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論文題目	Development of gonad-stimulating substance-like peptide system and spatiotemporal expression property in adult organs in relation to the breeding season in the sea cucumber, <i>Apostichopus japonicus</i> . (マナマコにおける生殖腺刺激物質様ペプチドシステムの発生と生殖時期に関連した成体内での発現特性)
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Abstract

Development of gonad-stimulating substance-like peptide (GSSL) system during larval development and its dynamic change of spatiotemporal expression pattern and molecular conformation in adult organs in relation to the breeding season were examined using immunochemistry in the sea cucumber, *Apostichopus japonicus*. GSSL has been isolated and identified in the radial nerve extract and promotes egg maturation *in vitro* (Katow *et al.* 2009). However, its developmental origin and potential occurrence of dynamic change of GSSL expression property during breeding season have been remained unknown. The present study was aimed to disclose the answers to these questions.

According to the present immunohistochemistry GSSL was first detected in 2-day post-fertilization (-dpf) auricularia larvae at both posterior and anterior tip regions being exclusively associated with cells that resemble morula cells (GSSL cells) along the ciliary bands. In 7-dpf auricularia, GSSL cells were increased in number and more extensively distributed along the ciliary bands, except along the post-oral transverse ciliary band that has no GSSL cells. According to whole-mount immunohistochemistry with anti-serotonin antibodies, the serotonergic nerve cells appeared only in the anterior region of the ciliary band (pre-oral ciliary band). Furthermore, the nervous system stained by anti-synaptotagmin monoclonal antibody (a pan nervous cell marker in echinoderm) was not associated with GSSL distribution in larvae. GABAergic cells also were not associated with GSSL distribution. Thus, GSSL system is distinctive from known nervous systems to date.

However, in 45-dpf doliolaria larvae, GSSL cells spread to inter-ciliary bands region on the body surface in addition to the area along the ciliary band. By 70-dpf pentactula larva stage, GSSL cells disappeared from the body surface, and localized exclusively to the tips of the primary podia and primary (buccal) tentacles through 3-month post-fertilization (-mpf) juvenile stage. Since these organs are not involved in reproductive activity, it is suggested that GSSL may have another function in addition to promoting egg maturation.

According to the immunoblotting anti-GSSL antiserum bound to a single faint band at 17 kDa region in 20-hpf gastrulae and as two bands at 30 kDa and 17 kDa regions in auriculariae. In 45-dpf doliolariae, GSSL-containing polypeptide (GSSLP) was detected as two faint broad bands at 30 kDa and 18 kDa regions. On the other hand, GSSLP was detected only at 18 kDa region in 70-dpf

pentactula larva stage. In adult organs, GSSLP was detected in the gonads from shortly before empirical breeding season (May and June) to July. In the gonads GSSLP showed considerable polymorphism, which was associated with *N*-glycosylation and the occurrence of intra-molecular disulfide bonds. In the ovary, GSSLP was expressed from March to June and corresponded to two bands at 113 and 100 kDa regions. In July, only the larger band remained weakly. In the testis, GSSLP was detected first in April as two bands at 245 kDa and 190 kDa regions. The number of bands increased to five in June but decreased to three smeared bands in July. The GSSLP in the radial nerve and circumoral nerve ring corresponded to a single peptide of 170 kDa with little *N*-glycosylation and its expression level remained unchanged throughout a year with no correlation with the breeding season.

According to immunohistochemistry, like in larvae, GSSL was detected mainly in the morula cells in all the adult organs examined in the present study. However, in addition to these cells, it was detected in the follicle cells of the ovary and, for a brief period, in the jelly space on the oocytes as well, but never in the ooplasm. In the testis, the morula cells were localized at the place close to the invaginated inner epithelium, but never in the male gametes. In July animals, gonadal morula cells were rarely observed. The GSSLP was also examined in other adult organs and according to immunoblotting analysis it was detected as a single band at approximately 170 kDa region in the tube feet and tentacles. However, it was not detected in the respiratory trees, stomach or intestine. According to immunohistochemistry analysis using Polywax sections, consistent with above immunoblotting results, GSSL-positive cells were detected only in the tube feet and tentacles. Overall, these observations suggested that GSSL system is distinctive from known nervous systems and plays an important role in the spawning of sea cucumber.

論文審査結果の要旨

本論文は従来不明であったマナマコの卵成熟誘起作用をもつ生殖腺刺激物質様ペプチド(GSSL)の個体発生中の発現機序と成体における生殖期間での特異発現の有無を免疫化学的に明らかにすることを目的として行われた課題研究の成果を報告するものである。

本研究において、Hamdy Omar Ahmed は GSSL が初期オーリクラリア幼生期に幼生後端部で繊毛帯に近接して発現することを発見した。GSSL は成体の放射神経組織に集中的に存在する桑実の形をしたモルラ細胞の細胞質に見られるが、オーリクラリア幼生においても発生に伴って GSSL をもつモルラ細胞 (GSSL 細胞) が繊毛帯にそって分布を広げる。GSSL 細胞の繊毛帯にそった分布経路は同様の配向をもつセロトニン神経系、シナプトタグミン神経繊維系、ドーパミン細胞分布、GABA 細胞分布とも異なった独自のものであった。このことから、GSSL システムは固有の経路をもつことが分かった。このような GSSL 細胞の分布はドリオラリア幼生から新たに形成されてくる管足や触覚の先端部に集中する。変態前のペンタクチュラ幼生にも同様の分布形態が維持され、変態後の稚ナマコに引き継がれる。このような免疫組織化学的解析からは一見多様な機能をもつ GSSL が考えられるが、実際は一貫して幼生や稚ナマコの運動器官に関連している。変態後の成体では放射神経に密接して GSSL 細胞が分布する。放射神経における GSSL は生殖期間に関係なく通年で発現していることから、この組織での GSSL 発現は生殖には関係しないと考えられる。しかし、卵巣及び精巣では生殖期間にのみ GSSL を含むペプチドを発現する。これらのペプチドは卵巣型と精巣型に二分される。卵巣型は相対分子量 113 kDa と 100 kDa の 2 種類のペプチドからなり、生殖期間にのみ見られる。一方、精巣型は顕著なタンパク多型を示す。生殖初期の 4 月には 240/245 kDa と 190 kDa の 2-3 個のペプチドとして現れ、5 月には 3 個、6 月には 4 個のペチドにまで増加する。しかし、この期間が終了する 7 月には各ペプチドは不明瞭な幅の広いバンドとして免疫ブロッキングで観察される。これ以外の季節には検出されない。このような生殖期間に限定した GSSL ペプチドの発現との分子形態変化は棘皮動物では世界的にも初めて観察された事象である。

以上の研究への取り組みは自立して研究活動を行うに必要な高度の研究能力と学識を有することを示している。したがって、Hamdy Omar Ahmed 提出の論文は、博士 (生命科学) の博士論文として合格と認める。