Developmental Biology 405 (2015) 304–315

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

Evolution of Developmental Control Mechanisms

Evolution of the chordate regeneration blastema: Differential gene expression and conserved role of notch signaling during siphon regeneration in the ascidian *Ciona*

Mayuko Hamada [a], Spela Goricki [b], Mardi S. Byerly [b], Noriyuki Satoh [a], William R. Jeffery [b,*]

[a] Marine Genomics Unit, Okinawa Institute of Science and Technology Graduate University, 1919-1 Tancha, Onna, Okinawa 904-0495, Japan
[b] Eugene Bell Center for Regenerative Biology and Tissue Engineering, Marine Biological Laboratory, Woods Hole, MA 02543, USA

ARTICLE INFO

Article history:
Received 19 December 2014
Received in revised form
27 June 2015
Accepted 19 July 2015
Available online 20 July 2015

Keywords:
Ciona intestinalis
Regeneration
Differential gene expression
Notch pathway
Cell
Proliferation
Blastema
Muscle differentiation

ABSTRACT

The regeneration of the oral siphon (OS) and other distal structures in the ascidian *Ciona intestinalis* occurs by epimorphosis involving the formation of a blastema of proliferating cells. Despite the long-standing use of *Ciona* as a model in molecular developmental biology, regeneration in this system has not been previously explored by molecular analysis. Here we have employed microarray analysis and quantitative real time RT-PCR to identify genes with differential expression profiles during OS regeneration. The majority of differentially expressed genes were downregulated during OS regeneration, suggesting roles in normal growth and homeostasis. However, a subset of differentially expressed genes was upregulated in the regenerating OS, suggesting functional roles during regeneration. Among the upregulated genes were key members of the Notch signaling pathway, including those encoding the delta and jagged ligands, two fringe modulators, and to a lesser extent the notch receptor. *In situ* hybridization showed a complementary pattern of *delta1* and *notch* gene expression in the blastema of the regenerating OS. Chemical inhibition of the Notch signaling pathway reduced the levels of cell proliferation in the branchial sac, a stem cell niche that contributes progenitor cells to the regenerating OS, and in the OS regeneration blastema, where siphon muscle fibers eventually re-differentiate. Chemical inhibition also prevented the replacement of oral siphon pigment organs, sensory receptors rimming the entrance of the OS, and siphon muscle fibers, but had no effects on the formation of the wound epidermis. Since Notch signaling is involved in the maintenance of proliferative activity in both the *Ciona* and vertebrate regeneration blastema, the results suggest a conserved evolutionary role of this signaling pathway in chordate regeneration. The genes identified in this investigation provide the foundation for future molecular analysis of OS regeneration.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction

Understanding the mechanisms of tissue regeneration and their evolutionary modifications are among the most important challenges in modern biology. Whereas the body parts of most adult vertebrate species show limited regenerative abilities, the amphibians and teleosts are able to completely replace appendages, tails, and even the lens of the eye (Tsonis, 2000; Brockes and Kumar, 2008; Poss, 2010). Vertebrates with strong regenerative capacities exhibit pluripotent stem cells that are set-aside during development to replenish injured tissues and organs (Bryant et al., 2002; Sanchez-Alvarado and Tsonis, 2006). In these animals, regeneration is dependent on the formation of a blastema of proliferating cells that contributes newly differentiated cells to re-forming structures. Notch signaling appears to be important for the maintenance of cell proliferation in the vertebrate regeneration blastema (Grotek et al., 2013; Münch et al., 2013). Along with many other functions, the Notch signaling system is also involved in vertebrate cardiac and skeletal muscle regeneration (Conboy and Rando, 2002; Raya et al., 2003; Zhao et al., 2014).

In contrast to most vertebrates, invertebrate chordates (cephalochordates and tunicates) have extensive regeneration capacities (Berrill, 1951; Tiozzo et al., 2008; Somorjai et al., 2010), suggesting that regeneration is an ancestral chordate trait that has been modified during vertebrate evolution. The phylogenetic status of the tunicates as the probable sister group of vertebrates (Delsuc et al., 2006; 2008; Bourlat et al., 2006) renders them attractive for studying the comparative biology of chordate regeneration. Regeneration is best known in the ascidian tunicates (Berrill, 1951; Tiozzo et al., 2008). After the removal of zooids and

* Corresponding author.
E-mail address: Jeffery@umd.edu (W.R. Jeffery).

http://dx.doi.org/10.1016/j.ydbio.2015.07.017
0012-1606/© 2015 Elsevier Inc. All rights reserved.

immature buds, the entire body of Botryllid colonial ascidians can be replaced by totipotent stem cells residing in the residual basal vasculature (Rinkevich et al., 1995; Brown et al., 2009). Regeneration is probably more limited in solitary ascidians, such as *Ciona intestinalis*, in which distal body parts can be replaced from proximal parts, as long as the latter contain a portion of the branchial sac (Hirschler, 1914; Jeffery, 2015a). In *Ciona*, the oral siphon (OS), a muscular tube leading into the mucus-forming pharynx, and the neural complex, which includes the brain and the associated neural gland, are able to regenerate with complete fidelity within about a month after their removal (Sutton, 1953; Whittaker, 1975; Bollner, et al., 1992; 1993; 1995; Dahlberg et al., 2009; Auger et al., 2010). The OS pigment organs (OPO), which are sensory receptors rimming the siphon opening (Dilly and Wolken, 1973), and the circular muscle fibers lying immediately below them, reappear more rapidly, within about 5–10 days after amputation (Auger et al., 2010). Nerve tracts from the siphon stump are also re-extended into the regeneration blastema during the early stages of OS regeneration (Auger et al., 2010).

Recent studies have focused on the source and deployment of stem cells for *Ciona* distal regeneration (Jeffery, 2015b). The stem cells involved in OS replacement are located in lymph nodes lining the transverse vessels of the branchial sac, thus explaining why the latter is required for regenerative activity (Hirschler, 1914; Jeffery, 2015b). The same or closely related cells were previously identified as hematogenic stem cells in *Ciona* and other solitary ascidians (Ermak, 1975; 1976). A subset of these hemocytes is also the precursor of body muscle cells in colonial ascidians (Berrill, 1941; Sugino et al., 2007). The stem cells of the branchial sac initiate proliferation in response to distal injuries and invade the wounded areas to form the blastema (Jeffery, 2015b). Subsequently, new OPO and siphon muscle fibers are formed, and the regenerating OS re-grows to full length (Auger et al., 2010). As adult *Ciona* age, the pool of stem cells may decline or lose potency, resulting in reduced regeneration capacity (Jeffery, 2015b). *Ciona* has served as a model for understanding the molecular aspects of embryonic development (Satoh, 1994; 2014) and benefits from extensive molecular tools (Stolfi and Christiaen, 2012), including a sequenced genome (Dehal et al., 2002), EST collections (Satou et al., 2002; Tassy et al., 2010), and microarrays (Yamada et al., 2005; Azumi et al., 2003, 2007). However, these exceptional resources have yet to be exploited in regeneration studies.

In this investigation, microarray analysis and quantitative real time RT-PCR have been employed to identify differentially expressed genes during *Ciona* OS regeneration. Analysis of gene expression profiles showed that while most genes are down-regulated, consistent with roles in normal growth and physiology and temporary suppression during an injury response, a smaller subset of genes is upregulated, suggesting potential roles in the regenerating OS. The upregulated genes include some key members of the Notch-signaling pathway, such as those encoding the ligands delta1 and jagged, two of the fringe modulators, and to a lesser extent the notch receptor. Chemical inhibition of Notch signaling suppressed cell proliferation in the branchial sac and regeneration blastema and prevented OPO replacement and siphon muscle cell differentiation. These results suggest that Notch signaling has a conserved role in formation of the chordate regeneration blastema and constitute the first molecular analysis of OS regeneration in the ascidian *Ciona intestinalis*.

2. Materials and methods

2.1. Biological materials and procedures

Ciona intestinalis, Type B, was collected in the vicinity of Woods Hole, MA, USA and maintained in tanks of running seawater. Some animals were also obtained from fertilized eggs cultured in a closed system as described by Jeffery (2015b). Animals were relaxed by treatment with 200 μg/ml tricaine methanesulphonate (MS222; Sigma Aldrich, St. Louis, MO, USA) and the OS was amputated with micro-scissors and watchmaker's forceps as described previously (Auger et al., 2010). Following amputation the regenerating animals were placed in running natural seawater or Millipore filtered seawater (MFSW) for recovery.

2.2. Preparation of oral siphons for RNA extraction

The procedure for obtaining control and regenerating OS is summarized in Fig. 1A. We used the siphons from five animals of equal size (8 cm in length) for each RNA extraction, which was carried out at 0 (control), 3, 6, and 9 days post-amputation (dpa). The tunic was removed by dissection prior to OS amputation (Auger et al., 2010). The OS of each animal was excised by cutting through a plane perpendicular to its long axis. The amputation plane was located about three quarters of the way from the distal rim to the siphon base. Each excised OS was immediately placed in RNA Later (Sigma-Aldrich). These amputations yielded the control OS (0 dpa) samples (Fig. 1A). The animals subjected to the first OS amputation were separated into three groups, which were allowed to regenerate for 3, 6, or 9 dpa respectively. At the desired time, the regenerating OS was amputated again as described above and immediately immersed in RNA Later. The second amputation yielded samples of regenerating OS at 3, 6, and 9 dpa (Fig. 1A).

2.3. Microarray procedures

To identify differentially expressed genes in regenerating oral siphons, total RNA was purified from the OS samples using the RNeasy micro kit (Qiagen, Valenica, CA, USA) following the manufacturer's protocols. RNA was quantified using a NanoDrop-1000 spectrophotometer, and quality was monitored with the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Two microarray experiments were performed for each set of samples (control, 3, 6 and 9 dpa). cRNA targets labeled with cyanine-3 were synthesized from 200 ng total RNA using a Quick Amp Labeling Kit for one color detection (Agilent Technologies). A set of fluorescently labeled cRNA targets was employed in a hybridization reaction with the *Ciona intestinalis* 44 k Oligoarray ver.2 (Agilent Technologies; NCBI GEO Accession no. GPL5576). The chip contains 42,034 oligonucleotide probes representing 19,964 genes. Hybridization and washing were performed using the GE Hybridization Kit and GE Wash Pack (Agilent Technologies) and then scanned on an Agilent Technologies G2565BA microarray scanner system with SureScan technology. The protocols for the above procedures were used according to the manufacturer's instructions. The intensity of probes was determined from scanned microarray images using Feature Extraction 10.5 software (Agilent Technologies). The algorithms and parameters in this analysis were used in the default condition of the software (Yamada et al., 2005). Some probes that were judged as beyond analysis by Feature Extraction 10.5 software were eliminated from the following analysis. The data are available at NCBI GEO under accession number GSE59280. The raw data were normalized using the 75th percentile signal intensity. The genes differentially expressed between control OS and regenerating OS were determined at each stage by one-way ANOVA ($P < 0.05$) and fold change (cut offs > 1.5, 5.0, and 10.0) using GeneSpring GX (Agilent Technologies). The differentially expressed genes were linked to established gene models and ESTs (Satou et al., 2008) and the best-hit NCBI human genes by BLAST.

Download English Version:

https://daneshyari.com/en/article/2172849

Download Persian Version:

https://daneshyari.com/article/2172849

Daneshyari.com