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Review

Measuring time during early embryonic development

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ABSTRACT

In most metazoans, embryonic development is orchestrated by a precise series of cellular behaviors. Understanding how such events are regulated to achieve a stereotypical temporal progression is a fundamental problem in developmental biology. In this review, we argue that studying the regulation of the cell cycle in early embryonic development will reveal novel principles of how embryos accurately measure time. We will discuss the strategies that have emerged from studying early development of *Drosophila* embryos. By comparing the development of flies to that of other metazoans, we will highlight both conserved and alternative mechanisms to generate precision during embryonic development.

Contents

1. Regulation of the cell cycle in early embryos: the need for speed and precision

When an egg develops into an organism, cells undergo an extraordinary population expansion and they obtain a spatial and temporal identity that will then determine their fate. This process poses a high risk for error amplification resulting from stochastic cellular decisions and unusually fast time scales [1]. Nonetheless, embryonic development is a precise process. It is thought that there must be complex developmental programs with correcting mechanisms in place to avoid the transmission of errors [2]. The *Drosophila* embryo provides a good model to study these problems since development is controlled in a highly stereotypical and reproducible pattern. Moreover, tools and methodologies for *Drosophila* are extensive and allow for careful interrogation of the dynamics of regulatory pathways quantitatively. In this review, we will propose that studying the cell cycle of early *Drosophila* embryos provides a unique system to dissect the molecular mechanisms ensuring precise temporal control of development.

A widespread phenomenon in the development of living organisms is the remodeling of the cell cycle to allow for remarkably fast cell cycles prior to gastrulation [3]. This pattern is particularly conserved in organisms that lay eggs, which develop externally, such as insects, amphibians and fish. The need for such exceptionally rapid cell cycle programs is most likely linked to the fact that eggs that develop externally subsist entirely on the maternal nutrients contained within the egg [4]. To make sure that embryos have sufficient nutrients, mothers lay very large eggs. However, such big size poses severe challenges for the regulation of embryonic

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development, since it is probably difficult for a single diploid nucleus to transcribe genes so efficiently to keep up with the vast demand of a very large cytoplasm. Therefore, embryos remain transcriptionally silent while they undergo several rounds of extremely rapid cleavage divisions [3]. The end result of this phase is an embryo with thousands of cells, which are now ready to take on developmental programs and gastrulation through transcriptional regulation.

The developmental strategy outlined above highlights the need for speed and synchrony in the regulation of the cleavage divisions, as the proper execution of the developmental programs that drive morphogenesis requires that these programs be initiated at very similar time across large spatial scales (Fig. 1A). How such features are achieved remain largely uncharacterized, although recent studies have started to shed light on this important problem. Before discussing these insights, we need to quickly review the early steps of embryonic development as well as our molecular understanding of cell cycle regulation.

2. Cell cycle dynamics during early *Drosophila* development

The *Drosophila* egg is an oval-shaped cell about 500 μm long and 150 μm in diameter. Despite its large size, development of the *Drosophila* embryo follows a precisely timed dynamic program [5]. After fertilization, the egg goes through 13 rapid and synchronous divisions, which take place in a syncytium (*i.e.*, a common cytoplasm not divided by membranes) [6]. These early cell cycles are exceptionally fast: nuclei undergo 13 mitotic divisions in 2–2.5 h, whereas an average tissue culture cell takes 8–24 h to go through one cell cycle [7]. These unusual speeds are achieved by omitting gap phases, having very short S-phases, and depending on maternally-loaded gene products to direct development. Nuclei in the early embryo therefore alternate between S-phase and mitosis during the early cycles. Gap phases (G_1 and G_2) canonically serve as pauses in the cell cycle during which cells grow or exit the cell cycle in the presence of unfavorable growth conditions or inhibitory signals from other cells [8]. However, the nuclei in the syncytial fruit fly embryo have all of the nutrients needed for development and embryos do not grow in size. Therefore, gap phases are dispensable in these initial cycles, which allows for a faster cycling times.

3. Cell cycle regulation and the mitotic switch

The embryonic cell cycle is driven by a regulatory network of proteins centered on the cyclin-dependent kinase (Cdk1) and the anaphase promoting complex (APC) [9]. Even though concentration of Cdk1 is constant, Cdk1 activity oscillates as nuclei go through several rounds of cell cycles due to oscillations in the levels of its regulatory subunits. These oscillations result in the phosphorylation of downstream components of the cell cycle machinery, which then lead to the initiation of cell cycle events [10].

Given its essential role in cell cycle progression, Cdk1 has many regulators of its kinase activity. To be activated, Cdk1 first requires binding of regulatory proteins called cyclins. Once Cdk1 is bound by a cyclin partner, it can be phosphorylated at Thr161 by Cdk1-activating kinase (CAK) which is required for enzymatic activity [11]. Surprisingly, CAK activity is not regulated by any known cell-cycle control pathway and it is maintained at high levels throughout the cell cycle. Therefore, the activating phosphorylation of Cdk1 is not rate limiting. Even though the activating phosphorylation of Cdk1 is not regulated, two inhibitory phosphorylations in Cdk1 are highly regulated and play an important role in the dynamics of Cdk1 activity [8]. One of them is found at a conserved tyrosine residue (Tyr15) and the other is found in animal cells at a threonine residue (Thr14). Tyr15 and Thr14 are located near the ATP-binding pocket and most likely block Cdk1 activity by interfering with the orientation of ATP phosphates [8].

The kinases that are responsible for adding these inhibitory phosphorylations are Wee1 and Myt1. Cdc25 phosphatases (String and Twine in *Drosophila*) are in charge of removing the inhibitory phosphorylations. Hence, there are four Cdk1 isoforms and the active form of Cdk1 is phosphorylated on Thr161 but not on Tyr15/Thr14. Finally, Cdk1 can be indirectly regulated by the regulators of Cdc25 and Wee1. For example, Chk1 kinase (Grapes in *Drosophila*) can indirectly inhibit Cdk1 by activating the Cdk1 inhibitor, Wee1, and inhibiting the Cdk1 activator, Cdc25 [7,8].

The described cell cycle control system generates robust, switch-like and adaptable changes in Cdk activity which lead to all-or-none transitions of cell cycle events. This is because the Cdk1 regulatory network includes feedback loops and other regulatory interactions that lead to irreversible activation and inactivation of cyclin-Cdk1 complexes [12]. Wee1 and Cdc25 provide the basis for the rapid activation of the mitotic switch. Both enzymes are regulated by active cyclin-Cdk1 complexes: Wee1 is inhibited and Cdc25 is activated [13,14]. Thus, active Cdk1 activates its activator and inhibits its inhibitor, generating a positive feedback loop and a double negative (positive) feedback loop, respectively. These feedbacks have the important property of generating a bistable system, which rapidly transitions from a low state of Cdk1 activity to a high state [15–18]. Bistability also provides hysteresis, *i.e.*, the activity of Cdk1 is dependent of its history, a property which helps with the irreversible nature of entry into mitosis [16,17].

Mitotic exit is driven by a negative feedback loop. Active cyclin-Cdk1 complexes activate the APC, which results in the polyubiquitination and degradation of cyclin [19–21], resetting Cdk1 complexes to their inactive, interphase state. The Cdk1-APC system behaves as a time-delayed negative feedback, a property which plays an important role in regulating the oscillatory activity of Cdk1 [22]. Other feedback mechanisms have been described that play a role in ensuring the proper abrupt regulation of anaphase [23,24].

Proteins phosphorylated by Cdk1 during mitosis must be dephosphorylated to reset the cycle to the next interphase. In metazoans, PP2A phosphatases play a crucial role in these dephosphorylation events. Importantly, Cdk1 has an active role in downregulating the activity of PP2A through a negative feedback mechanism, mediated by the activity of the Greatwall kinase and the endosulfine inhibitor [25,26]. This feedback mechanism seems to significantly contribute to changing the phosphorylation-dephosphorylation balance of Cdk1 substrates during the embryonic cycles [26].

4. Regulation of the early cell cycles

The early nuclear cycles of the *Drosophila* embryo demonstrate several specialized mechanisms by which very rapid cell cycles can be implemented [7,27]. First, all the required cell cycle components are loaded in the embryo at extremely high levels maternally. Specifically, the high Cdk1 activity is able to drive DNA replication of the several nuclei present in the embryo with extreme speed [7,27]. Experiments analyzing the activity of Cdk1 have suggested that these early nuclear cycles could proceed in the presence of very little oscillations in Cdk1 activity [27]. However, cyclin degradation is still required for mitotic exit events during syncytial cycles [28]. How can cell cycle events be triggered in the absence of oscillations in Cdk1 activity, but still require cyclin degradation? One possibility is that Cdk1 activity oscillates only locally in regions surrounding nuclei and spindles to regulate mitosis, so that analysis of its activity by biochemical methods (which report total activity in the embryo) would not show oscillations. *Drosophila* embryos also undergo cortical contractions during the early cycles which span