Structure of IZUMO1-JUNO Reveals Sperm-Oocyte Recognition during Mammalian Fertilization

Fertilization, the key step in sexual reproduction, occurs when haploid sperm and egg fuse to create a genetically distinct individual. IZUMO1 on the sperm surface and JUNO on the egg surface have been proven to be the only protein pair indispensable for fertilization. The crystal structures of human IZUMO1, JUNO and the IZUMO1-JUNO complex provide the first step toward understanding the sperm–egg interaction. IZUMO1 and JUNO form a stable 1:1 complex by utilizing the central domain of IZUMO1 and the region behind the hydrophobic pocket of JUNO. These structures reveal the molecular mechanism of mammalian gamete recognition, and provide insights for the development of infertility treatment and new contraceptive agents.

In mammalian reproduction, fertilization is a fundamental process to generate a new individual, achieved by specific interaction between male and female gametes and by their fusion to create a zygote [1]. As the culminating event of fertilization, a robust and precise mechanism is required for gamete membrane fusion. Sperm surface protein IZUMO1 [2] and its counterpart oocyte receptor JUNO [3] are the only factors proven to be essential for gamete membrane fusion. IZUMO1 is a type I transmembrane protein and its extracellular region comprises an N-terminal IZUMO domain and a C-terminal immunoglobulin-like domain, and a short cytoplasmic tail. JUNO is a glycosylphosphatidylinositol-anchored protein belonging to the folate receptor (FR) family but lacks the ability to carry folic acid [3]. Because IZUMO1 and JUNO play important roles in fertilization, they could be potential candidates for contraceptive agents. However, the mechanism of their specific recognition remains elusive.

Size-exclusion chromatography and sedimentation velocity analytical ultracentrifugation analysis revealed that both proteins exist as monomers in solution. IZUMO1 binds to JUNO with high affinity in a 1:1 binding mode at pH7.5 as confirmed by isothermal titration calorimetry analysis. The crystal structures of human IZUMO1, JUNO, and the IZUMO1-JUNO complex have been determined at 2.1, 2.0, 2.9 Å resolutions, respectively, using synchrotron X-rays [4]. The rod-shaped structure of IZUMO1 is composed of an N-terminal α-helical IZUMO domain, a central β-hairpin region, and the C-terminal immunoglobulin-like domain (**Fig. 1a**). IZUMO1 has five disulfide bonds with ten Cys residues conserved among mammalian species, which fix the orientations of the IZUMO and immunoglobulin-like domains relative to the central β-hairpin region.

JUNO exhibits a globular architecture stabilized by eight disulfide bonds, and has a hydrophobic pocket that corresponds to the folate binding pocket in FRs (**Fig. 1b**). Although most of the hydrophobic residues involved in the interaction between FRs and folate are conserved in JUNO, JUNO does not exhibit any affinity to folate [3]. In addition to the hydrophobic interactions, FRβ recognizes folate pterin moiety via several polar residues (**Fig. 1b**), which have been shown to be important for folate binding in FRs [5, 6]. These residues are not conserved in JUNO except for S190 (**Fig. 1b**). Furthermore, since the side chain of W190 of JUNO exhibits distinct conformation as FRs, the hydrophobic pocket of JUNO becomes narrower than that of FRs. These structural differences explain why JUNO is unable to bind folate.

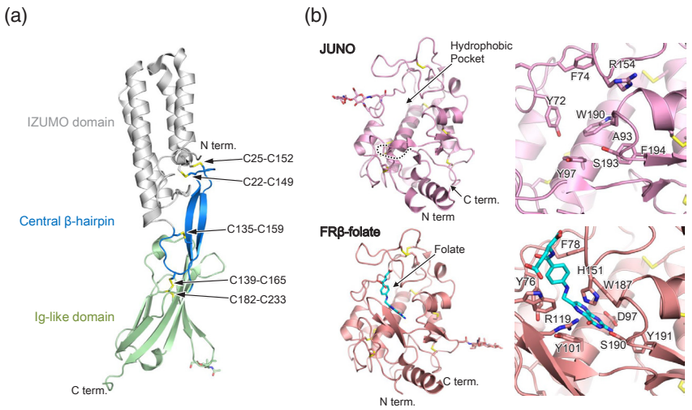

Figure 1: Crystal structures of IZUMO1 and JUNO. **(a)** Structure of human IZUMO1. **(b)** Structures of JUNO (top) and FRβ-folate complex (PDB ID, 4KMZ) (bottom). The overall structures (left panels) and the magnified view of the hydrophobic pocket (right panels) are shown.

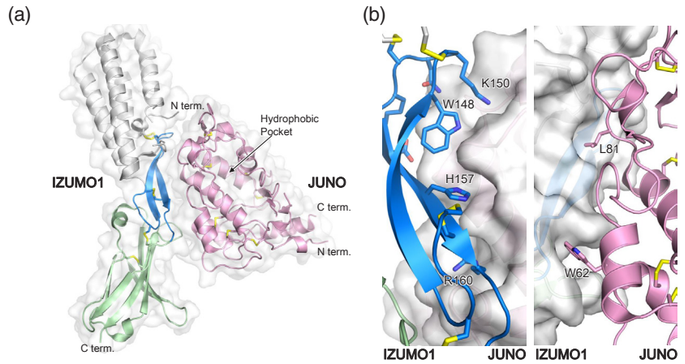

Figure 2: Structure of the IZUMO1-JUNO complex. Overall structure of the complex **(a)** and the binding interface **(b)** showing the JUNO surface (left) and IZUMO1 surface (right).

IZUMO1-JUNO exists as a 1:1 complex in the crystals (**Fig. 2a**). Structural comparison of IZUMO1 and JUNO alone with those in the complex revealed that there are no major structural differences upon complexation. IZUMO1 interacts with JUNO mainly via the central β-hairpin region, while JUNO interacts with IZUMO1 behind the hydrophobic pocket (**Fig. 2a**). The hydrophobic and van der Waals interactions together with the six hydrogen bonds comprise the binding interface with good surface complementarity (**Fig. 2b**). In particular, two Trp residues in the interface, W148 of IZUMO1 and W62 of JUNO, make major contributions to the binding (**Fig. 2b**). These Trp residues are highly conserved among all species. Indeed, mutations of the interface residues resulted in reduced affinity.

The structure of IZUMO1-JUNO complex would represent the initial gamete recognition state and further structural conversion of the complex might occur during fertilization. Our biochemical experiment indicated that the IZUMO1-JUNO interactions are affected by environmental factors such as reducing conditions and pH values: the reduced condition and acidic pH diminish the binding, which might be involved in the regulation of IZUMO1-JUNO interaction. Meanwhile, in the subsequent process of fertilization, the mediation of unidentified factor(s) on the oocyte may also be considered. Further investigations will be required to reveal the processes following the initial encounter of IZUMO1 and JUNO.

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BEAMLINES

BL-1A and AR-NE3A

H. Ishida, U. Ohto and T. Shimizu (The Univ. of Tokyo)