Developmental Biology ■ (■■■■) ■■■–■■■

Contents lists available at ScienceDirect

Developmental Biology

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

Delayed transition to new cell fates during cellular reprogramming

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ARTICLE INFO

Article history:
Received 25 April 2013
Received in revised form
19 April 2014
Accepted 21 April 2014

Keywords:
Reprogramming
Regulative development
Gene regulatory network
Sea urchin embryo
Cell fate
Differentiation
Cell fate specification

ABSTRACT

In many embryos specification toward one cell fate can be diverted to a different cell fate through a reprogramming process. Understanding how that process works will reveal insights into the developmental regulatory logic that emerged from evolution. In the sea urchin embryo, cells at gastrulation were found to reprogram and replace missing cell types after surgical dissections of the embryo. Non-skeletogenic mesoderm (NSM) cells reprogrammed to replace missing skeletogenic mesoderm cells and animal caps reprogrammed to replace all endomesoderm. In both cases evidence of reprogramming onset was first observed at the early gastrula stage, even if the cells to be replaced were removed earlier in development. Once started however, the reprogramming occurred with compressed gene expression dynamics. The NSM did not require early contact with the skeletogenic cells to reprogram, but the animal cap cells gained the ability to reprogram early in gastrulation only after extended contact with the vegetal halves prior to that time. If the entire vegetal half was removed at early gastrula, the animal caps reprogrammed and replaced the vegetal half endomesoderm. If the animal caps carried morpholinos to either *hox11/13b* or *foxA* (endomesoderm specification genes), the isolated animal caps failed to reprogram. Together these data reveal that the emergence of a reprogramming capability occurs at early gastrulation in the sea urchin embryo and requires activation of early specification components of the target tissues.

Introduction

It is widely thought that the differentiation potential of a cell decreases as the cell becomes increasingly specified (Cherry and Daley, 2012). However, this view has been challenged by recent findings that combinations of defined factors can revert the fate of a differentiated cell to a pluripotent state in mammals, leading to induced pluripotent stem cells (iPSCs) (Liu et al., 2008; Park et al., 2008; Takahashi et al., 2007; Takahashi and Yamanaka, 2006; Yu et al., 2007). Thus, studying the reprogramming process will yield insights into how the gene regulatory system buffers perturbations and faithfully carries out the developmental program, and in addition provide useful information for regenerative medicine and disease modeling (Cherry and Daley, 2012).

The sea urchin embryo provides an excellent platform for studying reprogramming. It exhibits high developmental plasticity in that an isolated blastomere from the 4-cell stage embryo develops into a complete pluteus larva, a phenomenon that led to the original concept of "regulative" development (reviewed by Hörstadius, 1973). Later in development, when cells specified toward one fate were surgically removed, cells of other fates reprogrammed to replace the missing cells, a process referred to at the time as "transfating" (Ettensohn and McClay, 1988; McClay and Logan, 1996; Ettensohn et al., 2007). Those cell fate switches during sea urchin embryonic development altered the gene regulatory networks (GRNs) governing specification (Davidson, 2006; Davidson et al., 2002), and resulted in a dynamic and qualitative transition to a different GRN state. The remarkable advance in knowledge of the sea urchin GRN provides an excellent platform for a systems analysis of this reprogramming mechanism.

In the sea urchin, the skeletogenic lineage arises from four large micromeres formed at the vegetal pole of the embryo as a result of two consecutive asymmetric cleavages at the 16-cell and 32-cell stages. At the mesenchyme blastula stage, descendants of large micromeres undergo an epithelial-mesenchymal transition (EMT)

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http://dx.doi.org/10.1016/j.ydbio.2014.04.015
0012-1606/

and ingress into the blastocoel to form the primary mesenchyme cells (PMCs). The PMCs migrate to specific locations in the blastocoel, fuse via syncitial cables, and secrete the calcium carbonate skeleton of the larva. When skeletogenic cells were removed, either at the 16-cell stage as micromeres or at the mesenchyme blastula stage as PMCs, non-skeletogenic mesoderm cells (NSM) reprogrammed to assume a skeletogenic fate (Ettensohn and McClay, 1988; Sweet et al., 1999). If the PMCs and the archenteron tip (all mesoderm) were removed, the remaining presumptive endoderm reprogrammed to assume skeletal and other mesodermal fates

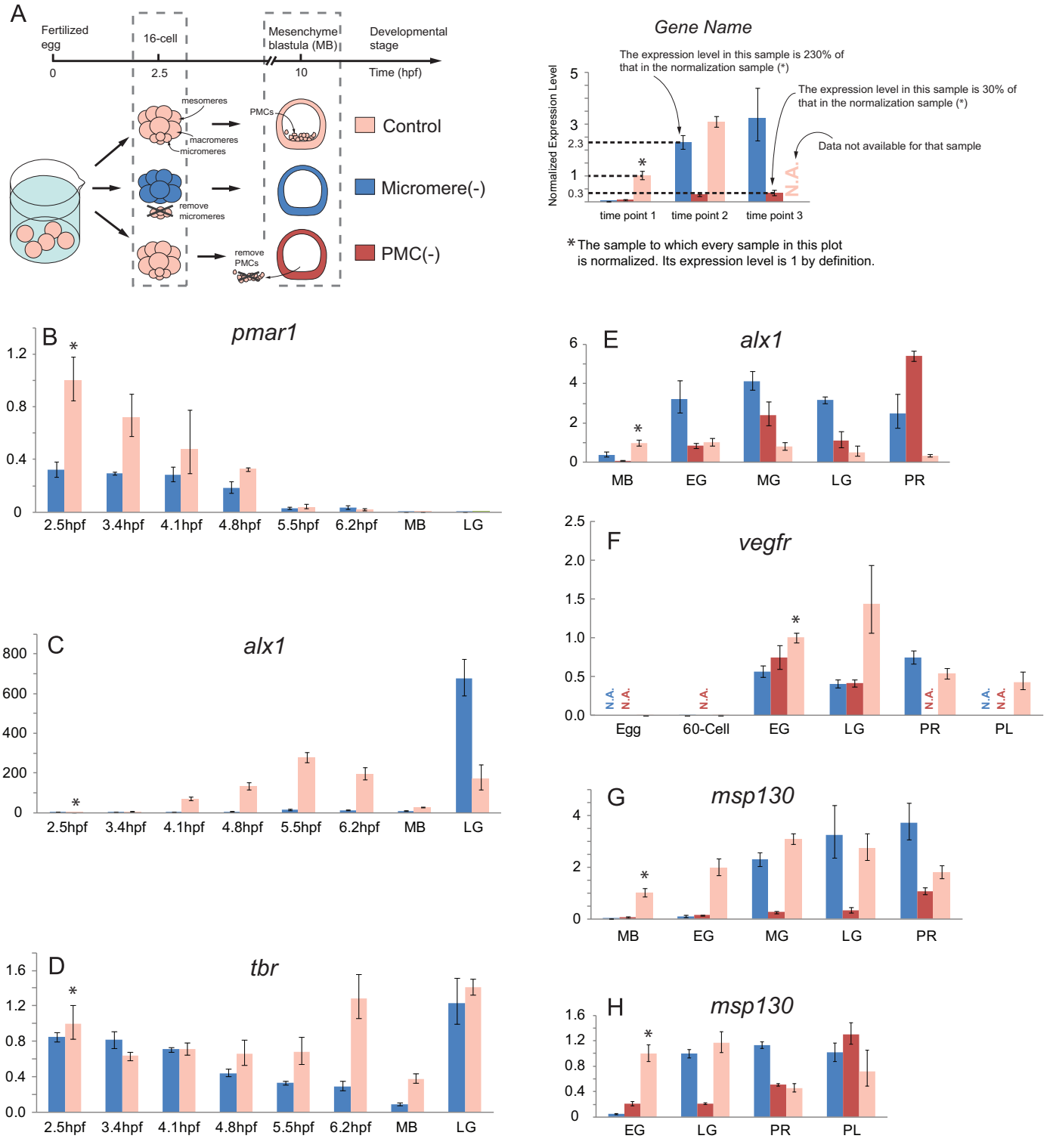

Fig. 1. Skeletogenic reprogramming in micromere (-) embryos occurs after a long delay. (A) (Left panel) Illustration of the experiment. At the 16-cell stage micromeres were removed (micromere(-), blue), or at the mesenchyme blastula stage PMCs were removed (PMC(-), red). (Right panel) Explanation of the qPCR plots. (B–H) Analysis of (B) *pmar1*, (C) *alx1*, (D) *tbr*, (E) *alx1*, late, (F) *vegfr*, (G) *msp130*, early, and (H) *msp130* late. Horizontal axes give hours post fertilization (hpf) until morphogenesis: MB, mesenchyme blastula; EG, early gastrula; MG, mid-gastrula; LG, late gastrula; PR, prism; PL, pluteus. In each plot, the normalization standard sample is indicated by a * sign, and has the relative expression value of 1.

Please cite this article as: Cheng, X., et al., Delayed transition to new cell fates during cellular reprogramming. Dev. Biol. (2014), http://dx.doi.org/10.1016/j.ydbio.2014.04.015