Clocked gene expression in somite formation

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Summary

A recent paper[1] describes a striking expression pattern during somite formation for a chick ortholog of the fly *hairy* gene. Before segmentation, *c-hairy1* mRNA oscillates in the presomitic mesoderm such that three distinct spatial patterns are seen. The authors use a series of ingenious manipulations to show that these phases follow each other in time, adding up to a 90-minute periodicity in *c-hairy1* expression. The discovery of this clock of gene expression emphasizes the importance of temporally regulated events in the establishment of spatial patterns. *BioEssays* **20**:528–531, 1998. © 1998 John Wiley & Sons, Inc.

Different modes of segmentation

The body axis of most higher organisms is made up of serially repeated elements, or segments. In *Drosophila*, where segmentation is set up by gradual subdivision of the blastoderm without an increase in size, most of the gene products regulating this process have been identified. They form a hierarchy initiated from gradients of maternal gene products, then zygotic genes are activated in progressively smaller domains (originally classified into gap, pair-rule, and segment-polarity gene classes),[2] and finally each domain acquires a unique combination of gene expression that defines its identity. The early events in this cascade occur within the continuous cytoplasm of the egg and syncytial blastoderm, and it is therefore not surprising that many of these genes encode transcriptional activators and repressors.[3–6]

Other invertebrates (including annelids and most other arthropods) as well as vertebrates use an apparently different strategy, whereby the body segments are generated at the caudal end of a multicellular embryo.[7–9] In higher vertebrate embryos, the somites form as epithelial balls of cells every 90 minutes, budding from the rostral ends of bilateral rods (the segmental plates) of mesenchymal cells that flank the neural tube.[9,10] The number of somites formed has, therefore, been used extensively as an accurate method of staging embryos, but we still know very little about some of the most fundamental aspects of the biology of somite formation. What mechanisms ensure that each species always produces the same number of somites, despite great variation between species?[10] How do animals regulate the number of cells that come together to form each somite (which in turn determines its size)? How is the speed of somite formation controlled such that the left and right sides of the embryo develop coordinately and keep pace with the development of neighboring structures? How are regional differences specified such that somites in different parts of the axis develop into their appropriate cervical, thoracic, lumbar, etc., derivatives? How is the spatial periodicity of the somites coordinated with the segmentation of other tissues (the segmental blood vessels, motor nerves, and sensory and sympathetic ganglia)?

We know just as little about the gene products involved in the regulation of somite formation, and, given the differences in their biology, it was no surprise that true vertebrate orthologs of the maternal and gap genes expressed in the segmenting mesoderm could not be found.

Vertebrate ortholog of the pair-rule gene *hairy*, with segment-polarity-like expression

Palmeirim and colleagues[1] used RT-PCR to isolate a chick ortholog of the *Drosophila* pair-rule gene *hairy*, which encodes a bHLH transcriptional repressor of the *hairy/Enhancer of Split* (HES) family.[5,6,11] Unlike a recently identified zebrafish ortholog of *hairy* (*Her1*), which is expressed in a pair-rule like manner in alternating somite primordia[12] the new chick ortholog is expressed more like a segment-polarity gene, in the posterior (caudal) half of each somite from the time of its formation.[1]

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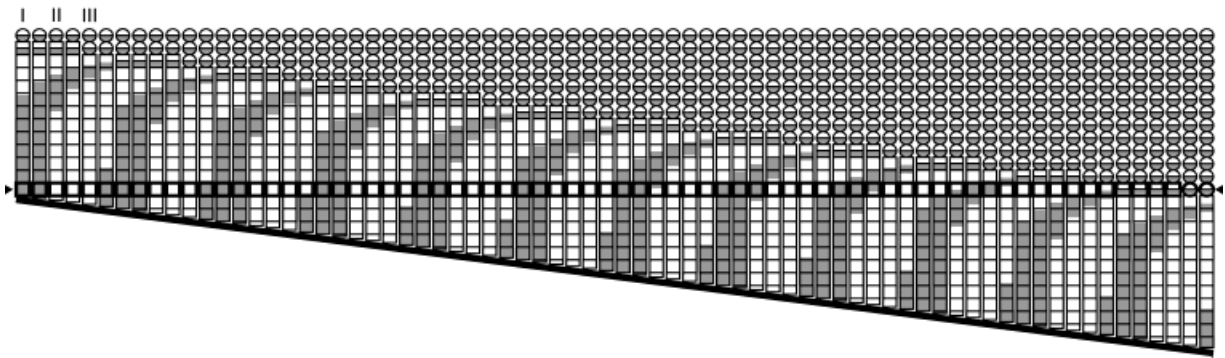
Figure 1. Schematic diagram showing *c-hairy1* expression pattern in the segmental plate over an 18-hour period (from the time a cell enters the segmental plate to the time it becomes incorporated in the newly forming somite). During the 18-hour period, a total of 12 somites are produced. Each column represents the mesoderm of the same embryo at 15-minute intervals. Somites are shown as circles, and the segmental plate is subdivided into squares, each representing a prospective somite. Somites form every 90 minutes (six columns) from the anterior (top) end of the segmental plate. At the same time, cells leave the primitive streak (shown as a thick line along the bottom of the figure) and are continuously added to the caudal (bottom) end of the segmental plate. *c-hairy1* is expressed (gray shading) in the posterior halves of the somites and as a wave that progresses anteriorly through the segmental plate. One row of squares is shown in bold; following this row from left to right, the history of this particular prospective somite can be followed. Cells in the upper part of this territory undergo 10 pulses of *c-hairy1* expression and eventually become part of the anterior half of the somite, whereas cells in the lower part undergo 11 pulses of expression, and the last pulse is maintained as cells become incorporated in the posterior half of the same somite. Note that cells enter the segmental plate at the correct phase of the *c-hairy1* expression cycle. The roman numerals indicate the three types of patterns that Palmeirim and colleagues[1] used to classify different stages of c-hairy expression. A moving computer simulation of this expression pattern by Dr. Julian Lewis may be viewed at the website: http://www.cell.com/supplemental/91/5/639/

Periodic expression of *c-hairy1* before somite formation

By whole-mount in situ hybridization, Palmeirim and colleagues[1] noticed that the pattern of *c-hairy1* expression in the segmental plate was not always identical even in embryos with the same number of somites. They observed variations that could be classified into three types of patterns (Fig. 1) with about equal frequency, but both sides of the embryo always display the same pattern. They reasoned that this variation could be due to dynamic changes in gene expression, in which the three patterns observed would follow each other within the time required to generate one somite (90 minutes). To test this, they designed an ingenious experiment. They fixed one side of the embryo and cultured the other side for 30, 60, 90, and up to 180 minutes. By comparing the pattern of expression on the two sides, they confirmed that each apparent phase of expression exists for about 30 minutes, that the phases follow each other in time, and that all three phases together make up a cycle of 90 minutes. They then demonstrate that these changes in gene expression are not due to cell movements by labeling cells with a fluorescent dye and comparing the patterns of expression with the position of the labeled cells after 30 minutes. Therefore, *c-hairy1* transcripts oscillate in the presomitic

mesoderm with a 90-minute period, before acquiring stable expression in the caudal half of each somite as it forms.

c-hairy1 clock as seen by prospective somite cells

During segmentation, prospective somite cells are generated from the remnants of the primitive streak at the tail of the embryo. These cells remain in the segmental plate for about 18 hours before becoming part of a somite. During this time, 12 somites form sequentially by budding from the anterior end of the segmental plate, whereas more cells are continuously added to the posterior end.[9] Figure 1 shows a representation of *c-hairy1* expression as it may be experienced by cells after they leave the primitive streak and become displaced anteriorly along the segmental plate. Each cell experiences 10 pulses of *c-hairy1* expression before reaching the tip of the segmental plate. At that point the expression of *c-hairy1* stabilizes: cells in the anterior half of the next budding somite stop expressing *c-hairy1*, whereas cells in the posterior half turn it on for the 11th time and maintain its expression thereafter. The reason for the "disappearance" of one *c-hairy1* oscillation could be due to an expression clock with a cycle slightly longer than 90 minutes, or with a cycle that gradually slows down as the cell

progresses along the segmental plate. An additional observation that becomes apparent from the representation in Figure 1 is that some cells enter the segmental plate and start expressing *c-hairy1* immediately, whereas others enter at the low point of *c-hairy1* expression. This finding raises the possibility that the clock that drives *c-hairy1* expression in the segmental plate is already operating in the primitive streak.

Models and functional speculations

For many years, developmental biologists have been fascinated by the problem of how vertebrate embryos regulate somite number, size, and the timing of their formation. Based on the results of teratology and experimental manipulations, several models have been proposed.[13–19] Of these models, Cooke and Zeeman[13] were the first to propose a cellular clock, and that this clock interacts with a slowly progressing wave that moves along the axis of the embryo. Interaction between the wave and the oscillator were proposed to allocate cells to individual somites in regular fashion along the axis.

But the findings of Palmeirim et al.[1] are strikingly reminiscent of a different model, proposed by Meinhardt.[14,15] He suggested that overt somite formation is preceded in the segmental plate by regular oscillations ("like the pendulum of a grandfather clock") between two states that will eventually correspond to the anterior (rostral) and posterior (caudal) halves of the future somites, and that these oscillations stop when a spatial alternation of anterior and posterior half-segments is generated. Meinhardt predicted: "this model would obtain strong support if the postulated oscillations in the mesoderm before somite formation could be detected. One full cycle of this oscillation should take precisely the same time as that required for the formation of one somite . . ." (ref. 15, page 188). This is exactly what Palmeirim et al.[1] observed: *c-hairy1*, which is a marker for the posterior (caudal) half of the somite after it forms, oscillates in the segmental plate with a period similar to that required for one somite to form, and the oscillations stop, generating stripes in alternating somite halves, when somite formation occurs.

It is indeed very tempting to speculate that these oscillations in *c-hairy1* expression in the segmental plate are somehow connected with the mechanism responsible for allocating cells to individual somites before their formation, as suggested in models like Cooke and Zeeman's, Meinhardt's, or others. They could also represent part of the mechanism that establishes the identity of cells in one or the other half of the future somite and, as Meinhardt suggested, this process could be inextricably linked with the formation of boundaries between somites. It is also possible that the oscillations in *c-hairy1* expression are part of a clock that contributes to impart somite cells with information about their position along the rostrocaudal axis of the whole embryo, leading to the acquisition of region-specific characteristics (such as the shape of cervical, thoracic, etc., vertebrae).

What drives the clock?

One of the challenges for the future will be to uncover the mechanism that drives the clock. Although the WRPW sequence in c-Hairy1 suggests that it is a transcriptional repressor,[5,6] Palmeirim et al.[1] argue that c-Hairy1 protein itself is not the clock mechanism because inhibition of protein synthesis with cycloheximide does not prevent the oscillations of gene expression, at least over a 60-minute period. One might look for some of the transcription factors that regulate the expression of *Drosophila hairy*, such as Bicoid, Caudal, Giant, Hunchback, Krüppel, Knirps, or Tramtrack. Apart from *caudal*, none of these correspond directly to known vertebrate genes, and none of the *caudal* orthologs identified so far are expressed in the somitic mesoderm or its precursors.

Lessons to be learned

The study of Palmeirim et al.[1] highlights how gene expression during development is probably much more dynamic than is often assumed based on the techniques generally used to study it. Despite the obvious advantages of techniques such as RT-PCR, its abuse can conceal both the spatial and the temporal features. Even in situ hybridization and immunohistochemistry, which are able to reveal the subtlety of spatial organization of domains of gene expression, can lead to a static view and to the false assumption that expression of a particular gene by a cell is an indication of its fate. Most recently, new methods for visualizing changes in gene expression with fine spatial and temporal resolution in the living embryo have been introduced, such as the expression of fluorescent proteins or substrates for reporter genes, but their use is still limited to very early stages of development in higher organisms.

The results of Palmeirim et al.,[1] therefore, offer several lessons. They include reminders that development is dynamic, that gene expression patterns are not necessarily markers of cell lineage or fate, that theoretical models for developmental processes do sometimes make useful predictions, and how much more there is to learn about the mechanisms that generate pattern and form in development.

References

1 Palmeirim I, Henrique D, Ish-Horowicz D, Pourquié O (1997) Avian *hairy* gene expression identifies a molecular clock linked to vertebrate segmentation and somitogenesis. *Cell* **91**:639–648.
2 Nüsslein-Volhard C, Wieschaus E (1980) Mutations affecting segment number and polarity in *Drosophila. Nature* **287**:795–801.

3 Akam M (1987) The molecular basis for metameric pattern in the Drosophila embryo. *Development* **101**:122.

4 Tautz D, Sommer RJ (1995) Evolution of segmentation genes in insects. *Trends Genet* **11**:23–27.

5 Fisher AL, Ohsako S, Caudy M (1996) The WRPW motif of the hairy-related basic helix-loop-helix repressor proteins acts as a 4-aminoacid transcription repressor and protein-protein interaction domain. *Mol Cell Biol* **16**:2670–2677.

6 Jiménez G, Pinchin SM, Ish-Horowicz D (1996) In vivo interactions of the *Drosophila* Hairy and Runt transcriptional repressors with target promoters. *EMBO J* **15**:7088–7098.

7 Weisblat DA, Wedeen CJ, Kostriken RG (1994) Evolution of developmental mechanisms: Spatial and temporal modes of rostrocaudal patterning. *Curr Top Dev Biol* **29**:101–134.

8 Stern CD (1990) Two distinct mechanisms for segmentation? *Sem Develop Biol* **1**:109–116.

9 Keynes RJ, Stern CD (1988) Mechanisms of vertebrate segmentation. *Development* **103**:413–429.

10 Richardson MK, Allen SP, Wright GM, Raynaud A, Hanken J (1998) Somite number and vertebrate evolution. *Development* **125**:151–160.

11 Paroush Z, Finley RLJ, Kidd T, Wainwright SM, Ingham PW, Brent R, Ish-Horowicz D (1994) Groucho is required for *Drosophila* neurogenesis, segmentation, and sex determination and interacts directly with hairy-related bHLH proteins. *Cell* **79**:805–815.

12 Müller M, v Weizsäcker E, Campos-Ortega JA (1996) Expression domains of a zebrafish homologue of the *Drosophila* pair-rule gene *hairy* correspond to the primordia of alternating somites. *Development* **122**:2071–2078.

13 Cooke J, Zeeman EC (1976) A clock and wavefront model for control of the number of repeated structures during animal morphogenesis. *J Theor Biol* **58**:455–476.

14 Meinhardt H (1982) *Models of Biological Pattern Formation.* London: Academic Press.

15 Meinhardt H (1986) Models of segmentation. In R. Bellairs, D.A. Ede, and J.W. Lash (eds): *Somites in Developing Embryos.* NATO ASI Series 118. New York: Plenum Press, pp. 179–189.

16 Bellairs R, Veini M (1984) Experimental analysis of control mechanisms in somite segmentation in avian embryos: II. Reduction of material in the gastrula stages of the chick. *J Embryol Exp Morphol* **79**:183–200.

17 Stern CD, Fraser SE, Keynes RJ, Primmett DRN (1988) A cell lineage analysis of segmentation in the chick embryo. *Development* **104 Suppl**:231–244.

18 Primmett DRN, Norris WE, Carlson GJ, Keynes RJ, Stern CD (1989) Periodic segmental anomalies induced by heat-shock in the chick embryo are associated with the cell cycle. *Development* **105**:119–130.

19 Polezhaev AA (1992) A mathematical model of the mechanism of vertebrate somitic segmentation. *J Theor Biol* **156**:169–181.