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Molecular interaction of zp3 to zp3r reveals a cross–species fertilization mechanism

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ABSTRACT

Objective: To evaluate the role of ZP3R in the species-specific fertilization mechanism.

Methods: ZP3/ZP3R protein sequences of *Mus musculus*, *Rattus norvegicus*, and *Cavia porcellus* were downloaded from UNIPROT. Percentage of amino acids that was calculated by using the SIAS program. Protein sequences modeled was established by using the Modeller 9.14 program and glycosylation of the ZP3 using GlyProt program. Docking simulation of the ZP3R-ZP3 was performed between the same species and different species with PatchDock program. **Results:** Comparison of the ZP3R and ZP3 structure between species showed that ZP3 in these three species was more similar than ZP3R. Docking simulations of protein showed that changes in the pattern of the ZP3-ZP3R domain for interaction on cross-species compared to the same species. Changes in the pattern of binding ZP3R-ZP3 made sperm-egg binding was not functional and could inhibit cross-fertilization. **Conclusions:** ZP3R-ZP3 interaction is species-specific, and the role of ZP3R is greater than ZP3 in determining the species-specific recognition stage and sperm-egg binding.

1. Introduction

Mammalian fertilization involves a series of interactions between sperm with egg to produce a zygote. The prevention of fertilization between species induces reproductive isolation that is important to maintain the identity of the species[1]. Reproductive isolation may occur in the prezygotic or postzygotic stage. Prezygotic isolation mechanisms between species may be a barrier to mating or prevent the sperm from fertilizing the egg. Postzygotic blockage occurs after fertilization[2].

The specificity of fertilization is the result of molecular interactions in both types of gametes at various stages of fertilization[1].

Recognition and sperm-egg binding are mediated by cell surface proteins[3]. The surface of mammalian egg cells is surrounded by a relatively thick extracellular matrix known as the zona pellucide (ZP). ZP3 is one constituent ZP protein that acts as a binding partner for sperm proteins in the sperm-egg recognition stage[4]. The sperm proteins that bind to mouse ZP3 are β -1, 4-galactosyltransferase, tyrosine phosphorylated and ZP3R (sp56)[5].

ZP3R has previously been known as a mouse sp56 sperm surface protein. ZP3R has a high affinity for ZP3[6]. The structure of

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primary sequence indicates that ZP3R is a member of a superfamily of protein receptor. The mouse's ZP3R polypeptide consists of a signal peptide at the N-terminal region, six short consensus repeats are called the Shusi domain sequence, followed by the specific sequence of a seventh Shusi domain and the C-terminal region. ZP3R is a lectin since it has a carbohydrate recognition domain that specifically binds to the ZP3 functional domain oligosaccharide[7].

The first stage of fertilization is sperm binding to the ZP, this stage is commonly species-specific. Glycosylation of ZP3 contributes to the specific interaction. Variations in the structure of the ZP3 oligosaccharide may affect binding between sperm and egg[8]. ZP3 is known to be a conserved protein in various species of mammals[9]. Other studies have shown that anti-ZP3 antibodies of one species can bind to ZP3 in other species, *ie.* anti-bZP3 binds to rabbit ZP3[10]. The fact that ZP3 is conserved in mammals raises doubts that ZP3 is a determinant of the species-specific properties in fertilization. There have been reports on the role of sperm proteins in species-specific sperm-egg interactions; *eg.* zonadhesin plays a role in species-specific sperm-egg adhesion[11]. In this study, we examined ZP3R-ZP3 interactions by protein-protein molecular docking simulations to determine whether ZP3R-ZP3 is species-specific and to assess the role of ZP3R in this interaction.

2. Materials and methods

2.1. Protein sequence assessment

This study used ZP3/ZP3R protein sequences of *Mus musculus* (*M. musculus*) P10761/9Q60736, *Rattus norvegicus* (*R. norvegicus*) P97708/Q7TSY4 and *Cavia porcellus* (*C. porcellus*) H0WAZ1/O08569, downloaded from UNIPROT (<http://www.uniprot.org>). Identical percentage calculation was done by the SIAS program.

2.2. Modeling

Protein samples were modeled using the homology modeling approach by Modeller 9.14 via CHIMERA. Ramachandran plot structure was carried out using RAMPAGE (<http://mordred.bioc.cam.ac.uk>) to determine the validity of the modeled structure. Low quality model was refined using MODREFINER (<http://zhanglab.ccmb.med.umich.edu/ModRefiner/>) to obtain valid ZP3 and ZP3R protein structures for further analysis.

2.3. Protein glycosylation

The interaction model of ZP3R and ZP3 involved glycosylated groups for the specific reaction. Therefore, the ZP3 protein model was glycosylated in advance using GlyProt (<http://www.glycosciences.de>).

2.4. Molecular protein-protein docking

The interaction between ZP3R and ZP3 of the three species were performed using the PatchDock by rigid docking based on the complementary shape approach. (<http://bioinfo3d.cs.tau.ac.il/PatchDock/>). ZP3R-ZP3 interaction does between the same species and different species. To obtain a more accurate result, a refinement process was carried out from the result of rigid docking using FireDock (<http://bioinfo3d.cs.tau.ac.il/FireDock/>).

2.5. Visualization and interactions

The docking results of the ZP3R-ZP3 interaction complex were analyzed using the PyMOL program to determine the amino acids involved in the interaction between ZP3R and ZP3. All protein models were visualized by Chimera v.1.8.1.

3. Results

3.1. Protein sequence assessment

The protein sequence assessment showed that the sequence of ZP3 was more similar between species than ZP3R. For example, ZP3 sequence of *M. musculus* 91.03% identical to ZP3 of *R. norvegicus*, whereas only identical sequences ZP3R 11:43% (Table 1). In addition, the number of non-identical amino acid residues for all three species was higher in ZP3R than in ZP3 (Figure 1).

Table 1

Comparison of the identical protein sequences of ZP3 and ZP3R in three species.

Species	<i>M. musculus</i>		<i>R. norvegicus</i>		<i>C. porcellus</i>	
	ZP3	ZP3R	ZP3	ZP3R	ZP3	ZP3R
<i>M. musculus</i>	100.00%	100.00%	-	-	-	-
<i>R. norvegicus</i>	91.03%	11.43%	100.00%	100.00%	-	-
<i>C. porcellus</i>	7.28%	5.81%	7.03%	5.25%	100%	100%

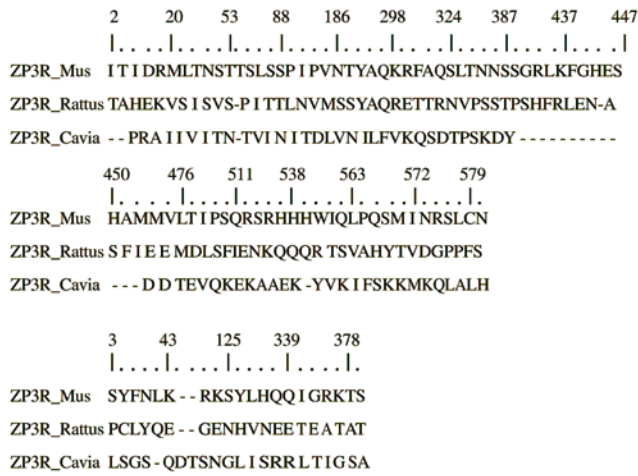


Figure 1. Comparison of non-identical amino acid residues between ZP3R and ZP3 in three species analyzed using the Bioedit program.

3.2. Three dimensional (3D) model and structure

The 3D model of ZP3R and ZP3 from each species were constructed using the modeling approach (Figure 2 and 3). ZP3R interacted with ZP3 oligosaccharide groups. Thus, the ZP3 models were glycosylated. The superimposed results were compared with the 3D structure of ZP3 and ZP3R of the three species. The 3D models of ZP3 were more similar compared to the 3D models of ZP3R. No significant differences were seen in superimposed ZP3 models of three different species (Figure 2B). But in ZP3R models of three species looks different. There is no specific sequence in ZP3R *C. porcellus* (Figure 3A). The superimposed 3D structure of ZP3R from the three species seen that Sushi Domain seventh of *C. porcellus* deviated from the other two models. (Figure 3B marked with a red circle).

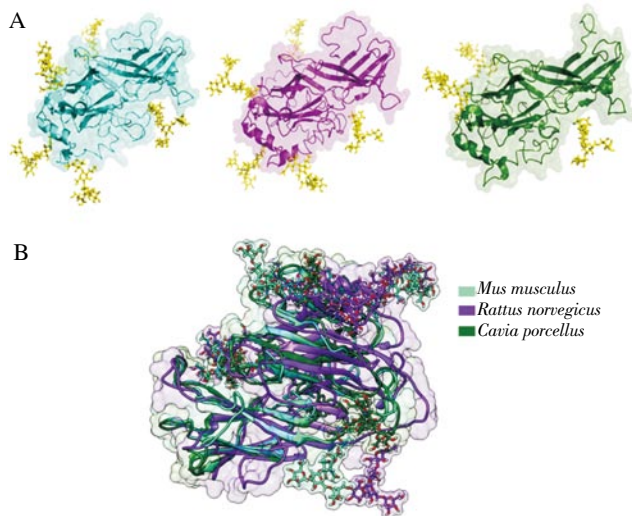


Figure 2. 3D model of ZP3 protein from the three species: *M. musculus*, *R. norvegicus*, *C. porcellus* (A) and the superimposed 3D structure of ZP3 protein of the three species (B).

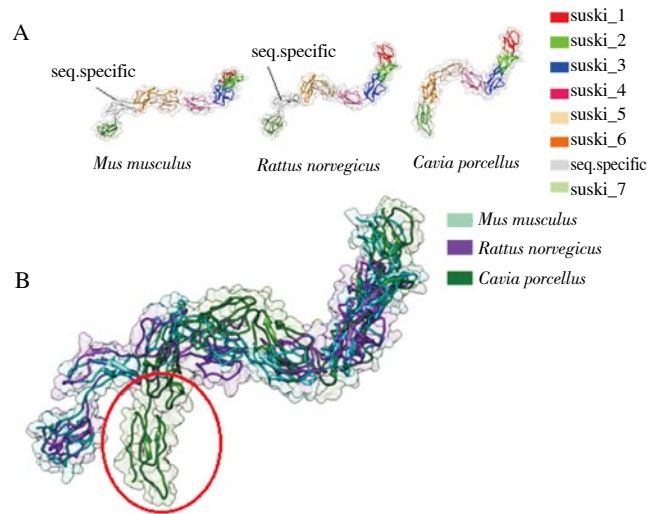


Figure 3. A: D structure of ZP3R protein from the three species. No specific sequences were found in the 3D structure of ZP3R in *C. porcellus*. B: Superimposed 3D structure of ZP3R from the three species. Red circle is the seventh Domain Shushi *C. porcellus*.

3.3. ZP3 and ZP3R interaction

One of the parameters that indicates molecular docking is the docking score or free energy binding; the more negative of the value of the docking score, the more favorable it is that the two proteins with form an interaction. On the other hand, a positive docking score indicates that the complex is not favorable. Docking scores between ZP3 and ZP3R showed that proteins derived from similar species (marked with *) had a lower docking score compared to proteins derived from different species. The interaction between ZP3R *R. norvegicus*-ZP3 *C. porcellus* (marked with **) is not significantly different from *R. norvegicus* - *R. norvegicus* (Table 2).

Visualization of ZP3R-ZP3 and the interaction analysis of domains/ amino acids showed that ZP3R interacts with the glycosylation of ZP3 with different domain patterns (Table 2 and Figure 4). The docking of the ZP3R-ZP3 complex between similar species was used as a benchmark to assess whether changes occur in the pattern of domain interactions. There were alterations in the pattern of domain interactions in all ZP3R-ZP3 protein docking simulations with different species. For example, *M. musculus*-*M. musculus* ZP3R-ZP3 docking had an interaction pattern between Shusi-5 and Shusi-6 with glycosylation (N327, N330 and S332) on ZP3. There were changes in the binding pattern for ZP3R *M. musculus*-ZP3 *R. norvegicus* and *M. musculus* ZP3R-ZP3 *C. porcellus*. In ZP3R *M. musculus*-ZP3 *R. norvegicus*, the interacting domain was Shusi-5 with glycosylation

(N304) on ZP3, while in ZP3R *M. musculus*-ZP3 *C. porcellus*, the interacting domain was Shusi-1 with glycosylation (N263) on ZP3 (Table 3).

Table 2

Docking scores of ZP3R-ZP3.

ZP3R	ZP3	Docking score
	<i>M. musculus</i>	-9.44*
<i>M. musculus</i>	<i>R. norvegicus</i>	-1.06
	<i>C. porcellus</i>	0.10
	<i>M. musculus</i>	-4.04
<i>R. norvegicus</i>	<i>R. norvegicus</i>	-23.07*
	<i>C. porcellus</i>	-23.74**
	<i>M. musculus</i>	2.82
<i>C. porcellus</i>	<i>R. norvegicus</i>	-4.94
	<i>C. porcellus</i>	-29.13*

*Proteins derived from similar species had a lower docking score compared to proteins derived from different species. **Not significantly different from *R. norvegicus*-*R. norvegicus*.

Table 3

Domain/amino acid interactions during ZP3-ZP3R docking.

Interaction		Domain/Amino acid	
ZP3R	ZP3	ZP3R	ZP3
Mus	Mus	Shusi-5 (N303, F307, K312, L313, K314, Q316, C317) and Shusi-6 (R420, Q418)	Glycosylation (N327, N330, S332)
	Rat	Shusi-5 (N343, T342, S341, Q334, T338)	Glycosylation (N304)
	Cav	Shusi-1 (R67)	Glycosylation (N263)
Rat	Mus	Shusi-6 (Y373, Y375, R364, E363, I360)	Glycosylation (N330)
	Rat	Shusi-1 (L40, S44, H66), Shusi-2 (E152, V154) and Shusi-3 (I155, N146, T157)	Glycosylation (N304, N146)
	Cav	Shusi-5 (F304, W294, Y 315, L313, R302)	Glycosylation (N263)
Cav	Mus	Shusi-5 (P321, R325, W335, K334, E330)	Glycosylation (N146, E144)
	Rat	Shusi-7 (L413, E425, D416, I417, L418)	Glycosylation (N304)
	Cav	Shusi-5 (D307, P319) and Shusi-6 (A396, M399, Q395)	Glycosylation (N263)

Description: Mus: *Mus musculus*; Rat: *Rattus norvegicus* and Cav: *Cavia porcellus*.

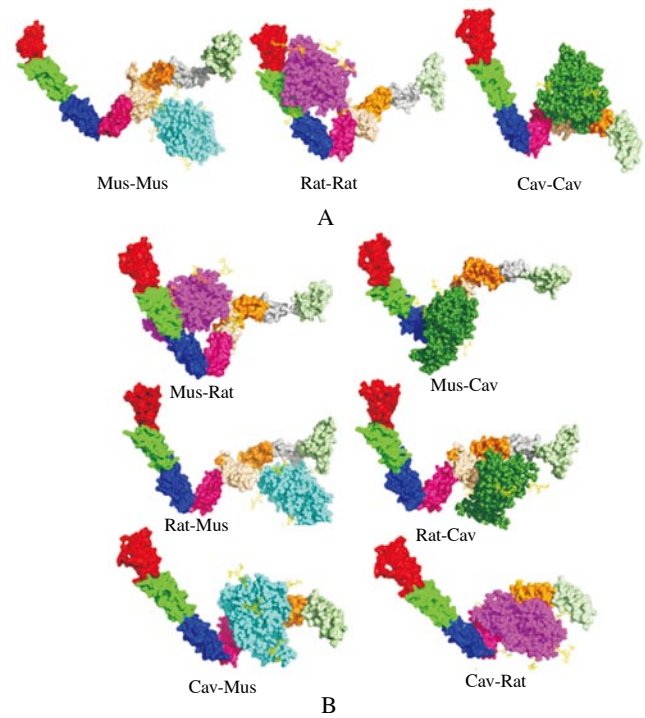


Figure 4. Results of ZP3 and ZP3R docking between same species (A). between different species (B).

Mus: *M. musculus*; Rat: *R. norvegicus*; Cav: *C. porcellus* (B). Visualization of 3D models using Chimera v.1.8.1.

4. Discussion

The sequence assessment and the 3D structures of three species showed that ZP3 was more similar between species than ZP3R. The significant difference is that there were no specific sequences in ZP3R of *C. porcellus*. If the protein structure from one species is similar to that of another species, it may interact with the partner protein of a different species. This finding suggests that ZP3R plays a greater role in determining the species-specific sperm-egg interaction. This is contradictory to previous studies showing that ZP3 determines the species-specific sperm-egg interaction[8,12].

When compared among related species, ZP3 and ZP3R of *M. musculus* were more similar to *R. norvegicus* compared with *C. porcellus*, indicated by the percentage of identical amino acid residues and the superimposed 3D structure. This is understandable because of the close kinship between *M. musculus* and *R. norvegicus*. All three species belonged the same order (Rodentia), but *M. musculus* and *R. norvegicus* belong to same family (Muridae), while *C. porcellus* is included in the Caviidae family[13]. However, the structural similarities in ZP3R and ZP3 between *M. musculus* and *R. norvegicus* were not enough to allow for a functional interaction.

A negative docking score indicates that the protein can interact to form a favorable complex, but it does not guarantee that the complex will be functional. The binding pattern of domains/amino acids is important for identifying the formation of protein complexes. For example, the docking score of *R. norvegicus*-*C. porcellus* ZP3R-ZP3 was -23.74, lower than that of *R. norvegicus*-*R. norvegicus* at -23.07, indicating that binding occurs in both interactions, but there was a change in the domain/amino acid binding pattern. The binding pattern of *R. norvegicus*-*R. norvegicus* ZP3R-ZP3 was between Shusi-1 to -3 with glycosylation (N304, N146), while in *R. norvegicus*-*C. porcellus* the interaction was between Shusi-5 with glycosylation (N263). Although the *R. norvegicus*-*C. porcellus* ZP3R-ZP3 complex formed, there is a possibility that the complex is not functional. Morrison *et al.*[14] found that a protein interaction is determined by the physical interaction between protein domains that match like a lock and key.

Variations in the binding pattern occurred in all docking simulations with ZP3R-ZP3 from different species, even though the docking score had a negative value. If the ZP3R-ZP3 complex that forms between species is not functional, this indicates that their interaction is species-specific and will prevent cross-fertilization between species at the sperm-egg binding recognition stage.

Sperm binding to the zona pellucida involves multimolecules expressed on the surface of sperm[15]. Disruption of these proteins like zonadhesin (Zan)[11], β 1,4-galactosyltransferase-I[16], and ZP3R[17] do not make the null individual sterile. However, Zan-null mice sperm exhibit a loss of species-specific properties; thus, these sperm can bind to the egg cells of other species. This indicates an important role of Zan in the species specificity of sperm-egg adhesion[11].

Based on result of this study, we conclude that the ZP3R-ZP3 interaction is species-specific, and that the role of ZP3R is greater than that of ZP3. Therefore, ZP3R can be added to the list of sperm proteins involved in species-specific sperm-egg binding.

Conflict of interest statement

The authors declare that they have no competing interest.

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