Journal of Oral Biosciences ■ (■■■■) ■■■–■■■

Contents lists available at ScienceDirect

Journal of Oral Biosciences

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

Review

Unveiling a rhythmic regulatory mode hidden in developmental tissue growth by fluorescence live imaging-based mathematical modeling

Tadahiro Iimura, D.D.S, Ph.D[a,b,c,d,*], Ji-Won Lee [a]

[a] Division of Bio-Imaging, Proteo-Science Center (PROS), Ehime University, Ehime 791-0295, Japan
[b] Division of Analytical Bio-Medicine, Advanced Research Support Center (ADRES), Ehime University, Ehime 791-0295, Japan
[c] Artificial Joint Integrated Center, Ehime University Hospital, Ehime 791-0295, Japan
[d] Graduate School of Medicine, Ehime University, Ehime 791-0295, Japan

ARTICLE INFO

Article history:
Received 10 August 2016
Received in revised form
29 August 2016
Accepted 30 August 2016

Keywords:
Cell cycle
Embryo
Development
Imaging
Tissue

ABSTRACT

Background: Fluorescence live imaging and mathematical modeling have been used in biomedical fields as a novel combined approach in the post-genomic era. The combined approach can potentially reveal novel biological functions that would not be uncovered by conventional molecular gain- and loss-of-function approaches.
Highlight: We used live imaging-based mathematical modeling and revealed a rhythmic regulatory mode of embryonic tissue development hidden in the stochastic cellular behavior of cell cycle progression.
Conclusion: The scope of this review is to describe the experimental and theoretical evidence for cell cycle progression as a stochastic molecular and cellular process. In addition, we elucidate the rhythmic mode of tissue development that endows "biological robustness" for reproducible tissue development. Finally, we discuss the potential of the live imaging-based mathematical approach for understanding pathophysiological development in tissues.

Contents

1. Introduction

Molecular expression and cellular behavior are often not synchronous even in a population of cells that are functionally and/or developmentally homogenous. In particular, cell cycle progression has been well described as a stochastic molecular and cellular process. On the other hand, embryonic tissue development is a highly organized process, indicating that biological robustness confers reproducibility in the

* Corresponding author at: Division of Bio-Imaging, Proteo-Science Center (PROS), Ehime University, Shitsukawa, Toon-City, Ehime 791-0295, Japan.
Fax: +81 89 960 5059.
E-mail address: iimura@m.ehime-u.ac.jp (T. Iimura).

http://dx.doi.org/10.1016/j.job.2016.09.001
1349-0079/

Please cite this article as: Iimura T, Lee J-W. Unveiling a rhythmic regulatory mode hidden in developmental tissue growth by fluorescence live imaging-based mathematical modeling. J Oral Biosci (2016), http://dx.doi.org/10.1016/j.job.2016.09.001

formation of morphologically and functionally proper tissues. Theoretically, cyclic or periodic waves, such as a molecular clock, are proposed to contribute toward such robustness.

Therefore, the identification of spatiotemporal regulatory modes, such as rhythmic actions hidden in heterogeneous cellular behavior, is an important task. To overcome this issue, we recently used a combined approach of live imaging and mathematical modeling and successfully identified a possible rhythmic action in developing zebrafish embryo as a model system of growing tissue [1]. This short review describes the experimental and theoretical approaches used to understand asynchronous cell cycle progression, the rhythmic growth mode of tissue development, and the potential of the live imaging-based mathematical approach for biomedical research. Quantitative imaging and successive mathematical modeling can be compatible with molecular omics, which will lead to a deeper understanding of pathophysiological development in tissues.

2. Stochastic behavior of molecular interactions and cellular behavior

It is well understood that each single cell exhibits a stochastic nature of gene expression and molecular interactions. The nature of each cell is intrinsically associated with fluctuations in cellular response observed in a given population of cells exposed to the same environmental conditions [2–6]. In multicellular conditions, extrinsic noise arises from heterogeneous cellular properties, such as size, shape, and status of cell cycle phases including mitosis [2,7–11]. Therefore, stochastic cell behavior or function can also be explained by intrinsic and extrinsic noises.

3. Cell cycle progression, an exemplified model of stochastic cellular behavior

Stochastic cell cycle progression has been phenomenologically described in several studies [12–16]. Furthermore, mathematical models have been proposed to account for the variable transition timing in cell cycle progression [13,17–24]. Based on observations of *in vitro* cell cycle progression, "the restriction point" of the G1/S transition has been proposed, which is well recognized as a conceptual framework for the stochastic nature of the G1/S transition [16]. The restriction point separates the G1 phase into the G1-post-mitosis phase (G1-pm) and the G1-pre-S phase (G1-ps). The earlier phase, G1-pm, is highly constant in time length (approximately 3 h), in which cells can proliferate depending on mitotic stimuli. On the other hand, the duration of G1-ps considerably varies, and cells in this phase transition into S phase independent of mitotic stimuli. At the molecular level, the restriction point is currently understood to extend the timing of the phosphorylation of Rb proteins by Cyclin D1. Phosphorylated Rb releases E2F to initiate S phase entry.

Mathematical modeling has suggested a bistable model of G1 and S phases that can regulate the restriction point in mammalian G1/S transition [25–29]. By temporally monitoring E2F transcriptional activity with stimuli of various magnitudes, Yao et al. experimentally demonstrated that bistable E2F activation directly correlates with the mammalian G1/S transition; thus, the experimental study successfully confirmed that the RB-E2F pathway and related multiple positive feedback loops can generate bistability, specifically, by forming a Rb-E2F bistable switch [30] (Fig. 1). The stochastic cellular behavior of the G1/S transition and varying pace of cell cycle progression in distinct cells are the likely causes of fluctuations in cellular response and behavior under multicellular conditions. Therefore, this stochastic nature of the G1/S transition model takes into account both intrinsic cellular noises and extrinsic noises in tissue development and homeostasis.

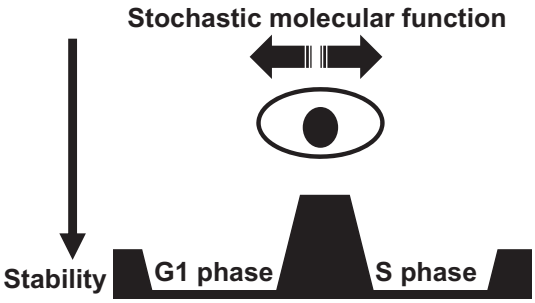

Fig. 1. Bistable model of the G1/S transition. The RB-E2F pathway and related multiple positive feedback loops can generate bistability, in which a single cell in the G1 phase can enter into either the G1 phase or S phase under intrinsic and extrinsic stimuli. This model thoroughly demonstrates that the stochastic cellular behavior of the G1/S transition and varying pace of cell cycle progression in distinct cells are the likely causes of fluctuations in cellular response and behavior in multicellular tissues.

4. Tissue and organ development, a highly coordinated biological process implying biological robustness

In contrast to the molecular nature and cellular behavior described above, embryonic development appears to be a spatiotemporally well-coordinated process that involves sequential cell proliferation and functional cell differentiation in which properly patterned tissues and organs are eventually formed and functionally developed. Therefore, the concept of "intrinsic time" concept has been postulated to explain the precision and reproducibility of the development of this tissue or organ [31,32].

Cell cycle progression during embryonic development has long been considered as an intrinsic cellular time-counting machinery that ensures the proper morphogenesis and patterning of tissues. Temporal activation of mitotic activity in mesenchymal tissues of the developing limb is reported to correlate with the future segmented skeletal pattern, thus ensuring the proper positioning of bones and joints in the limbs [33]. In addition, clustered mitotic activity in the developing endoderm is proposed to be responsible for morphogenetic folding to form the mature digestive tract [34]. Furthermore, periodic activation of mitosis in the paraxial mesoderm, which contains the precursor population of axial skeletal cells, has been repeatedly observed in concert with segmental somite formation in the developing tissue]. Since somites principally endow a segmented architecture to the axial skeleton and its associated muscles and neurons, the timed machinery of somite formation has been suggested to be a fundamental system for implementing the body plan and establishing/maintaining anatomical structure [35–37].

Experimental approaches in molecular and genetic embryology and human pathogenetics have established that the periodic formation of somites is regulated by a segmentation clock that exhibits an oscillatory expression of signaling molecules related to Notch, Wnt, and Fgf, which is principally established by the negative feedback loops of these signaling pathways [38–41]. However, current molecular findings seem to disagree with the idea that the cell cycle clock mentioned above functions as an oscillator of the segmentation clock [42–44].

It is well established that many of the biological functions required for homeostasis are regulated by circadian rhythms. Molecular insights into the circadian clock revealed that the negative feedback loops of transcriptional factors are operated with a 24-h periodicity in the brain and other peripheral tissues and organs [45]. Therefore,

Please cite this article as: Iimura T, Lee J-W. Unveiling a rhythmic regulatory mode hidden in developmental tissue growth by fluorescence live imaging-based mathematical modeling. J Oral Biosci (2016), http://dx.doi.org/10.1016/j.job.2016.09.001