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# A mechanical perspective on vertebral segmentation

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ABSTRACT

Segmentation is a characteristic feature of the vertebrate body plan. The prevailing paradigm explaining its origin is the 'clock and wave-front' model, which assumes that the interaction of a molecular oscillator (clock) with a traveling gradient of morphogens (wave) pre-defines spatial periodicity. While many genes potentially responsible for these processes have been identified, the precise role of molecular oscillations and the mechanism leading to physical separation of the somites remain elusive. In this paper we argue that the periodicity along the embryonic body axis anticipating somitogenesis is controlled by mechanical rather than bio-chemical signaling. Using a prototypical model we show that regular patterning can result from a mechanical instability induced by differential strains developing between the segmenting mesoderm and the surrounding tissues. The main ingredients of the model are the assumptions that cell–cell adhesions soften when overstretched, and that there is an internal length scale defining the cohesive properties of the mesoderm. The proposed mechanism generates a robust number of segments without dependence on genetic oscillations.



## 1. Introduction

Segmentation, the repetitive division of the body axis in modular units, is a ubiquitous motif in biology (Bhat & Newman, 2009; Cooke, 1988, Ten Tusscher, 2013). In vertebrates, segmentation is established early in embryogenesis by the formation of somites, blocks of tissue that bud off periodically from the anterior part of the pre-somitic mesoderm (PSM). Somites are transient structures that eventually give rise to a variety of tissues, including the spine, skeletal muscles, and the dorsal skin (Brent & Tabin, 2002; Christ, Huang, & Scaal, 2007). Unveiling the mechanism of somite formation is one of the major challenges in developmental biology (Bénazéraf & Pourquié, 2013; Dias, de Almeida, Belmonte, Glazier, & Stern, 2014; Herrgen et al., 2010; Hester, Belmonte, Gens, Clendenon, & Glazier, 2011; Pourquié, 1999, 2011; Stern & Vasiliauskas, 1999).

The process by which the somites are formed can be viewed as a subdivision of an initially continuous cylinder into a row of separate blocks. Segmentation appears as a sequential self-slicing and an adequate theory of somite formation must provide an explanation for the physical process of cell clustering and cleavage. These mechanical phenomena are driven by internally generated active tractions. Externally driven processes of this type are ubiquitous in non-animate Nature with

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formation of cracks in drying mud and stress induced fracturing of coating films as some of the most well known examples (Hutchinson & Suo, 1992).

The prevailing paradigm for vertebrate segmentation, does not address the issue of cleavage and mechanical separation is viewed as of secondary importance. It is believed that the principal role is played by a cellular oscillator which interacts with a traveling wave of morphogens and in this way produces a periodic biochemical pattern (Baker & Schnell, 2009; Cooke & Zeeman, 1976, Meinhardt, 2008; Murray, Maini, & Baker, 2011; Rué & Garcia-Ojalvo, 2013). The underlying biochemical mechanism, known as the 'clock and wave-front' model (see Fig. 1), has been substantiated by the identification of both: genes that oscillate (Li, Fenger, Niehrs, & Pollet, 2003; Palmeirim, Henrique, Ish-Horowicz, & Pourquié, 1997; Schröter et al., 2012), and diffusion gradients of morphogens that propagate along the body axis (Dubrulle & Pourquié, 2002; Kicheva, Bollenbach, Wartlick, Jülicher, & Gonzalez-Gaitan, 2012). The long range synchronization issue for independent genetic oscillators has also been addressed and various components have been integrated into a comprehensive network model (Baker, Schnell, & Maini, 2008; Goldbeter & Pourquié, 2008; Hester et al., 2011). Even though the 'clock and wave-front' model does not specify how the finite blocks of cells undergo synchronized consolidation into somites, it is supported by the observations that mutations to some of the proposed genetic candidates alter the period of somitogenesis and affect the total number of somites in the body (Harima et al., 2013; Herrgen et al., 2010; Kim et al., 2011; Schröter et al., 2012).

The ambiguity, however, remains because it has not been yet possible to smoothly and predictably tune the period of the proposed pacemakers. More importantly, the 'clock and wave-front' mechanism appears to be incompatible with some experimental observations (Kondo, 2014). In particular, it does not explain why despite a nearly twofold fluctuation in the overall size of the presomitic mesoderm during embryonic development, a relatively constant number of somitomeres is found in tandem sequences: these observations suggest that without any changes in the temporal periodicity, the spatial scale of somites can be affected by the size of the PSM (Tam, Meier, & Jacobson, 1982). It is also alerting that the 'clock and wave-front' mechanism does not rely on the concommitency of somitogenesis and the elongation of the body axis (Gomez et al., 2008).

In this paper we discuss an alternative *hypothesis* that somitogenesis is largely driven by the mechanical stresses induced by growth and active contraction (cf. Beloussov, 2001). We developed a simple model showing how the emerging periodicity can result from mechanical self-organization. Our model suggests that the hypothetical segmentation clock invoked in the 'clock and wave-front' mechanism may have *spatial* rather than *temporal* nature.

The paper is organized as follows. In Section 2 we review the experimental evidence showing that mechanical signaling plays an important role during vertebrate segmentation. Various theoretical approaches to the mechanical modelling of morphogenetic instabilities are discussed in Section 3. Our mathematical model is formulated in Section 4, where we also review the related work in non-biological setting. The linear problem capturing the pre-patterning stage of the somitogenesis process is discussed in Section 5. Some remarks about the actual separation of somites are collected in Section 6. Finally, our conclusions and some future perspectives are presented in Section 7.

## 2. Mechanical signaling

A general limitation of the 'clock and wave-front' mechanism is that it focuses exclusively on genes and biochemical pathways, thereby neglecting the mechanical stresses in the growing embryo. It is well known, however, that cells can extract as much information from the mechanical cues as they do from diffusing factors (Mammoto & Ingber, 2010; Schwarz & Safran, 2013). It would be then rather natural for the embryo to employ mechanical forces as long-range communication means to guide morphogenesis and to trigger the appropriate response of the genome (Beloussov, 2012; Davidson et al., 2010). This

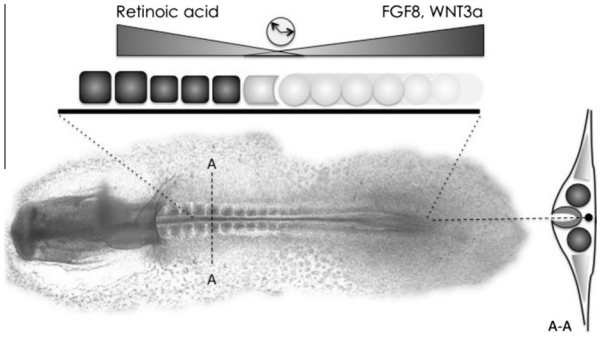


**Fig. 1.** Convenional picture of the vertebral segmentation in a chicken embryo. Bottom: Physical image of somites (black blocks) sequentially budding off from the PSM. The growing notochord is shown by the black line and by the dot in the cross section A-A. Within the non-differentiated PSM the incipient periodic pattern can be readily identified with somitomeres appearing as white blocks. Top: Schematics of the 'clock and wave-front' mechanism. Cells at the growing tail produce FGF8 and WNT3a signaling which keep them in a non-differentiated state. Retinoic acid, produced by the newly formed somites, facilitates differentiation into epithelial cells. This morphogenetic profile is traveling from head to tail with a constant speed while the genetic oscillation clock at the moving differentiation front sets the boundaries of the somites.