Current Biology, Vol. 13, R632–R634, August 19, 2003, ©2003 Elsevier Science Ltd. All rights reserved. DOI 10.1016/S0960-9822(03)00567-0

Segmentation Clock: Insights from Computational Models

Dispatch

Olivier Pourquié[1] and Albert Goldbeter[2]

Two new theoretical models show how negative feedback loops incorporating a time delay can account for a variety of transcriptional oscillations, such as the mechanism of the segmentation clock in zebrafish and a number of recently identified transcriptional oscillators.

The study of biological oscillators has for long been a major focus of interest for theoretical biologists [1]. Complex models of the cell cycle [2] or circadian clocks [1] have been elaborated in the past few years. In developmental biology, few examples of oscillators have been identified [3]. The best characterized example so far is the segmentation clock, a transcriptional oscillator involved in the control of the segmentation of the body axis [4]. While a wealth of data has accumulated on this oscillator over the past few years, no modelling attempts based on these data have been reported — until now.

A paper by Lewis [5] in this issue of *Current Biology* proposes a theoretical model which integrates the different components of the zebrafish oscillator. The proposed model is based on a negative feedback loop with a transcriptional delay. It accounts for the transcriptional oscillations produced by the segmentation clock. This study is complemented by a report by Monk [6], which extends this modelling approach to other oscillations based on transcriptional loops recently uncovered. The articles by Lewis and Monk illustrate the usefulness of theoretical models for comprehending the dynamics of regulated cellular processes. Both studies show that mathematical models provide an important tool for analyzing dynamic phenomena that cannot be predicted on the basis of sheer intuition.

The body of a vertebrate animal is formed by a series of repeated blocks called segments, which include structures such as vertebrae, muscles and peripheral nerves. This segmental pattern of the body axis is established early in embryogenesis through the rhythmic production of the somites, paired blocks of paraxial mesoderm which bud off sequentially from the anterior extremity of the presomitic mesoderm. The segmentation clock drives the periodic transcription in the presomitic mesoderm of so-called 'cyclic genes', most of which are related to the Notch signalling pathway (see [4] for a review).

The cyclic genes include downstream targets of Notch signalling, such as genes of the *hairy* and *Enhancer of Split* family — including *c-hairy1*, *c-hairy2*, *hes1*, *hes7*, *her1*, *her7* and *hey* in chick, mouse and zebrafish — and also genes encoding regulators of Notch signalling, such as the ligand DeltaC in zebrafish and the glycosyl-transferase Lunatic fringe in mouse and chick. One proposed role of the clock is to drive the periodic activation of Notch signalling in the rostral presomitic mesoderm, thus setting the pace of boundary formation. The Notch pathway plays a central role in the core mechanism of the oscillator, and it has been suggested that it coordinates oscillations between neighboring presomitic mesoderm cells [7–13].

The theoretical model proposed by Lewis [5] entirely relies on Notch signalling (Figure 1). It accounts for the generation of the cyclic gene oscillations, and for their coordination in the presomitic mesoderm. This model is based on two interacting loops: a negative feedback loop established by the basic-helix–loop–helix transcription factors HER1 and HER7 on their own promoters, and an intercellular loop involving regulated Notch activation [10,11,13]. Lewis [5] models first the HER1/HER7 direct negative auto-regulatory loop. He shows that transcriptional oscillations with a period similar to that seen *in vivo* (around 30 minutes) can be obtained with parameters in a physiological range, if the delays in production of Her mRNA and protein are taken into account. The oscillations are robust even when one allows for the noisy, flickering character of gene regulation, as the regulatory protein binds to and dissociates from its site in the DNA.

By varying the parameter values, Lewis [5] shows that the model is compatible with a number of experimental observations, some of which are counter-intuitive. For instance, the model indicates that oscillations can be maintained, with period practically unchanged, even if the protein synthesis rate is drastically reduced. This prediction of the model is compatible with the published effects of cycloheximide treatments, in which *c-hairy1* oscillations were not completely halted [14]. Another prediction of the model is the progressive dampening or loss of regularity of the oscillations if Notch signalling is impaired — dampening if noise effects are small, loss of regularity if noise effects are larger. This may account for the observation that, in zebrafish or mouse *notch* mutants, the severity of the segmentation defects observed increases along the antero-posterior axis, with the cells — in the zebrafish mutants at least — becoming progressively uncoordinated so as to create a random, pepper-and-salt pattern of expression of the oscillatory genes [9].

The second feedback loop is based on Notch activation by Delta in an adjacent cell, regulating the expression of the *her* genes in this cell. This loop drives the periodic expression of *deltaC*, resulting in rhythmic activation of Notch and of its downstream target *her* genes. Physiological parameters can be found for which synchronized oscillations of the HER-based loop can be triggered by this circuitry in adjacent cells. The Notch-based loop is able to sustain

[1]Stowers Institute for Medical Research, 1000E 50th street, 64110 Kansas City, Missouri, USA. [2]Faculté des Sciences, Université Libre de Bruxelles Campus Plaine, C.P. 231, B-1050 Brussels, Belgium.

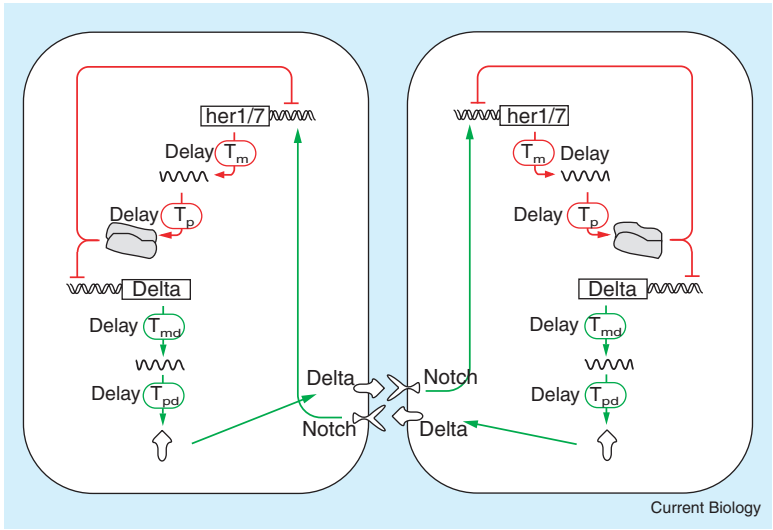

Figure 1. A schematic representation of the molecular network constituting the segmentation clock in two adjacent cells in zebrafish.

The circuitry constituting the HER1/HER7-based oscillator — which also requires components of the Notch pathway — is shown in red while the pure Notch-based oscillator is shown in green. (Adapted from [5].)

oscillations on its own and to synchronize these oscillations between adjacent cells. But these oscillations have a much longer period than those driven by the HER-based loop, closer to the period seen in the chick or mouse somite clock (around 2 hours). Remarkably, the Notch-based synchronous oscillations can occur even if there is no direct HER autoregulation: that is, they can be generated by the standard Delta–Notch signalling circuitry which is usually assumed to mediate lateral inhibition [15].

The model simulations thus show that, in the zebrafish somitogenesis clock, oscillatory gene expression may result either from direct negative autoregulation of *her1/her7* expression within each cell of the presomitic mesoderm, or from intercellular communication via the Delta–Notch pathway. A slight change in parameter values modulating the respective weights of the two types of regulation can produce an abrupt change in the period of oscillatory gene expression, as the mechanism switches from a 'pure' internal one to one based primarily on the intercellular mechanism. This leads Lewis [5] to speculate that, during evolution, the use of one or the other loop might have varied between species, thus accounting for the diversity of speed of somite formation seen among vertebrates.

A parallel can be made between the work reported by Lewis [5] and models for circadian oscillations, which have recently uncovered the possibility that there are multiple sources of oscillatory behavior in the genetic regulatory network of the circadian clock in *Drosophila* and mammals [16]. Wnt signalling has recently been shown to act upstream of Notch in the segmentation clock mechanism in the mouse embryo [17]. Such a role for the Wnt pathway has not been established yet in the zebrafish clock, but it is certainly possible that it has one. If this is the case, the model will have to be refined and its complexity will drastically increase.

A second report by Monk [6] nicely complements the theoretical approach of Lewis [5] on the zebrafish somitogenesis oscillator. Monk describes models in which a transcriptional delay is introduced into negative feedback loops to explain the recently described oscillations of the mRNAs coding for the transcription factors HES1, p53 and NFκB. These oscillations have been reported to occur with a period of 2–3 hours in cultured cells [18–20]. Monk's simulations based on such a modeling approach nicely fit the experimental observations in the original papers.

In simple models based on a negative feedback involving two variables, sustained oscillations can be obtained only if there is a time delay in the negative feedback loop. This time delay is a critical feature of the models presented by Lewis [5] and Monk [6]. A system with three or more variables, however, can show sustained oscillations even in the absence of such delays. Accordingly, in a recent model describing *hes1* oscillations in cell cultures, Hirata *et al.* [18] invoked the existence of an unknown intermediate to obtain oscillations. The third variable need not be a new molecular species: it suffices to distinguish the cytoplasmic and nuclear forms of the regulatory protein, as shown by a study [1] of models for circadian oscillations based on negative autoregulation of gene expression.

A highly useful aspect of the studies by Lewis [5] and Monk [6] is their attempt to provide experimentally based estimations for the parameter values, including the time delays in transcription and translation. Both authors estimate that the transcription delay is of the order of 10–20 minutes, and show that when the half-life of the protein and mRNA is sufficiently short, the period of the oscillations is largely set by this delay. With regard to oscillatory gene expression controlled by negative feedback, there is a continuum of possible situations: the oscillations are either primarily timed by the transcriptional delay or, at the other extreme, they can arise in the absence of this type of delay, when the chain of events forming the feedback loop is sufficiently long — this constraint is already satisfied in the presence of three variables — and when the degree of cooperativity of repression is sufficiently large. Transcriptional or translational delays probably become negligible when the period of the oscillations becomes very long, as in the case of circadian rhythms. The

incorporation of delays may be important, but the modeller pays a price as they make the numerical integration of the equations more cumbersome.

An interesting point emphasized by these two studies [5,6] is the likelihood of the wide occurrence of oscillatory gene expression resulting from transcriptional delays in regulated genetic networks. The question is: why have such oscillations not been reported more often? A possible explanation is that, given the possibility of cell desynchronisation, it might be necessary to resort to measurements in individual cells to uncover further evidence for the occurrence of oscillatory gene expression. The results of these delay-driven oscillator models show that it might be critical to incorporate delays in the description of genetic regulatory networks. This is important for predicting the impact of time delays on both the dynamic behavior of these networks and the parameter values predicted by the models.

References

1. Goldbeter, A. (2002). Computational approaches to cellular rhythms. Nature *420*, 238-245.
2. Chen, K.C., Csikasz-Nagy, A., Gyorffy, B., Val, J., Novak, B., and Tyson, J.J. (2000). Kinetic analysis of a molecular model of the budding yeast cell cycle. Mol. Biol. Cell *11*, 369-391.
3. Pourquie, O. (1998). Clocks regulating developmental processes. Curr. Opin. Neurobiol. *8*, 665-670.
4. Pourquie, O. (2003). The Segmentation clock: converting embryonic time into spatial pattern. Science *301*, 326-328.
5. Lewis, J. (2003). Autoinhibition with transcriptional delay: a simple mechanism for the zebrafish somitogenesis oscillator. Curr. Biol. this issue.
6. Monk, N.A.M. (2003). Oscillatory expression of Hes1, p53 and NF-kB driven by transcriptional time delays. Curr. Biol. this issue.
7. Morales, A.V., Yasuda, Y., and Ish-Horowicz, D. (2002). Periodic Lunatic fringe expression is controlled during segmentation by a cyclic transcriptional enhancer responsive to Notch signaling. Dev. Cell *3*, 63-74.
8. Dale, J.K., Maroto, M., Dequeant, M.L., Malapert, P., McGrew, M., and Pourquie, O. (2003). Periodic Notch inhibition by Lunatic Fringe underlies the chick segmentation clock. Nature *421*, 275-278.
9. Jiang, Y.J., Aerne, B.L., Smithers, L., Haddon, C., Ish-Horowicz, D., and Lewis, J. (2000). Notch signalling and the synchronization of the somite segmentation clock. Nature *408*, 475-479.
10. Holley, S.A., Julich, D., Rauch, G.J., Geisler, R., and Nusslein-Volhard, C. (2002). her1 and the notch pathway function within the oscillator mechanism that regulates zebrafish somitogenesis. Development *129*, 1175-1183.
11. Oates, A.C., and Ho, R.K. (2002). *Hairy/E(spl)-related* (*Her*) genes are central components of the segmentation oscillator and display redundancy with the Delta/Notch signalling pathway in the formation of anterior segmental boundaries in the zebrafish. Development *129*, 2929-2946.
12. Takke, C., and Campos-Ortega, J.A. (1999). *her1*, a zebrafish pair-rule like gene, acts downstream of notch signalling to control somite development. Development *126*, 3005-3014.
13. Henry, C.A., Urban, M.K., Dill, K.K., Merlie, J.P., Page, M.F., Kimmel, C.B., and Amacher, S.L. (2002). Two linked *hairy/Enhancer of split*-related zebrafish genes, *her1* and *her7*, function together to refine alternating somite boundaries. Development *129*, 3693-3704.
14. Palmeirim, I., Henrique, D., Ish-Horowicz, D., and Pourquié, O. (1997). Avian hairy gene expression identifies a molecular clock linked to vertebrate segmentation and somitogenesiss. Cell *91*, 639-648.
15. Artavanis-Tsakonas, S., Rand, M.D., and Lake, R.J. (1999). Notch signalling: cell fate control and signal integration in development. Science *284*, 770-776.
16. Leloup, J.C., and Goldbeter, A. (2003). Toward a detailed computational model for the mammalian circadian clock. Proc. Natl. Acad. Sci. U.S.A. *100*, 7051-7056.
17. Aulehla, A., Wehrle, C., Brand-Saberi, B., Kemler, R., Gossler, A., Kanzler, B., and Herrmann, B.G. (2003). Wnt3a plays a major role in the segmentation clock controlling somitogenesis. Dev. Cell 4, 395-406.
18. Hirata, H., Yoshiura, S., Ohtsuka, T., Bessho, Y., Harada, T., Yoshikawa, K., and Kageyama, R. (2002). Oscillatory expression of the bHLH factor Hes1 regulated by a negative feedback loop. Science *298*, 840-843.
19. Lev Bar-Or, R., Maya, R., Segel, L.A., Alon, U., Levine, A.J., and Oren, M. (2000). Generation of oscillations by the p53-Mdm2 feedback loop: a theoretical and experimental study. Proc. Natl. Acad. Sci. U.S.A. *97*, 11250-11255.
20. Hoffmann, A., Levchenko, A., Scott, M.L., and Baltimore, D. (2002). The IkappaB-NF-kappaB signaling module: temporal control and selective gene activation. Science *298*, 1241-1245.