Interspecies comparison of sea star adhesive proteins – Supplemental material

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Materials and Methods

(a) Tube foot transcriptome of Asterina gibbosa

Tube feet from one adult individual of *A. gibbosa* were amputated and total RNA was extracted using TRIzol (Life Technologies, Carlsbad, CA). After DNase digestion with DNase I, the RNA was purified with Quiagen RNAeasy Mini kit according to the manufacturer’s protocol. RNA quality was assessed using an Agilent 2100 Bioanalyzer. Library preparation, sequencing and de novo assembly were performed at the Beijing Genomics Institute (Hong Kong). Shortly, libraries were prepared from the RNA pool by mRNA enrichment, fragment interruption, addition of adapters, size selection and PCR amplification. High-throughput sequencing was conducted using the Illumina HiSeq™ 2500 platform to generate 100-bp paired-end reads. Sequencing was performed according to the manufacturer's instructions (Illumina, San Diego, CA). After reads filtering, Trinity [1] was used for de novo assembly of clean reads. Tgicl [2] was used to remove abundance and obtain Unigenes. About 33.15 Gb bases were generated in total on the Illumina HiSeq sequencing platform. Assembling and filtering resulted in 62,950 Unigenes, with a total length of 81,832,015 bp, average length of 1,299 bp, N50 of 2,718 bp, and GC content of 44.06%.
(b) **In situ hybridization**

**RNA probe synthesis.** Primers were designed with Primer3 and for antisense/sense probe production a T7/SP6 promoter region was added at the 5’ end of the reverse/forward primers. Primer sequences are listed in Suppl. Table 2. PCR reactions were performed with Q5® High-Fidelity DNA Polymerase (NEB) using tube foot specific cDNA. The PCR products were purified with Wizard® SV Gel and PCR Clean-Up System (Promega). To synthesize single stranded digoxigenin-labelled RNA probes, T7/SP6 polymerase (Promega) and DIG labelling mix (Roche) were used. RNA probes were purified with Micro Bio-Spin® Chromatography Columns (Biorad) and their concentration was determined with a DS-11 UV-Vis Spectrophotometer (DeNovix). RNA probes were used at a concentration of 0.1-0.2 ng/µl.

**Whole mount in situ hybridization.** Tube feet were amputated and fixed in 4 % PFA in PBS for 2 h at room temperature. After several washes in PBS, they were dehydrated in a methanol series. After rehydration and several washes in PBS, they were permeabilised with a Proteinase K treatment (20 µg/ml in PBS) for 35 min (A. gibbosa tube feet) or 45 min (A. rubens tube feet) at room temperature. The reaction was stopped in a glycine solution (0.4 % in PBS). After several washes in PBS, the tube feet were transferred to 0.1 M triethanolamine (pH 8) containing acetic anhydride at a concentration of 1:400 and 1:200 for 10 min each. Afterwards the tube feet were postfixed in 4 % PFA in PBS for 20 min, washed several times in PBS and heated in 80 °C preheated PBS for 20 min. After 10 min in 50 % hybmix in PBS, tube feet were transferred and stored in 100% hybmix at -20 °C until usage. Prehybridization was performed for 2 h in hybmix at 55 °C under agitation (350 rpm). Probes were denatured and added at a final concentration of 0.1-0.2 ng/µl. Hybridization was performed overnight at 55 °C under agitation (350 rpm). The next day the tube feet were transferred into hybmix preheated at 62 °C and subsequently hybmix was replaced with 2xSSC in a series of 75 %, 50 %, 25 % hybmix in 2xSSC at 62 °C. After two times 30 min in 2xSSC containing 0.1 % CHAPS (v/v) at 62 °C, tube feet were twice washed in MAB at room temperature. Tube feet were blocked in blocking reagent (Roche) for 2 h at 4 °C and incubated in anti-digoxigenin-AP Fab fragments (Roche) 1:2000 in blocking reagent overnight at 4 °C. After several washes with MAB, the signal was developed using...
the NBT/BCIP system (Roche) at 37 °C. The colour reaction was stopped with 100 % ethanol. After several washes in PBS, tube feet were gradually transferred to glycerol and images were taken with a Zeiss Axioscope A1 microscope.

Paraffin section in situ hybridization. For section in situ hybridization, PFA-fixed and paraffin-embedded tube feet were cut into 14 µm thick longitudinal sections and placed on Superfrost ultra plus® slides (Thermo scientific). Slides were heated in an oven at 60 °C for 1 h and allowed to cool down. After dewaxing in xylene and rehydration, sections were postfixed in 4% PFA in PBS for 20 min, washed several times in PBS and treated with 0.2 M HCl for 10 min. Following washes in PBS, sections were treated with Proteinase K (20 µg/ml) for 20 min. Sections were postfixed in 4 % PFA in PBS for 5 min, washed in PBS and placed in 0.1 M triethanolamine (pH 8) containing 0.5 % acetic anhydride for 10 min. Afterwards sections were washed and gradually dehydrated with ethanol. To dry the sections, Chloroform was dropped on the sections and allowed to evaporate. Slides were placed in a humidity chamber and incubated with hybmix at 55 °C for 30 min. Sections were incubated with RNA probes diluted in hybmix (0.2 ng/µl) overnight at 55 °C. The following day the sections were incubated in 50 % formamide in 2x SSC at 62 °C (3 x 20 min). After washes with 1x SSC and PBS, sections were blocked in blocking reagent (Roche) for 1 h at 4 °C. Sections were incubated with Anti-digoxigenin-AP Fab fragments (Roche) 1:2000 in blocking reagent overnight at 4 °C. Following several washes, the signal was developed using the NBT/BCIP system (Roche) at 37 °C. Colour reaction was stopped with ethanol and sections washed several times with PBS. Sections were mounted in Mowiol® 4–88 (Roth, Germany), prepared according to the manufacturer's protocol and images were taken with a Zeiss Axioscope A1 microscope.

References

Suppl. figures

Suppl. Figure 1: Anti-Sfp1 beta antibody staining on tube foot sections of *Marthasterias glacialis* and *Henricia sp.* (a-d) Anti-Sfp1 beta antibody staining and (e-h) negative control, skipping the primary antibody and using the secondary antibody alone.
**Suppl. Figure 2:** Histological staining of tube foot sections from various sea star species. Symbols indicate the tube foot morphology with □ simple-disc ending, ■ reinforced-disc ending and ▽ knob-ending.
Suppl. Figure 3: Footprint proteins with exclusive mRNA expression in secretory cells of the disc adhesive epidermis in tube feet of *A. rubens.*
Suppl. Figure 4: Footprint proteins with mRNA expression in secretory cells of the disc adhesive epidermis and in the stem epidermis in tube feet of *A. rubens.*
Suppl. Figure 5: Expression of conserved footprint proteins in *Asterina gibbosa* tube feet.