrate-recognition Modules. The two CRD modules (Fig. 2 *B*, *boxed region*, positions 72–190 and 205–324) are separated by a 14-residue spacer (*overlined*; Fig. 2 *B*). These modules are characteristic of an "intron plus" C-type lectin (Taylor et al., 1990). Alignments with representative CRD modules retrieved by BLAST and FASTA searches of GenBank (Fig. 5) show the similarity of these sequence elements. A quantitative comparison using pairwise alignment scores is shown in Table III; numbers below the diagonal line are in SD units, and above the diagonal, percent identities. The consensus sequence of the intron plus C-type lectin CRD (Taylor et al., 1990) is shown below the alignments (Fig. 5). Identity of both CRDs of REJ to the consensus sequence is found in 12 out of 29 positions. The classification of intron plus and intron minus CRDs is also based on genomic structure, of which we know nothing regarding REJ.

The REJ Module. Comparing REJ to GenBank detected similarity to the human PKD1 protein. Closer examination revealed a novel module of 707 amino acids here named "the REJ module" (50% of the mature REJ protein and 17% of PKD1). The alignment of the two REJ modules (Fig. 6; REJ positions 480–1,187; PKD1 positions 2,146–2,882) (International Polycystic Kidney Disease Consortium, 1995) yields 20% sequence identity and 40% sequence similarity. There are 10 conserved Cys residues in this alignment. The pairwise alignment score of 27 SD units shows the statistical significance of this alignment (Doolittle, 1987). The exact boundaries of the REJ module remain unknown until others are discovered. There is no evidence for repeating motifs within the 707 residues. In both sea urchin and human proteins, the REJ module is the closest module to the COOH terminus. A profile was made using the REJ modules of both proteins; it was compared to GenBank and no significant matches were found.

The portion of PKD1 encompassing the REJ module was originally reported to be nonhomologous to any sequence in GenBank (International Polycystic Kidney Disease Consortium, 1995). No repeating motifs were reported within this region. However, a second report of the PKD1 sequence (Hughes et al., 1995) claimed this region contained four fibronectin type-3 (Fn3) modules (Doolittle and Bork, 1993). We constructed a profile of the four putative Fn3 modules of the PKD1 protein (Hughes et al., 1995) and used it to search GenBank. No significant matches were found to proteins containing Fn3 modules. Next, we made a profile of 27 Fn3 modules and compared it to the four putative Fn3 modules of PKD1 protein (Hughes et al., 1995); no significant matches were found. This profile recognized a wide variety of proteins containing Fn3 modules. Based on these analyses, we do not believe that the PKD1 or REJ proteins contain Fn3 modules.

The original description of the PKD1 sequence (International Polycystic Kidney Disease Consortium, 1995) found only one transmembrane region close to the COOH

Table III. Quantitative Comparisons of CRD Modules

	REJ 1	REJ 2	HUM 8	MBRA	PPLA	RHANS	PKD1
REJ 1		50.4	35.7	35.1	24.8	23.9	25.7
REJ 2	39.6		31.0	33.3	28.1	24.6	21.2
HUM 8	15.5	14.9		33.6	23.9	29.6	24.6
MBRA	18.7	18.8	23.6		23.9	29.7	24.6
PPLA	14.5	12.5	17.2	13.1		24.0	14.8
RHANS	9.8	12.6	24.8	22.6	16.8		26.3
PKD1	9.1	9.3	12.2	11.6	5.0	12.1	

Values below the diagonal are pairwise alignment scores in SD units, and above the diagonal, percent identity in pairwise comparisons.

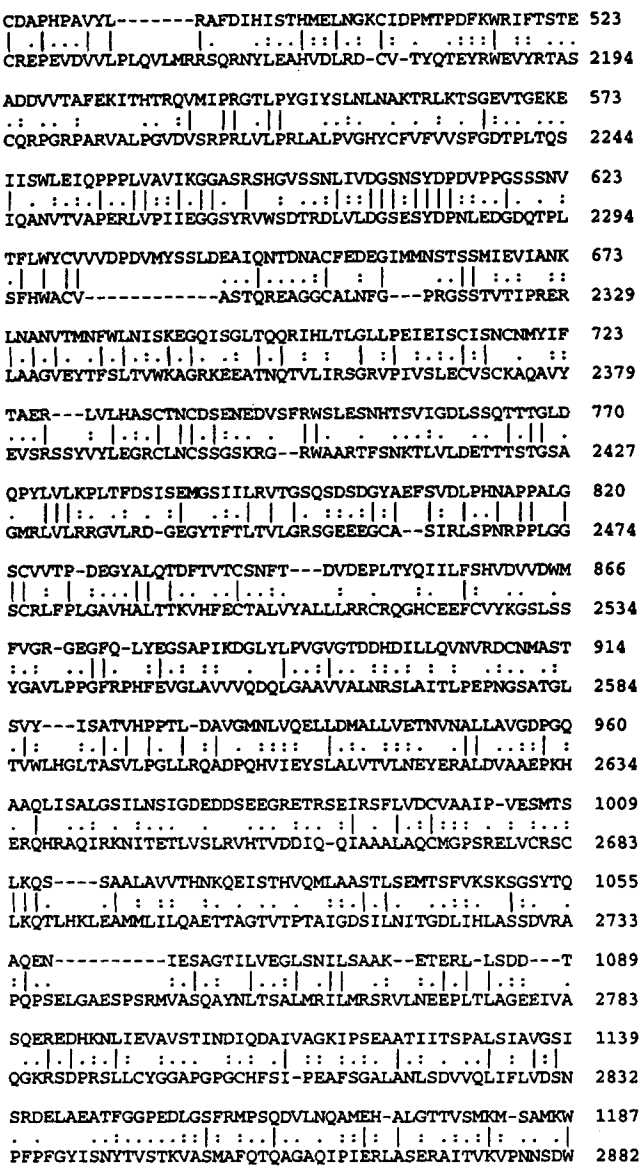

Figure 6. Alignments of the REJ modules of sea urchin REJ protein and human PKD1. In each pair of lines, sea urchin REJ (positions 480–1,187; Fig 2 *B*) is the upper sequence and human PKD1 (positions 2,146–2,882) is the lower sequence. Identical positions are connected by vertical lines, double dots show conservative replacements, and single dots show semiconservative replacements. Dashes are inserted for alignment.

terminus in the sequence of 4,304 amino acids. However, the second report of the PKD1 sequence (Hughes et al., 1995) claimed the existence of 11 transmembrane segments. Two of these putative transmembrane segments lie within the REJ module. Our analysis does not support the existence of transmembrane segments within the REJ module.

Discussion

REJ: Sperm Receptor for Egg Jelly

REJ had been previously identified as the major WGA-binding protein of sea urchin sperm. WGA blocked the EJ-induced AR, and the block could be overridden by the Ca^{2+} ionophore A23187 (Podell and Vacquier, 1984; Sendai and Aketa, 1989). Purified ^{125}I-labeled REJ bound to live eggs and species selectively to EJ dotted onto nitrocellulose (Podell and Vacquier, 1985). REJ is found on the entire cell surface of round spermatocytes, showing that it is expressed at the earliest stage of spermiogenesis (Nishioka et al., 1987). In mature spermatozoa, REJ localizes over the acrosome granule and on the entire flagellum (Trimmer et al., 1985; Longo et al., 1989). This previous work identified REJ as a potential candidate receptor for AR-inducing ligands of EJ (Keller and Vacquier, 1994*a*).

In the first report on mAb J10/14, the antibody blocked the EJ-induced AR (Trimmer et al., 1985). However, Fig. 1, *A* and *B*, show this mAb is a potent AR inducer. The contradiction can be explained by comparing the pH-dependency curves of AR induction by EJ vs mAb. mAb induction has a sharp pH-dependency curve between pH 7.5 and 8.0, whereas EJ is inductive below pH 7.5 (Fig. 1 *B*). In the first experiments using mAb J10/14, we did not know that the cells acidified the medium so rapidly when semen was diluted 100-fold into buffered seawater (Vacquier, 1986*b*). In Fig. 1, the cells were used within 1 min after dilution, which is before the drop occurs to the nonpermissive pH. This explains the difference in our previous results (Trimmer et al., 1985), compared to the results of Fig. 1, *A* and *B*. Results similar to Fig. 1 *A* have been obtained using sperm of a Japanese sea urchin in which the AR was induced by treatment with Fab of an IgG raised against the major WGA-binding membrane protein (260 kD) (Sendai and Aketa, 1989).

The most direct evidence that REJ is the sea urchin sperm receptor for EJ is the neutralization of EJ and mAb as inducers of the AR by preincubation with purified REJ protein (Table I; Figs. 1 *D* and 3 *B*). Inhibition is not caused by nonspecific interaction with added protein, because the AR and sperm attachment to eggs are quantitatively unaffected by 1 mg/ml soybean trypsin inhibitor (Vacquier et al., 1973).

Relationship of REJ to the Cell Membrane

The tentative hypothesis is that REJ is a type I membrane protein with the 18-residue segment near the COOH terminus (Fig. 2 *B*, *boxed*) spanning the cell membrane. Although this 18-residue segment is shorter than ideal, its flanking sequence elements agree with the charge difference rule for membrane-spanning domains (Sipos and von Heijne, 1993). Other experimental evidence indirectly supporting the hypothesis that REJ is an integral membrane protein is that it does not wash off the cells when they are suspended for 24 h in pH-8.0 seawater (Vacquier, V.D., unpublished observations). Additional indirect evidence is the eightfold increase in REJ density on the narrow belt of membrane over the acrosome, as compared with the remainder of the sperm head (Longo et al., 1989).

When sea urchin sperm are incubated 5–18 h in pH-9 seawater containing 5 mM benzamidine, right-side-out, tightly sealed, unilamellar plasma membrane vesicles bud from the cells and are sedimented at 40,000 *g* centrifugation (SMVs) (Podell et al., 1984) (Fig. 3 *A*). Approximately 30–40% of total REJ antigenicity remains tightly associated with SMV.

REJ is not released from the membrane vesicles by high salt washes or EDTA. Also, REJ is not stripped from SMVs by 0.2 M Na_2CO_3 at pHs ranging from 7–11. REJ is only released to the supernatant by detergent solubilization of the vesicles (Podell et al., 1984). However, during the preparation of SMVs, the prolonged exposure of sperm to pH-9.0 seawater/5 mM benzamidine releases ~60–70% of the total REJ antigenicity to the 200,000 *g* supernatant (Trimmer, 1987; Moy, G.W., and V.D. Vacquier, unpublished data). The combination of prolonged high pH plus benzamidine may cause the COOH terminus of REJ to pull out of the cell membrane, leaving the protein freely soluble. Conclusive evidence demonstrating the exact relationship of REJ to the membrane requires additional experiments. We previously demonstrated that REJ is not GPI-anchored to the cell membrane (Mendoza et al., 1993).

The CRD Module of REJ

The two CRD modules suggest REJ is involved in carbohydrate recognition of EJ. This agrees with data demonstrating that glycoproteins of EJ possess the AR-inducing activity. For example, harsh alkaline hydrolysis of EJ in the presence of borohydride (which should break all peptide bonds) and proteinase-K digestion release AR-inducing glycopeptides from the EJ complex. Digestion of EJ with peptide-N-glycosidase-F, followed by gel filtration chromatography, yields a glycopeptide peak with AR-inducing activity (Keller and Vacquier, 1994*b*). Furthermore, boiling *S. purpuratus* EJ in 5% SDS/10% mercaptoethanol, followed by precipitation and washing in 70% ethanol, does not decrease the AR-inducing potency of EJ (Keller and Vacquier, 1994*a*).

REJ as a Regulator of Sperm Ion Channels

Increases in both intracellular Ca^{2+} and pH are required for AR induction of sea urchin sperm (for reviews see Darszon et al., 1989; Schackmann, 1989; Garbers, 1989; Vacquier, 1986*a*). The interrelationship of the activations of the various ion transporters is not established. Blocking Ca^{2+} influx through the verapamil-sensitive Ca^{2+} channel also blocks the stoichiometric Na^+/H^+ exchange that elevates cellular pH (Schackmann, 1989; Darszon et al., 1989). When sperm are loaded with fura2 or indo1 and treated with mAbs to REJ, large increases in intracellular Ca^{2+} occur (Trimmer et al., 1986). Antibodies raised to REJ of a Japanese sea urchin show that the opening of sperm Ca^{2+} channels is a prerequisite for the Na^+/H^+ exchange that alkalinizes the cell and triggers the AR (Sendai and Aketa, 1991). Thus, the induction of the AR by antibodies to REJ shows that REJ is in some way linked to the regulation of ion transport. The primary structure of REJ does not suggest how it could be involved in this type of regulation.

REJ is also expressed on the surface of the sea urchin sperm flagellar membrane. The surface area of the flagellum is approximately five times the area of the sperm head. Immunofluorescence and immunogold localization show the majority of REJ resides on the flagellum. The response of fura2-loaded sperm to egg surface–derived molecules show that both the sperm head and the flagellum exhibit increased Ca^{2+} entry (Schackmann, 1989). If REJ functions as a regulator of ion transport, it would not be surprising to find it on the flagellar membrane. This membrane is extremely active in several ion transport activities required for maintenance of intracellular pH and ATP-dependent motility (Lee, 1984; Gatti and Christen, 1985).

Could REJ Modules Function in Ion Transport Phenomena?

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common human hereditary diseases. ADPKD is a systemic disease, affecting not only the kidneys, but other tubular organs such as the liver and pancreas. Heart valves are also abnormal, and ~10% of patients suffer cerebral aneurysms (for reviews see Aziz, 1995; Calvet, 1993; Carone et al., 1994, 1995; Gabow, 1993; Grantham, 1990; Ogborn, 1994). ADPKD is caused by mutations in the PKD1 gene on human chromosome 16. Although an enormous amount of research has been done on ADPKD, the primary molecular lesion in cellular physiology caused by mutant PKD1 protein remains unknown.

The surprising finding to report here is the homology shared between 50% of the sea urchin REJ protein and 17% of the human PKD1 protein (Fig. 6). The REJ module of REJ may be the functional portion involved in the regulation of ion transport in sea urchin sperm. Likewise, the REJ module of PKD1 may also function in ion transport homeostasis. Abnormal ionic flux could explain many of the molecular changes seen in ADPKD cells (see reviews cited above). Some of these abnormalities are increased cAMP concentrations, activation of oncogenes, cytoskeletal abnormalities, and increased cell proliferation.

Differences in ion transport between normal and ADPKD cells have been documented (see above reviews). In addition, ion transport in RBCs of ADPKD patients is altered (Guarena et al., 1993). Inhibition of Na^+/H^+ transport by amiloride suppresses renal cyst formation in vitro (Woo et al., 1994). Bioelectric properties of ADPKD cells suggest the involvement of anion channels in cyst formation (Grantham et al., 1995). Other ion transport systems implicated in ADPKD are: Na^+/K^+ ATPase, Na^+-dependent H^+ and Ca^{2+} transport, and Cl^-/HCO_3^- exchange (for review see Woolf and Winyard, 1995). REJ modules of REJ and PKD1 may function in the fine tuning of multiple ion transport phenomena. Future research on both REJ and PKD1 proteins should be aimed at identifying, isolating, and characterizing the proteins with which they are associated.

We thank Dr. R.F. Doolittle for consultations regarding the REJ module and Dr. J.S. Trimmer for helpful discussions.

This work was supported by National Institutes of Health grants HD-21379 to C.G. Glabe and HD-12986 to V.D. Vacquier, and a National Science Foundation Minority Predoctoral Fellowship to L.M. Mendoza.

Received for publication 15 January 1996 and in revised form 13 March 1996.

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