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Dynamics of two key maternal factors that initiate zygotic regulatory programs in ascidian embryos

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ABSTRACT

In animal embryos, transcription is repressed for a definite period of time after fertilization. In the embryo of the ascidian, *Ciona intestinalis* (type A; or *Ciona robusta*), transcription of regulatory genes is repressed before the 8- or 16-cell stages. This initial transcriptional quiescence is important to enable the establishment of initial differential gene expression patterns along the animal–vegetal axis by maternal factors, because the third cell division separates the animal and vegetal hemispheres into distinct blastomeres. Indeed, maternal transcription factors directly activate zygotic gene expression by the 16-cell stage; Tcf7/β-catenin activates genes in the vegetal hemisphere, and Gata.a activates genes in the animal hemisphere. In the present study, we revealed the dynamics of Gata.a and β-catenin, and expression profiles of their target genes precisely. β-catenin began to translocate into the nuclei at the 16-cell stage, and thus expression of β-catenin targets began at the 16-cell stage. Although Gata.a is abundantly present before the 8-cell stage, transcription of Gata.a targets was repressed at and before the 4-cell stage, and their expression began at the 8-cell stage. Transcription of the β-catenin targets may be repressed by the same mechanism in early embryos, because β-catenin targets were not expressed in 4-cell embryos treated with a GSK inhibitor, in which β-catenin translocated to the nuclei. Thus, these two maternal factors have different dynamics, which establish the pre-pattern for zygotic genetic programs in 16-cell embryos.

1. Introduction

Maternal factors in animal embryos activate transcription from genomes of zygotes shortly after fertilization, and subsequent developmental processes become dependent on zygotic transcripts (Langley et al., 2014; Tadros and Lipshitz, 2009). This process is called the maternal-to-zygotic transition, and the duration before transcription begins differs among different species.

Ciona intestinalis (type A), also known as *Ciona robusta*, is a tunicate, which belongs to the sister group of vertebrates. In *Ciona* embryos, the first four cell divisions occur synchronously and the cell cycle lengths are almost fixed (Dumollard et al., 2013; Hotta et al., 2007). Previous studies have revealed that a small number of genes initiate expression after the fourth cell division (at the 16-cell stage) by comprehensive expression assays (Imai et al., 2004; Matsuoka et al., 2013), although expression of two transcription factor genes, *Foxa.a* and *Sox1/2/3*, begins at the 8-cell stage (Miya and Nishida, 2003; Shimauchi et al., 2001). Maternal factors, including Gata.a and β-catenin, activate these genes in three distinct partially overlapping domains at the 16-cell stage (Bertrand et al., 2003; Hudson et al., 2013; Oda-Ishii et al., 2016; Rothbächer et al., 2007). Notably, *Foxd*, *Fgf9/16/20*, and *Tbx6b* are activated by β-catenin in the vegetal hemisphere [*Tbx6.b* is also regulated by Zic-r.a (Macho-1) and expressed only in the posterior vegetal cells]. *Efna.d* and *Tfap2-r.b* are activated by Gata.a in the animal hemisphere, and Gata.a activity is suppressed through its interaction with β-catenin in the vegetal hemisphere (Oda-Ishii et al., 2016). Since the animal and vegetal hemispheres do not segregate before the 8-cell stage, the transcriptional quiescence before the 8-cell stage is important for establishing these initial gene expression domains along the animal-vegetal axis. At subsequent stages, specific gene expression patterns are established on the basis of this initial setup (Bertrand et al., 2003; Hudson et al., 2013, 2016; Imai et al., 2006; Satou and Imai, 2015). Why are these genes activated at the 8- and 16-cell stages, but not before these stages?

In the present study, we analyzed the following points to understand the regulatory mechanisms of genes that are activated at the 16-cell stage: (1) when do the target genes of Gata.a and β-catenin precisely initiate expression? Is the timing of beginning of their expression tightly controlled? (2) When is β-catenin translocated into nuclei? (3) When and how much Gata.a is accumulated in nuclei of early embryos?

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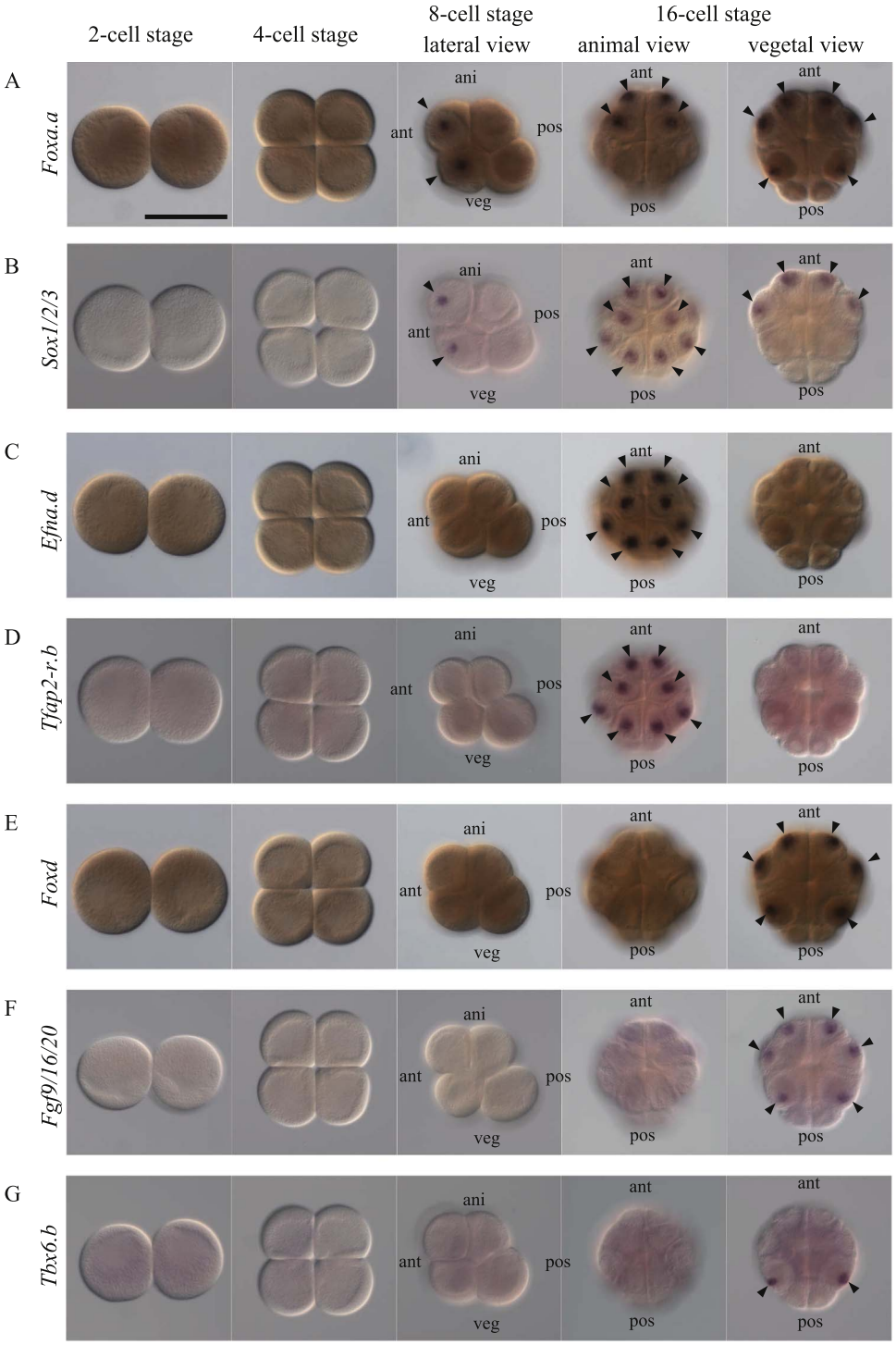

Fig. 1. Analysis of the onset of zygotic gene expression by *in situ* hybridization. Expression of (A) *Foxa.a*, (B) *Sox1/2/3*, (C) *Efna.d*, (D) *Tfap2-r.b*, (E) *Foxd*, (F) *Fgf9/16/20*, and (G) *Tbx6.b* at the 2- to 16-cell stages revealed by *in situ* hybridization. Arrowheads indicate expression. Ant, anterior side; pos, posterior side; ani, animal side; veg, vegetal side. Scale bar, 100 μm.

2. Results

2.1. Zygotic transcription of regulatory genes begins weakly at the 8-cell stage and markedly increases by the 16-cell stage

Only two genes, *Sox1/2/3* (also known as *Soxb1*) and *Foxa.a*, have been identified to be expressed zygotically at the 8-cell stage (Miya and Nishida, 2003; Shimauchi et al., 2001). Furthermore, according to our previous studies that examined zygotic gene expression comprehensively (Imai et al., 2004; Matsuoka et al., 2013), only a small number of genes, including *Efna.d* and *Foxd*, are expressed at the 16-cell stage. We first confirmed by *in situ* hybridization that expression of *Foxa.a* and *Sox1/2/3* was not observed at or prior to the 4-cell stage (Fig. 1A and B), and that expression of *Efna.d*, *Tfar2-r.b*, *Foxd*, *Fgf9/16/20* and *Tbx6.b* was not observed at or prior to the 8-cell stage (Fig. 1C–G).

To examine the expression quantitatively, we analyzed the expression levels of *Foxa.a*, *Sox1/2/3*, *Efna.d*, *Tfap2-r.b*, *Foxd*, *Fgf9/16/20*, and *Tbx6.b* using reverse-transcription and quantitative PCR (RT-qPCR) in three independent experiments (Fig. 2A–G). The expression level of maternal *Gata.a* was also measured as a control (Fig. 2H).