Cell time: How cells control developmental timetables

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An overview on the molecular and metabolic mechanisms behind individual cell differences in developmental timing in the segmentation clock and the central nervous system.

From the cell cycle to circadian rhythms, biology relies on precise timing. This includes the duration of a process, the order and direction of events, and the rate at which a process operates. Timing can depend on extrinsic mechanisms that guide the synchronous progression through development of a group of cells via systemic cues. However, timing also relies on intrinsic mechanisms that keep track of time within cells. A focus on developmental timing is gaining momentum, as researchers tease out the molecular and metabolic mechanisms responsible for it.

In evolutionary developmental biology, differences in genetically controlled temporal programs are well recognized and referred to as heterochronies. These include differences in the time of initiation, duration, or rate of a process in comparison with an organisms' ancestors or other species. Whereas shifts in the time of initiation or duration have been linked to genetic variation of regulatory sequences or differential expression dynamics (*1*, *2*), other heterochronies that emerge from changes in the rate of a process are distinct and usually involve the same genetic program operating at different speeds. This has been termed allochrony and does not seem to be explained by variations in regulatory sequences (Fig. 1, A to C) (*3*, *4*). However, less is known about the mechanisms driving allochronies.

MOLECULAR MECHANISMS OF INTRINSIC TIMERS

Developmental processes need to operate in harmony to synchronize cells, tissues, organs, and the whole organism. It is increasingly clear that a central element of this delicate dance is achieved by each cell using its own clock. In the laboratory, cells isolated from their embryonic environment and maintained in vitro normally recapitulate the timing of differentiation observed in vivo. This is surprising as the cues and spatially distributed chemical signals (morphogen gradients) that are essential for cell fate specification in development are usually missing or delivered exogenously in cell cultures. Moreover, cell-to-cell interactions that may coordinate events are often disrupted in these experiments, as isolated cells have lost the interaction with other cell types in their niche. Cells offer the most basic model to expose timing control processes and to investigate the intrinsic genetic mechanisms that control timing. Moreover, we can exploit interspecies comparative approaches to interrogate timing by using equivalent cells that develop at different rates.

THE SPEED OF BIOCHEMICAL REACTIONS AND THE RATE OF DEVELOPMENT

Comparative differentiation models of presomitic mesoderm (PSM) cells that give rise to bones and muscles and motor neurons in mouse and human provide insight on the mechanisms behind allochrony. They both are based on the premises that (i) in vitro models recapitulate intrinsic in vivo timing, (ii) differences exist in the tempo of the process between species, and (iii) the genetic network is conserved across species.

Periodic oscillations of the master regulator gene Hes7 in PSM cells serve as a model because the Hes7 period is species specific, ticking faster in mice than in humans. This two- to threefold difference in pace is equivalent to the delay observed in the pace of the embryogenic period of human development (~90 days) in comparison to mice (20 days). Whereas differences in gene regulation are a major source for heterochronic changes, experiments swapping mouse Hes7 locus for the human Hes7 ortholog did not change the Hes7 period in mouse embryos, suggesting that the species context determines the period of Hes7. By measuring and fitting degradation rates of Hes7 protein and mRNA and the delays in the feedback loop of Hes7, a mathematical model indicated that the interspecies period difference depends on the kinetics of transcription and translation (*3*). The relevance of protein turnover (production and degradation) was recently confirmed in treatments with translation inhibitors in human PSM cells where Hes7 oscillations were slowed down even further (*5*).

Spinal cord development is a highly conserved and well-characterized system. It undergoes a series of molecular and morphogenetic processes resulting in the formation of the range of neuronal cell types found in the adult. In mice and humans, the formation and differentiation of spinal cord neural progenitors run two to three times faster in a mouse in comparison with a human. Mouse and human stem cells differentiated to motor neurons in vitro mirror the differences in the speed of

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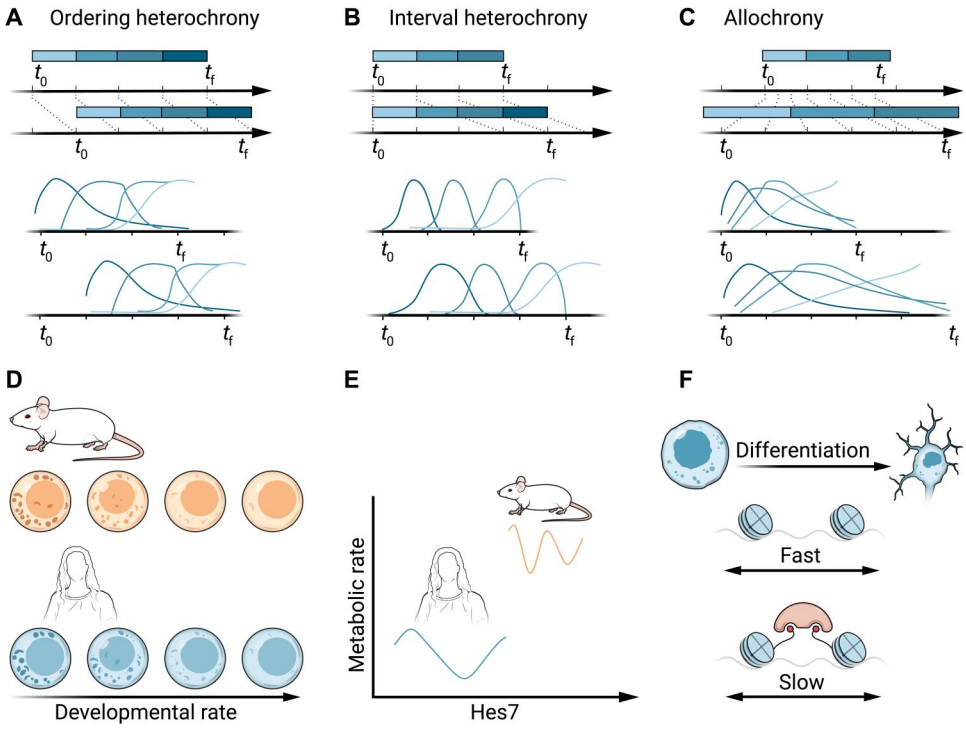

Fig. 1. Heterochronies in development and their mechanisms. (**A**) Ordering heterochronies. Shifts in the time of initiation of a conserved process leads to emergence of novel structures. (**B**) Interval heterochronies. Changes in the duration of specific phases of a process may alter the proportion of cell types and the overall timing of the process. (**C**) Allochrony. Proportional changes in the pace of the process. (**D**) Speed of biological reactions associates with the rate of development. (**E**) The tempo of Hes7 oscillations can be regulated by mass-specific metabolic rates. (**F**) Epigenetic regulators delay the speed of differentiation and maturation in corticogenesis. Credit: Austin Fisher, *Science Advances*.

differentiation observed in vivo, and human neural progenitors transplanted in chicks differentiate following the human time scale, thus limiting the pace of differentiation. This highlights the autonomous component controlling tempo (*6*). Neither differences in sensitivity to extrinsic signals nor differences in DNA sequence of key genes could explain the differences in tempo. Instead, a slower temporal progression in human associates with increased protein stability. This indicates that changes in protein stability across species may explain differences in tempo (*4*). The next milestone of discovery to explore relates to the consequences of modulating protein turnover and its relationship with basal metabolic rates.

METABOLIC RATE AND MITOCHONDRIAL ACTIVITY

As much as 25% of the metabolic expenditure in the body is due to constitutive protein turnover (translation and degradation), exceeding the energetic cost of other cellular processes such as DNA replication or transcription. Studies investigating protein turnover across mammals of different sizes have found that the proteome of larger animals is more stable, slower rates of protein turnover correlating with lower levels of ATP production (*7*). Therefore, differences in basal metabolic rates or energy production between species could explain the different time scales in development.

In PSM cells, human cells are twice as big as mouse cells, and the size-corrected mass-specific metabolic rate scales with the pace of development. Mouse PSM cells hold higher metabolic rates and faster Hes7 oscillations than human PSM cells. Pharmacological inhibition of the electron transport chain in human PSM cells slows down Hes7 period, and overexpression of the NADH oxidase lbNOX increases the translation rate and accelerates the segmentation clock (*5*). Metabolic rate measurements on cells treated with protein translation inhibitors do not alter metabolic rate. This indicates that translation rate does not operate upstream of the metabolic rate to regulate Hes7 period in human PSM cells (*5*). Can mass-specific metabolic rate be the master regulator for developmental tempo? It will be important to determine whether correlated differences in the segmentation period and metabolic rate are observed across a variety of species. Still to be investigated: Whether differences in Hes7 oscillations depend solely on the metabolic rate and whether the mechanisms that regulate the segmentation clock can regulate the overall differences in the pace of development.

The cerebral cortex represents a good example of how interval heterochronies contribute to developmental differences between species. The prolonged time scale in human cortical development contributes to the remarkable expansion of the neocortex and the complex morphology of human cortical neurons. Radial glial cells show species-specific heterochronies, as they manifest a longer mitotic phase, and the period in which differentiating cortical neurons remain plastic is species specific and associated with changes in mitochondria dynamics (*8*). Likewise, the structural and functional maturation of cortical neurons is a cell-intrinsic process with

different time scales across species. Mechanistically, the rates of mitochondrial activity can directly influence the developmental timeline of neuronal maturation in human. Stimulation of mitochondria respiration through blockade of pyruvate to lactate conversion, or by increasing the conversion of pyruvate into acetyl–CoA, accelerates neuronal maturation (*8*).

Overall, differences in metabolic rates could control the speed of biochemical reactions through the regulation of energy availability. Likewise, specific mitochondrial metabolites involved in posttranslational modifications could regulate the rate of development.

EPIGENETIC REGULATION

Epigenetic mechanisms are involved in the timely regulation of gene expression. Repressive chromatin modifications at individual gene loci fine-tune gene activation and allow for delays in gene expression. During mouse cortical development, polycomb repressive complex-2 inhibition in radial glia cells leads to an accelerated production of later-born neural cell types (*9*). This delay of cortical progenitor differentiation through epigenetic modulators could be linked to protein turnover. Single-cell RNA sequencing comparisons of cortical neural progenitors in the developing mouse brain identified a group of genes involved in translational regulation that may regulate temporal transitions. Specifically, fibrillarin, an rRNA methyltransferase, reduces translation of the epigenetic modifiers for H3K27me3 Ezh2 methyltransferase and Kdm6b demethylase and delays the differentiation of cortical neural progenitors (*10*).

In humans, down-regulation of epigenetic factors is associated with an increased maturation state of cortical neurons differentiating from pluripotent stem cells. Transient inhibition of the epigenetic regulators EZH2, EHMT1/2, or DOT1L in progenitors accelerates maturation properties later in human stem cell–differentiated neurons (*11*). It will be key to establish how such a barrier in progenitors is established before the onset of neurogenesis and transmitted to differentiated neurons.

While epigenetic regulators involved in gene repression seem to delay the speed of differentiation during corticogenesis, the precise mechanisms as to how does this happen remains to be further investigated. This is particularly important as epigenetic complexes are formed by context-specific subunits. Moreover, how do epigenetic factors differentially regulate the time scales of development between species remains to be determined.

A HANDFUL OF MECHANISMS FOR DEVELOPMENTAL TIMING

Today, we are in an exciting era for biological timing. Researchers are expanding our understanding of the molecular mechanisms that control different heterochrony types. Changes in the time of initiation or the duration of a process across species can be linked to changes in the regulatory landscape across species. In contrast, differences in the speed of biological reactions, basal metabolic rates, or epigenetic mechanisms affect developmental rates. Whereas initial evidence suggests that metabolic rate regulates the segmentation period upstream of protein turnover, the relationship between protein stability and metabolic rates remains to be determined in other tissues. It would also be interesting to determine whether mechanisms are conserved across developmental processes. Finally, epigenetic mechanisms are starting to emerge as novel candidates for heterochronies in development. These are only a handful of mechanisms to control timing in development, and we do not know whether they operate across tissues and organisms. Moving into the future, research will need to address the relationship and generality of these mechanisms during development and homeostasis.

REFERENCES

1. R. Aires, A. D. Jurberg, F. Leal, A. Nóvoa, M. J. Cohn, M. Mallo, Oct4 is a key regulator of vertebrate trunk length diversity. *Dev. Cell* **38**, 262–274 (2016).
2. I. T. Fiddes, G. A. Lodewijk, M. Mooring, C. M. Bosworth, A. D. Ewing, G. L. Mantalas, A. M. Novak, A. van den Bout, A. Bishara, J. L. Rosenkrantz, R. Lorig-Roach, A. R. Field, M. Haeussler, L. Russo, A. Bhaduri, T. J. Nowakowski, A. A. Pollen, M. L. Dougherty, X. Nuttle, M. C. Addor, S. Zwolinski, S. Katzman, A. Kriegstein, E. E. Eichler, S. R. Salama, F. M. J. Jacobs, D. Haussler, Human-specific NOTCH2NL genes affect notch signaling and cortical neurogenesis. *Cell* **173**, 1356–1369 (2018).
3. M. Matsuda, H. Hayashi, J. Garcia-Ojalvo, K. Yoshioka-Kobayashi, R. Kageyama, Y. Yamanaka, M. Ikeya, J. Toguchida, C. Alev, M. Ebisuya, Species-specific segmentation clock periods are due to differential biochemical reaction speeds. *Science* **369**, 1450–1455 (2020).
4. T. Rayon, D. Stamataki, R. Perez-Carrasco, L. Garcia-Perez, C. Barrington, M. Melchionda, K. Exelby, J. Lazaro, V. L. J. Tybulewicz, E. M. C. Fisher, J. Briscoe, Species-specific pace of development is associated with differences in protein stability. *Science* **369**, eaba7667 (2020).
5. M. Diaz-Cuadros, T. P. Miettinen, O. S. Skinner, D. Sheedy, C. M. Díaz-García, S. Gapon, A. Hubaud, G. Yellen, S. R. Manalis, W. M. Oldham, O. Pourquié, Metabolic regulation of species-specific developmental rates. *Nature* **613**, 550–557 (2023).
6. A. Dady, L. Davidson, P. A. Halley, K. G. Storey, Human spinal cord in vitro differentiation pace is initially maintained in heterologous embryonic environments. *eLife* **11**, e67283 (2022).
7. K. Swovick, D. Firsanov, K. A. Welle, J. R. Hryhorenko, J. P. Wise, C. George, T. L. Sformo, A. Seluanov, V. Gorbunova, S. Ghaemmaghami, Interspecies differences in proteome turnover kinetics are correlated with life spans and energetic demands. *Mol. Cell. Proteomics* **20**, 100041 (2021).
8. R. Iwata, P. Casimir, E. Erkol, L. Boubakar, M. Planque, I. M. Gallego López, M. Ditkowska, V. Gaspariunaite, S. Beckers, D. Remans, K. Vints, A. Vandekeere, S. Poovathingal, M. Bird, N. Corthout, P. Vermeersch, S. Carpentier, K. Davie, M. Mazzone, N. V. Gounko, S. Aerts, B. Ghesquière, S.-M. Fendt, P. Vanderhaeghen, Mitochondria metabolism sets the species-specific tempo of neuronal development. *Science* **379**, eabn4705 (2023).
9. L. Telley, G. Agirman, J. Prados, N. Amberg, S. Fièvre, P. Oberst, G. Bartolini, I. Vitali, C. Cadilhac, S. Hippenmeyer, L. Nguyen, A. Dayer, D. Jabaudon, Temporal patterning of apical progenitors and their daughter neurons in the developing neocortex. *Science* **364**, eaav2522 (2019).
10. Q. Wu, Y. Shichino, T. Abe, T. Suetsugu, A. Omori, H. Kiyonari, S. Iwasaki, F. Matsuzaki, Selective translation of epigenetic modifiers affects the temporal pattern and differentiation of neural stem cells. *Nat. Commun.* **13**, 470 (2022).
11. G. Ciceri, H. Cho, M. Kshirsagar, A. Baggiolini, K. A. Aromolaran, R. M. Walsh, P. A. Goldstein, R. P. Koche, C. S. Leslie, L. Studer, An epigenetic barrier sets the timing of human neuronal maturation. bioRxiv 490114 [Preprint]. 3 June 2022. https://doi.org/10.1101/2022.06.02.490114

10.1126/sciadv.adh1849