Developmental Biology 382 (2013) 280–292

Contents lists available at ScienceDirect

Developmental Biology

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

Genomes and Developmental Control

An ancient role for Gata-1/2/3 and Scl transcription factor homologs in the development of immunocytes

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ARTICLE INFO

Article history:
Received 22 January 2013
Received in revised form
7 June 2013
Accepted 12 June 2013
Available online 20 June 2013

Keywords:
Blastocoelar cell
Pigment cell
Sea urchin
GATA
Scl
Hematopoiesis

ABSTRACT

Although vertebrate hematopoiesis is the focus of intense study, immunocyte development is well-characterized in only a few invertebrate groups. The sea urchin embryo provides a morphologically simple model for immune cell development in an organism that is phylogenetically allied to vertebrates. Larval immunocytes, including pigment cells and several blastocoelar cell subtypes, emerge from a population of non-skeletal mesodermal (NSM) precursors that is specified at the blastula stage. This ring of cells is first partitioned into oral and aboral fields with distinct blastocoelar and pigment cell gene regulatory programs. The oral field is subsequently specified into several distinct immune and non-immune cell types during gastrulation. Here we characterize the oral NSM expression and downstream function of two homologs of key vertebrate hematopoietic transcription factors: *SpGatac*, an ortholog of vertebrate *Gata-1/2/3* and *SpScl*, an ortholog of *Scl/Tal-2/Lyl-1*. Perturbation of SpGatac affects blastocoelar cell migration at gastrulation and later expression of immune effector genes, whereas interference with SpScl function disrupts segregation of pigment and blastocoelar cell precursors. Homologs of several transcription regulators that interact with Gata-1/2/3 and Scl factors in vertebrate hematopoiesis are also co-expressed in the oral NSM, including *SpE-protein*, the sea urchin homolog of vertebrate *E2A/HEB/E2-2* and *SpLmo2*, an ortholog of a dedicated cofactor of the Scl–GATA transcription complex. Regulatory analysis of *SpGatac* indicates that oral NSM identity is directly suppressed in presumptive pigment cells by the transcription factor SpGcm. These findings provide part of a comparative basis to understand the evolutionary origins and regulatory biology of deuterostome immune cell differentiation in the context of a tractable gene regulatory network model.

Introduction

Specialized immune cells that guard against pathogens, regulate symbiosis and maintain tissue homeostasis are nearly ubiquitous among bilaterian animals. General features of these cell systems, such as division into phagocytic and granular subsets, are also widespread (Hartenstein, 2006), yet little is known of how these cells and their associated genetic circuitry are related across phyla. Immune recognition and effector mechanisms evolve rapidly (Clark et al., 2007; Hughes and Friedman, 2008) and are therefore problematic as comparative tools. Instead, a more fruitful approach to characterizing these cellular relationships may come from comparative investigations of developmental gene regulatory networks. These may contain conserved core elements of circuitry that can lend further insight to this problem (Davidson and Erwin, 2006).

The sea urchin embryo provides a powerful model for this purpose. It is well suited for characterizing developmental gene regulatory networks (Oliveri et al., 2008). Further, echinoderm larvae were the focus of classical studies that defined animal cellular immunity (Metchnikoff, 1891) and several recent studies have addressed immune functions in the larva (Furukawa et al., 2009; Silva, 2000; Yang et al., 2010). Our investigations in the

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http://dx.doi.org/10.1016/j.ydbio.2013.06.019

purple sea urchin, *Strongylocentrotus purpuratus*, indicate that the feeding larva is equipped with a well developed immune system. We have identified an expansive suite of immune receptors and effectors that are expressed in the larva and have characterized several distinct immune cell types on the basis of cell behavior and gene expression (Buckley and Rast, 2012; Hibino et al., 2006; E.C.H.H. and J.P.R., unpublished).

Larval immunocytes develop from two major derivatives of the embryonic non-skeletal mesoderm (NSM), also known as secondary mesenchyme cells (SMCs) because of their mesenchymal phenotype. The first of these, the pigment cells, emerges in early gastrulation and quickly differentiates into a uniform cell type (Gibson and Burke, 1985, 1987; Kominami, 2000a, 2000b; Ransick and Davidson, 2006). These cells are highly motile, engage in continuous surveillance-like activity and migrate to sites of bacterial infection (E.C.H.H. and J.P.R, unpublished). The second category of cells that contribute to larval immunity is collectively known as blastocoelar cells. These cells ingress from the tip of the archenteron about ten hours after pigment cells delaminate (Tamboline and Burke, 1992) and differentiate into a heterogeneous assemblage of cells with immune and non-immune functions by the onset of feeding (Hibino et al., 2006; Katow et al., 2004). Several of these blastocoelar cell types display immune-like motile behavior, are capable of efficient phagocytosis, and express immune effector genes (Hibino et al., 2006).

A series of regulatory events leading to the specification and initial subdivision of the NSM is well-characterized. The NSM originates from a ring of blastomeres that lie in direct contact with the skeletal mesoderm (SM). Notch signaling is required for specification of all NSM-derived cell types including pigment and blastocoelar cells (Sherwood and McClay, 1999). A Delta ligand expressed by the SM provides the source of this signal (Sweet et al., 2002). One of the first regulatory state changes that differentiates the NSM ring is the expression of the transcription factor glial cells missing (*SpGcm*) in a one-cell thick ring surrounding the skeletogenic precursors in the early blastula (Ransick et al., 2002). *SpGcm* is a direct target of Delta–Notch (D/N) signaling (Ransick and Davidson, 2006) and the timing of this D/N signal has been thoroughly characterized with respect to the early specification of the NSM relative to surrounding endoderm (Croce and McClay, 2010). A comprehensive study of downstream D/N signal targets in the NSM (Materna and Davidson, 2012) identifies *SpGcm* as the first spatially localized transcription factor activated by the D/N signal in the NSM lineage and the presence of a feed-forward circuit involving the transcription factor SpGataE, which at this stage shows an identical expression pattern in all the NSM cells.

At the mid-blastula stage, a set of pigment cell-specific genes are activated in the entire NSM ring (Calestani et al., 2003; Calestani and Rogers, 2010; Ransick and Davidson, 2006, 2012; Ransick et al., 2002). At the mesenchyme blastula stage, following ingression of the skeletogenic lineage, the NSM ring is subdivided into several regions that were initially characterized in cell fate mapping experiments (Ruffins and Ettensohn, 1993, 1996). Just prior to the ingression of the skeletogenic mesenchyme, the precursors of the blastocoelar cells, which are located on the oral side of the NSM ring, terminate expression of *SpGcm* and other pigment cell-specific genes (Calestani et al., 2003; Ransick et al., 2002) and begin to express a suite of oral NSM transcription factors (Materna et al., 2013). This oral–aboral division of the NSM is downstream of Nodal signaling (Duboc et al., 2010) and it is mediated by the expression of the *SpNot* gene which extends into the oral side of the NSM (Materna et al., 2013). Interestingly, perturbation analysis shows that at mesenchyme blastula *SpNot* expression is required for both up-regulation of oral NSM genes and down-regulation of aboral NSM genes. At this developmental stage the gene encoding the Delta ligand is the only characterized regulatory gene that remains expressed in the entire NSM lineage.

Here we are interested in the gene regulatory network that lies downstream of the initiation of the oral NSM regulatory program and, ultimately, how this transcriptional program contributes to the development of blastocoelar immune cell subtypes. Hence, we characterize the expression and downstream function of the sea urchin homologs of *Gata-1/2/3*, *SpGatac*, and of *Scl/Tal-2/Lyl-1*, *SpScl*, in the context of NSM and the development of larval immunocytes. Homologs of these genes are also important regulators of hematopoiesis in vertebrates (Anderson, 2006; Galloway et al., 2008; Laslo et al., 2008; Maeno, 2003). Both *SpGatac* and *SpScl*, along with other hematopoietic transcription factors, are transiently expressed in a spatial pattern consistent with a role in blastocoelar cell specification. Perturbation of these factors disrupts NSM patterning and immunocyte development. Perturbation of SpGatac results in an apparent defect of mesenchymal cell motility, whereas SpScl perturbation disrupts partitioning of the NSM. A negative feedback mechanism is also evident that blocks *SpGatac* expression in differentiating pigment cells.

Materials and methods

Animals and embryo culture

S. purpuratus specimens were obtained from Westwind Sealab Supplies, Victoria, BC, Canada and Point Loma Marine Invertebrate Lab, Lakeside, CA, USA. Adults were maintained in aquaria in artificial sea water (Instant Ocean) at 13 °C. Fertilized embryos were kept in 0.45 μM filtered Instant Ocean in Petri dishes or stirred cell culture flasks at 15 °C.

Reverse transcription quantitative PCR (RT-qPCR)

RT-qPCR analysis was performed as previously described (Fugmann et al., 2006; Rast et al., 2002). For injected embryos, 50–500 embryos were collected per sample and RNA was extracted with an RNeasy Micro kit with on-column DNase treatment (Qiagen). cDNA was synthesized using TaqMan Reverse Transcription Reagents (Applied Biosystems) with random hexamers. qPCR cycling was performed using SYBR Green PCR master mix (Applied Biosystems). The oligonucleotides that were used to quantify transcripts are listed in Table S1.

For some transcript prevalence measurements, coelomocytes were isolated from two *S. purpuratus* adults by mixing coelomic fluid with an equal volume of calcium–magnesium-free sea water containing 20 mM EDTA. RNA was isolated from cell pellets with Trizol (Invitrogen), DNase treated (DNA-free, Ambion) and used for reverse transcription (Superscript III, Invitrogen). RT-qPCR was carried out as described above.

Rapid amplification of cDNA ends (RACE) and cDNA analysis

Message sequences for *SpGatac* (short transcript) and *SpE-protein* were determined previously from coelomocyte cDNA (Pancer et al., 1999) and verified from embryonic cDNA. *SpScl*, *SpId* and *SpLmo2* were identified through BLAST analysis of the sea urchin genome (Hibino et al., 2006). Full transcript sequences were determined using a RACE strategy (GeneRacer, Invitrogen) using cDNA isolated from the mesenchyme blastula and early gastrula stage embryos. Transcript sequences for *SpScl*, *SpId* and *SpGatac* (long transcript) from our RACE data are consistent with transcriptome sequence available at SpBase (www.spbase.org; Tu et al., 2012). Sequence of the *SpGatac* (short transcript) was