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Progress of sperm Izumo1 relocation during spontaneous acrosome reaction

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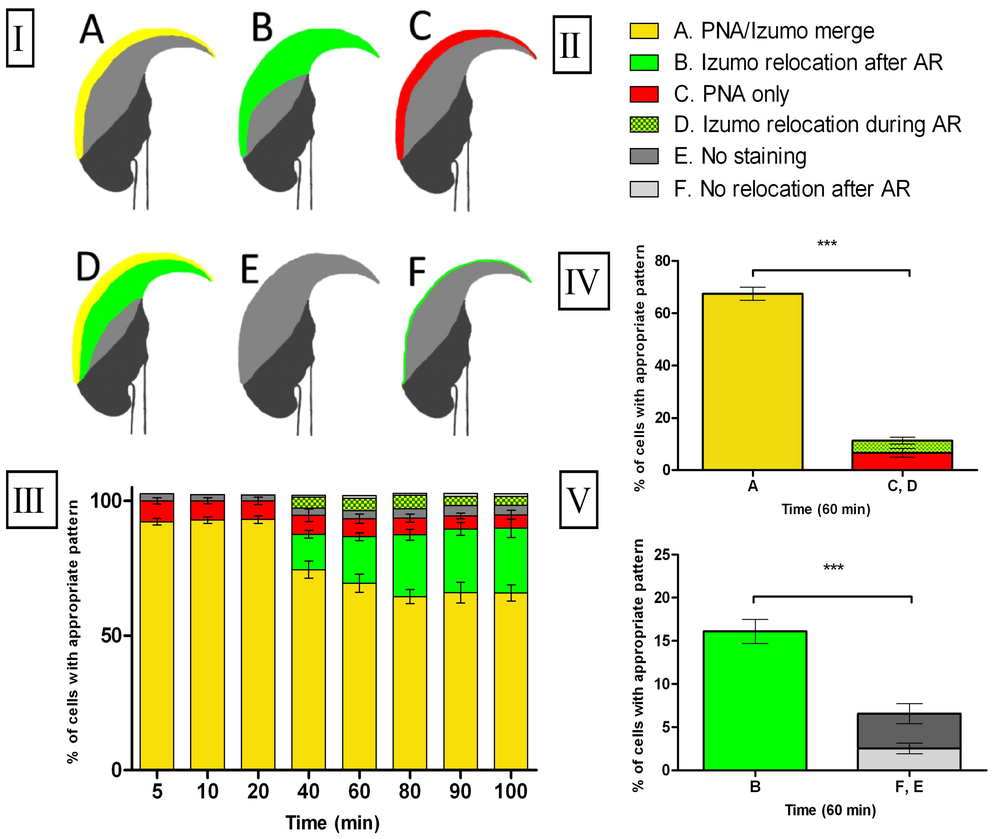
Abstract

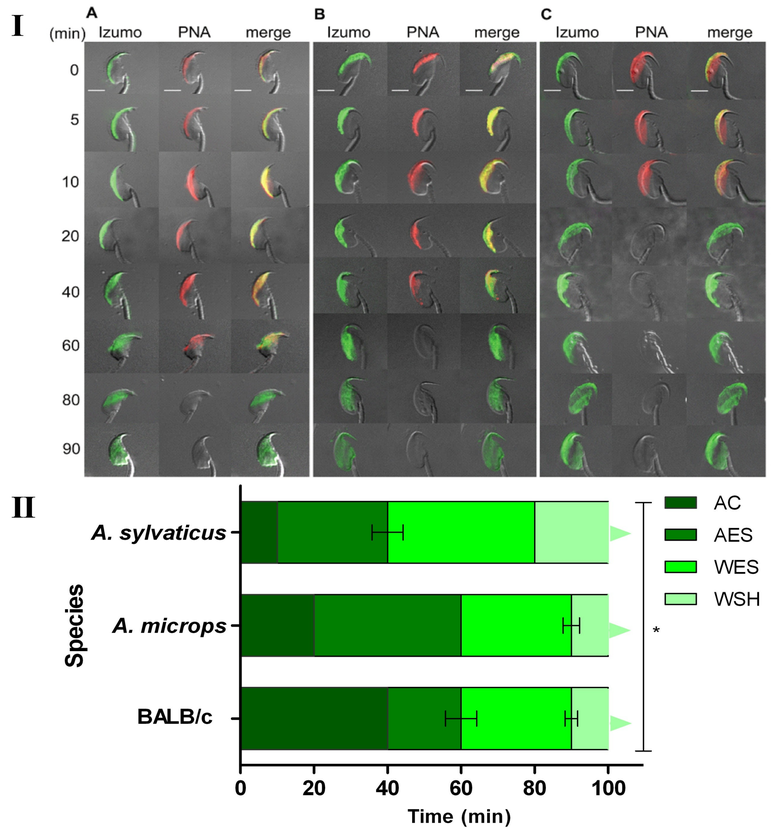
It has been recently shown in mice that sperm undergo acrosome reaction (AR) by passing through cumulus cells, furthermore the acrosome-reacted sperm can bind to *zona pellucida*, and consequently fertilize the egg. During AR, the relocation of the primary fusion protein Izumo1 into the equatorial segment is crucial for sperm-egg fusion. There is a high rate of spontaneous acrosomal reaction in rodents, with up to 60% in promiscuous species. The aim was to find out, whether the relocation of Izumo1 happens during the physiological spontaneous AR, or whether it occurs only in sperm with induced AR with further correlation to species-specific mating behaviour. Immunofluorescent detection of Izumo1 protein dynamics during the process of *in vitro* capacitation, spontaneous, calcium ionophore and progesterone induced AR was monitored. Our results show that during spontaneous AR there is a clear Izumo1 relocation from the acrosomal cap to the equatorial segment and further on to the whole sperm head. Additionally, there is positive tail tyrosine phosphorylation associated with hyperactive motility. Therefore, spontaneously acrosome reacted sperm have the same fertilizing potential as those after induced AR. Moreover, the beginning and progress of Izumo1 relocation and tail tyrosine phosphorylation positively correlates with the level of promiscuity and the acrosome instability in *Apodemus* species. The findings that crucial molecular changes essential for sperm-egg fusion represented by dynamic movements of Izumo1 also happen during spontaneous AR are vital for novel understanding of fertilization in mice. Moreover, this may represent a unique mechanism of accelerating the fertilizing process in a highly promiscuous environment under selective pressure of intra-specific sperm competition.

Figure 1



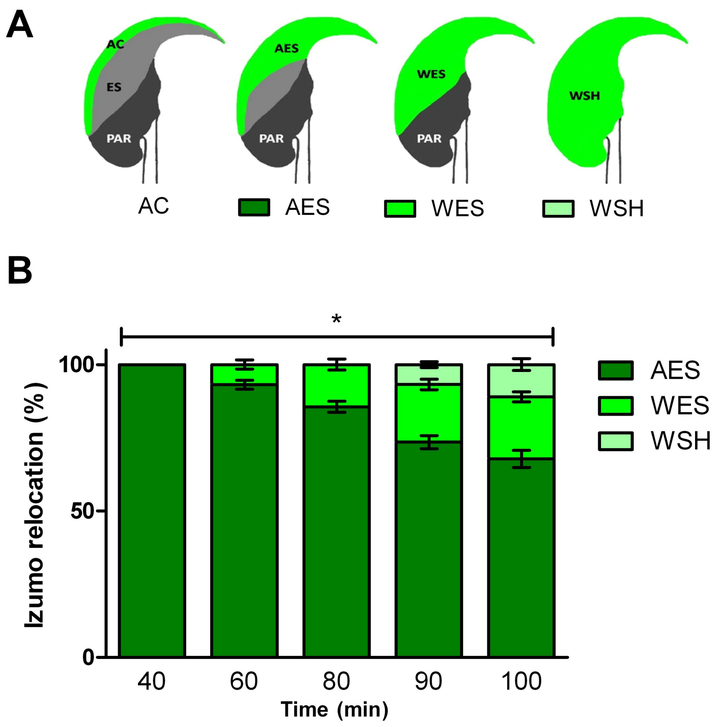
I and II Scheme of Izumo1 and PNA staining patterns distribution in BALB/c spermatozoa. Patterns detected during BALB/c spermatozoa *in vitro* incubation (detected in time 60 min). (A) PNA/Izumo1 merge. (B) Izumo1 relocation after AR. In other incubation times Izumo1 localization corresponds to Figure 1. (C) PNA staining only. (D) Izumo1 relocation during AR. (E) No sperm head staining. (F) No Izumo1 relocation. **III Changes of the PNA and Izumo1 staining patterns distribution during BALB/c sperm in vitro incubation.** Individual bars denote the distribution of Izumo1 and PNA staining patterns among individual times during sperm incubation. 200 cells were counted in 6 individual samples per one time. Error bars denote SD (SD are presented only for patterns 1,2,3). Statistical analysis of PNA and Izumo1 staining patterns prior and after AR in BALB/c spermatozoa.**IV Distribution of PNA and Izumo1 staining patterns prior to AR (counted in time 60 min).** 200 cells were counted in 6 individual samples. Error bars denote SD. The statistical significance of the differences among individual Izumo1 patterns abundances is indicated by asterisk (*p ≤ 0.001). **V Distribution of PNA and Izumo1 staining patterns after AR (counted in time 60 min).** 200 cells were counted in 6 individual samples. Error bars indicate the SD. The statistical significance of the differences among individual Izumo1 patterns abundances is indicated by asterisk (*p ≤ 0.001).

Figure 3



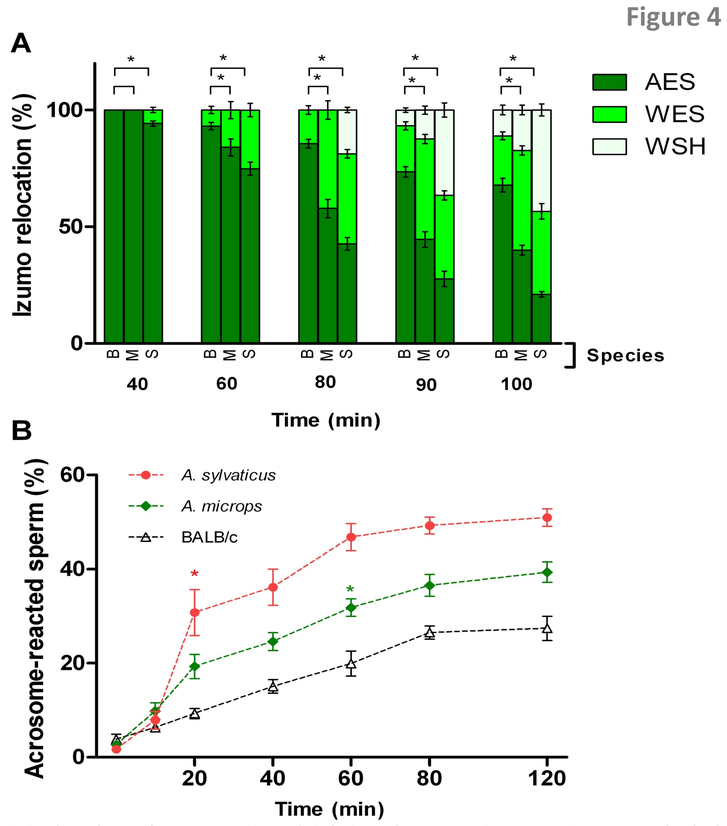
I The progression of Izumo1 relocation during spontaneous AR. (A) BALB/c. (B) *A. microps*. (C) *A. sylvaticus*. Immunofluorescent detection of Izumo11 (green) and acrosome integrity by PNA-lectin (red) during *in vitro* incubation (0, 5, 10, 20, 40, 60, 80 and 90 min). Representative results shown. Scale bar represents 4 µm. **II The timing of Izumo1 relocation correlates with the promiscuity behaviour among individual species.** Bars indicate the modus time (N=6), when more than 5% of sperm show the appropriate Izumo1 pattern for the first time, from the whole Izumo1 positive sperm population (AES) or from the population of spontaneously acrosome-reacted sperm (AES, WES, WSH), respectively. Error bars indicate the SEM. The statistical significance of the differences in the individual Izumo1 patterns abundances among individual strains is indicated by asterisk (*p ≤ 0.05). Arrows at the end of bars indicate the further progression of the Izumo1 relocation to the WSH in subsequent times of incubation. Acrosomal cap (AC), apical equatorial segment (AES), whole equatorial segment (WES), whole sperm head (WSH)

Figure 2



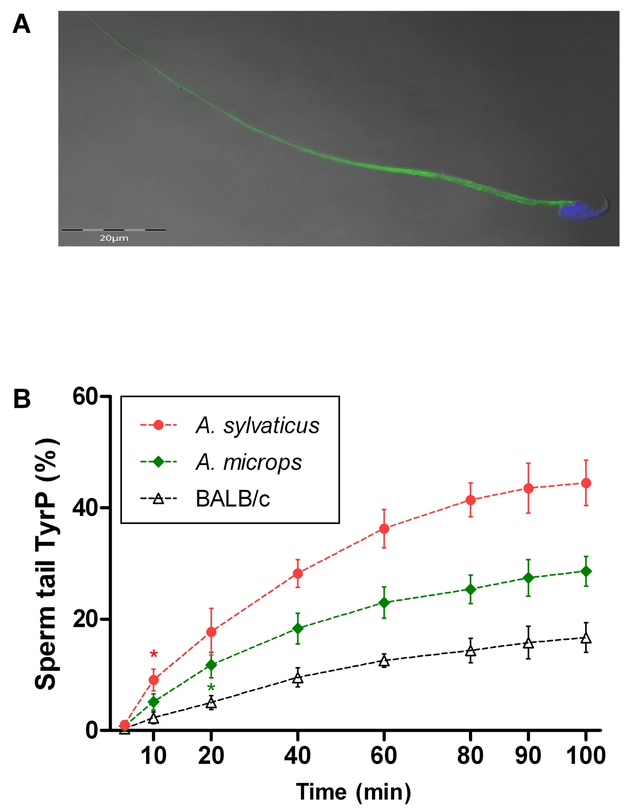
A Scheme of Izumo1 relocation across the mouse sperm head. Acrosomal cap (AC), equatorial segment (ES), post-acrosomal region (PAR), apical equatorial segment (AES), whole equatorial segment (WES), whole sperm head (WSH) **B Differences in the Izumo1 pattern proportions in BALB/c mouse spermatozoa during spontaneous AR.** 200 of described cells were counted and 3 specific Izumo1 patterns are presented per one time. Whiskers indicate the SD. The statistical significance of the differences among individual Izumo1 patterns abundances is indicated by asterisk (*p ≤ 0.05).

Figure 4



Statistical analysis of Izumo1 pattern distribution during spontaneous AR among individual species. A Differences in the Izumo1 pattern proportions (AES, WES, WSH) among species in selected times of incubation. Bars denote the arithmetic mean of individual Izumo1 pattern (AES, WES, WSH) in appropriate species and time of incubation (%). 200 cells were counted in 6 individual samples per one time. Error bars indicate the SEM. The statistical significance of the Izumo1 patterns abundance differences among individual species in different times of incubation is indicated by asterisk (*p ≤ 0.05). BALB/c (B), A. microps (M), A. sylvaticus (S). **B The species specific rate of spontaneous AR in the whole sperm population.** Points indicate the arithmetic mean of the number of acrosome-reacted sperm. Error bars denote SEM. Asterisks indicate the first time when the difference between individual species is significant (*p ≤ 0.05).

Figure 5



Statistical analysis of protein TyrP pattern distribution during spontaneous AR. A Immunofluorescent detection of tyrosine phosphorylation in A. microps spermatozoa. Tyrosine phosphorylation (green) over the mid and principal piece of the sperm tail. Nuclei are counterstained with DAPI (blue). Scale bar represents 20 µm. **B** The time progression of the tail protein TyrP among individual species in the spontaneously acrosome-reacted sperm. 200 cells were counted in 6 individual samples per one time. Points indicate the arithmetic mean of the tail protein TyrP (%). Error bars denote SD. Asterisk indicate the first time when the difference between individual species is significant (*p ≤ 0.05).

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