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Review Article

The Earthworm *Eudrilus Eugeniae*: a Model Organism for Regenerative Biology

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Abstract

The earthworm, *Eudrilus eugeniae* is an economical model system for cell and molecular biological experiments to study regeneration and stem cell biology. The purpose of this brief review is to summarize those published studies on the regeneration biology using *E. eugeniae* and to provide the advantages of the model system.

Keywords: Earthworm; *Eudrilus eugeniae*; Regeneration biology; Stem cell biology

Introduction

The earthworm, *Eudrilus eugeniae* belongs to the phylum Annelida, class: Clitellata, and family: Eudrilidae. The worm is commonly known as the "African nightcrawler". The worm is a great model for the cell and molecular biological experiments. Since it has an amazing ability of regeneration [1-6], the complex cell and molecular biological events of regeneration can be studied using the worm. As an experimental model for regenerative biology, *E. eugenia* has the following advantages: 1. It is economical and easy to maintain the earthworm in the laboratory but the cost of rearing fish, mouse and other animals are comparatively costly; 2. It has a higher growth rate as little as 5 weeks to reach maturity [7]; 3. It attains 12mg body weight per day; 4. It rapidly reproduces ; 5. it can tolerate temperature difference ranges from 15-30°C) [8,9]; since it has many well-developed organs such as ovary, testis, seminal vesicle, prostate gland, etc., it is possible to design the regenerative biological studies of different organs. Hence the model is better than hydra and planarians. They don't have well-developed organs as the worm does in it.

The review was prepared by all published articles regarding regeneration in *E. eugeniae*. It will be helpful to understand the advantages of the model system for regeneration studies.

Stem Cells and their Fluorescence in *Eudrilus eugeniae*

In 2002, from my lab, the first paper was published using *E. eugeniae* as a model [10]. The work describes the following: The stem cells of the worm are present in between the epithelial and circular muscle layers of the skin of body segments. The stem cells are fluorescent in nature. The cells have 2.3-fold higher fluorescence than the neighboring cells. We found 477.5 ± 15 µg/g of riboflavin in the tissues of the worm. The major causative fluorophores are riboflavin and its derivatives. Also, it was found that the worm gets the riboflavin from the gut microbes. Also, it was noted that stem cells migrate into the blastema from the adjacent segments. The above work was completed with the BrdU labeling technique. In 2015, we found the human Oct 4 antibody recognizes a single band at 48 KDa and the immunohistochemical analysis also showed the Oct 4 expressing cells are in between the epithelial and circular muscle layers of the skin of body segments [11]. The works confirmed the niche of stem cells in the worm.

Finding a Symbiotic Bacterium in *Eudrilus eugeniae*

In 2017, we found that the major supplier of the riboflavin to the *E. eugeniae* [4] is *Bacillus endophyticus* which is an endosymbiotic organism. Also, the work explains the following: Either the amputation of the anterior or posterior part of the worm triggers adaptational starvation in which the amputee does not take nutrients for 6 days. During the period, not only the worm but also the gut microbes undergo starvation. The starvation initiates sporulation in the population of the gut microbiome. During starvation, the gut microbiome produces good amount of riboflavin. We found 304.79 ±2.73 µg per gram of intestinal tissues of the worm in the control worm and the concentration of riboflavin increased three fold and reached 965.55± 0.67µg per gram of intestinal tissues in the 3rd day regenerating worm. The riboflavin produced by the microbiome is absorbed by the gut epithelial cells of the worm.

Riboflavin and Regeneration

The riboflavin is important for the regeneration of the worm and supplementation of riboflavin augments regeneration [10] and it is also found that the riboflavin augments the regeneration of planarians [12]. Also, our unpublished data confirm that the concentration of riboflavin in the lizard tail is higher than that of the other tissues. In vertebrates, the liver has more riboflavin concentration than that of other organs. The elevated concentration of riboflavin in the liver, lizard tail and earthworm has been documented. Also interestingly liver, lizard tail and earthworm have the powerful ability of regeneration. The data

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confirm that riboflavin is a major player in the complex regeneration process. Our experiments [13] and Christyrajet al., 2019 [14] confirm that the cellular and paracraine factors also are important factors for successful regeneration. The observation confirms the riboflavin and its derivates might support stem cell differentiation, self-proliferation and migration. Interestingly it has been reported that riboflavin is important for wound healing [15] and plant embryogenesis [16].

Role of the Nervous System in Regeneration

In 2016, to understand the neurohormone secreted from the neurosecretory cells of the Central Nervous System (CNS), the neuronal cellular changes were studied in *E. eugeniae* by Dipanwita Banik and Priyasankar Chaudhuri [3]. They observed the moderate to massive engorgement of neurosecretory cells at 72 h and 96 h of amputation.

Role of Clitellum in Regeneration

The clitellum of *E. eugeniae* resides in the 13-17 segments which are a slightly bulged, discolored organ that produces the cocoon enclosing the earthworm's eggs. In 2017, we found that major cellular changes happen in the clitellum during regeneration [1]. The clitellum has a pair of copulation setae and it secrets albumin. We found that the cells in the clitellar segments migrate during regeneration. Also, we already found stem cells reach blastema from adjacent segments [10]. The above two data suggest that the migrating clitellar cells should be the stem cells and they should reach the amputation site and eventually should enter into the posterior regenerative blastema. Then, they differentiate into varieties of cells to restore the lost organs. Amazingly the amputation at any of the clitellar segments kills the worm. The segment with intact clitellum can survive and successfully regenerates upon amputation at any other body parts but the portion of the worm which is detached from the clitellum dies. Also in the human body, the liver is the organ where the albumin is produced. Liver has amazing regenerative potential. The expression of genes and activations of signal transduction pathways are similar among cancerous tissues, embryogenesis and regeneration. Deposit of albumin around cancer cells has been noted much earlier [17] and also embryogenesis starts in the presence of albumin. Here the clitellum has the ability to synthesis albumin and the clitellum is important for regeneration. The correlation fascinates me.

The Function of TCTP in Regeneration

The Translationally Controlled Tumor Protein (TCTP) is conserved from the worm to human. The TCTP is a multifunctional protein which has role in cancer reversion, stem cell proliferation and stem cell differentiation [18,19]. In 2017,we found the maximum expression of TCTP at the tip of the blastema of posterior body part regeneration [1]. Also, the siRNA-based silencing of the TCTP blocked the regeneration process and the delayed wound healing process; also differentiation of epithelial granular cells and the migration of the cells from the clitellum were blocked in *E. eugeniae*.

The Social Ability of *E. eugeniae*

In the water, the earthworms coil together [20]. The social ability of the worm is called self-assemblage. The biological significance of the self-assemblage is not known but we predict that during the flood, the social ability might help them to survive. For example, if a group of worms are buried deep in the soil, the chance of survival for the group would be more than that of individuals. In 2016, we found that *E. eugeniae* amputated at the 10th segment survives and regenerates the brain of the worm in 8 days [5]. Also, the brain amputee lost social ability.Except for the social ability, we couldn't see any other difference in the activities of the worm. Hence, it is concluded that the tissues of the supraesophageal ganglion (brain) of the worm have the expression of specific genes which are essential for the social ability. Now the important question is "what are the specific gene(s) responsible for the social ability of the worm?". For answering the question, a systematic transcriptome analysis of the worm is essential.

A Model for Screening Anti-Mitotic Compounds

The cells in onion root divide faster. It is a good model for screening anti-mitotic compounds. The blastema appears on the 3rd day of amputation of the worm, *E. eugeniae*. The blastema has a rich population of undifferentiated cells. Also, those cells divide rapidly. In 2019, it was found that the regenerating worm is an accepted model for screening the anti-mitotic compounds [21]. The administration of antibiotic compounds in the amputated worm arrests the blastema formation.

RNA Molecules Sequencing of *E. eugeniae*

As a first step, we sequenced them RNA molecules expressed in the blastema of the 4th and 6th day of regeneration in 2020. The experiments helped us to understand the sequences of about 25,185 cDNA molecules of *E. eugeniae*. The genetic resource is much helpful to design the cell and molecular biological experiments to understand the complex regeneration process. It was found that the expression of TCTP in the anterior blastema was increased to 6.35 fold and 7.41 fold respectively on 4rd and 6th days of amputation [2]. We also found that the expression of TCTP is more in the anterior regenerative. The data confirms that the TCTP plays the function of the TCTP protein is the same in the regeneration of both anterior and post-regeneration.

Autotomy and Regeneration

Autotomy is the ability of certain animals to detach their part of the body that has been grasped by an external agent. The animals which can trigger autotomy also has the potential of regeneration. The lizard, crab, spider, octopus, also can trigger autotomy for escaping from the predators and then they can regenerate the lost body part. The autotomy has been reported in the earthworm by Walton, John Monteith Jr. in 1936 [22]. We found that the worm accepts any given dose of glucose by injection but it expels higher doses of sodium chloride by sequestering the extra concentration of sodium chloride into the posterior segments of the worm. Then, the worm detaches the sodium chloride enriched posterior segments [23]. After autotomy, the worm starts regeneration to restore the detached body part but the injection of several chemicals such as lead and arsenic compounds also triggers autotomy but that worm dies eventually. Hence, the ability of the autotomy and regeneration of *E. eugeniae* can be used for toxicological characterization of compounds and drugs.

Conclusion

The finding niche of stem cells in *E. eugeniae,* the fluorescent property, and their migration pave numerous ways to understand the biology of stem cell and also the complex regeneration process. Also, the available sequences of cDNA molecules of the worm will be supportive to explore molecular events of the regeneration. Since the

rearing cost of the worm is economical, with the support of minimum fund quality research can be carried out in the area of biology of stem cells and regeneration.

Future Direction

Our experiments suggest the focusing on posterior segments regeneration is useful to understand the regeneration process much easier than that of anterior body segments of *E. eugeniae* because the anterior segments have varieties of organs in the first 12 segments but the posterior parts of the worm from the 20th segment to anus have only skin, nerve cord, gut, blood vessels. In the region, the repetitive segments make the total body. Also, the worm takes 7 days to regenerate the functional anus and after the organogenesis of anus, the molecular biological events of regeneration should be the same every day till completion of the regeneration. The regeneration takes more than 60 days to complete. Hence, surplus opportunities are available to design the cell and molecular biological experiments.

Since the intact clitellum of *E. eugeniae* is essential for successful regeneration, understanding the function of the clitellum is important to reveal the secret of regeneration. Also, it is interesting to understand the social responsible genes in the brain of the worm.

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Conflict of Interest Statement

I do not have a conflict of interest on the work.

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