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Modeling the morphodynamic galectin patterning network of the developing avian limb skeleton

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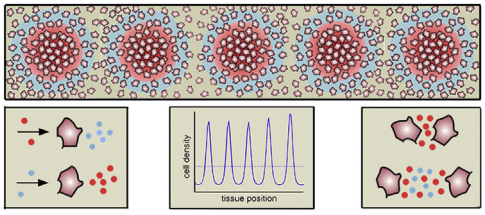
GRAPHICAL ABSTRACT



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ABSTRACT

We present a mathematical model for the morphogenesis and patterning of the mesenchymal condensations that serve as primordia of the avian limb skeleton. The model is based on the experimentally established dynamics of a multiscale regulatory network consisting of two glycan-binding proteins expressed early in limb development: CG (chicken galectin)-1A, CG-8 and their counterreceptors that determine the formation, size, number and spacing of the "protocondensations" that give rise to the condensations and subsequently the cartilaginous elements that serve as the templates of the bones. The model, a system of partial differential and integro-differential equations containing a flux term to represent local adhesion gradients, is simulated in a "full" and a "reduced" form to confirm that the system has pattern-forming capabilities and to explore the nature of the patterning instability. The full model recapitulates qualitatively and quantitatively the experimental results of network perturbation and leads to new predictions, which are verified by further experimentation. The reduced model is used to demonstrate that the patterning process is inherently morphodynamic, with cell motility being intrinsic to it. Furthermore, subtle relationships between cell movement and the positive and negative interactions between the morphogens produce regular patterns without the requirement for activators and inhibitors with widely separated diffusion coefficients. The described mechanism thus represents an extension of the category of activator–inhibitor processes capable of generating biological patterns with repetitive elements beyond the morphostatic mechanisms of the Turing/Gierer–Meinhardt type.

1. Introduction

The organization of cells and tissues into specific arrangements or patterns during embryogenesis, and the inheritance of these pattern-forming mechanisms, constitute important problems of both developmental and evolutionary biology (Müller et al., 2007). The patterning of the skeletal elements in vertebrate limbs is an experimental system within which these issues have received particular attention (Newman and Bhat, 2007).

The quasi-periodic arrangement of limb bones is well conserved across the tetrapods and consists of a progressive increase in element number along the proximal–distal axis (Saunders, 1948). Each skeletal element is preceded by a cartilage element,

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which in turn arises from condensations of limb mesenchymal cells (Hall and Miyake, 2000). The condensation of mesenchymal cells can also be observed in vitro in high-density micromass cultures. When precartilage mesenchymal cells are isolated from a developing chicken limb, dissociated and cultured at high densities on tissue culture plastic in serum-free conditions, they organize themselves into spot- or rod-like condensations of nearly uniform size and regularity of spacing surrounded by non-aggregated cells (Downie and Newman, 1994; Kiskowski et al., 2004; Christley et al., 2007). When packed into a limb bud ectodermal jacket the cells generate poorly formed, though discrete cartilaginous elements (Ros et al., 1994; Zwilling, 1964).

Aggregation results from random movement of cells occurring in an environment with local patches of increased adhesivity characterized by elevated levels of extracellular matrix (ECM) and adhesion molecules such as N-cadherin, NCAM, tenascin and fibronectin (Downie and Newman, 1995; Newman and Bhat, 2007). The determination of where the condensations form and where they do not, which determines the ultimate pattern of the skeleton, has proved to be a more difficult question.

The finding that regularly spaced condensations form from randomized cells in vitro, suggests that the mechanism of skeletal patterning is not dependent on stable gradients of diffusible molecules emanating from signaling centers as has been proposed in the form of the "positional information" hypothesis (Wolpert, 1969, 1989). Indeed, individual-based simulations of micromass cultures under experimentally constrained conditions (Kiskowski et al., 2004; Christley et al., 2007), analysis of the peculiarities of the limb skeletal patterns in certain mutant chicken embryos (Miura et al., 2006), and recently, examination of the response of skeletal pattern generation in mouse embryos in which Hox gene expression was manipulated in a semi-quantitative fashion (Sheth et al., 2012), all point to the underlying core mechanism of skeletogenesis being a reaction–diffusion-like process, as suggested earlier by Newman and Frisch (1979). This category of mechanism was originally discussed in chemical terms by Turing (1952), and in a biological context by Gierer and Meinhardt (1972), who characterized it as local autoactivation–lateral inhibition (LALI) (see Meinhardt and Gierer, 2000). The observation that the ECM molecule fibronectin is an important component of the condensation-associated adhesive patches and that one or more members of the TGF-β family of positively autoregulatory morphogens induces its synthesis, motivated mathematical and computational models of in vivo (Hentschel et al., 2004) and in vitro (Kiskowski et al., 2004; Christley et al., 2007) pattern formation, utilizing LALI mechanisms. (For exhaustive reviews on the mathematical models of limb pattern formation see Glimm et al., 2012; Newman et al., 2008; Zhang et al., 2013.)

The most general formulation of a Turing-type patterning process in limb bud mesenchyme is "morphodynamic" (in the sense of Salazar-Ciudad et al., 2003, in which cell–cell signaling and cell movement occur simultaneously), see Hentschel et al. (2004), Kiskowski et al. (2004) and Christley et al. (2007). However such a system is prohibitive to simulate in realistic geometries and thus a "morphostatic" approximation (where establishment of a stable "prepattern" of cell signals occurs on a faster time scale than cell movement, see Salazar-Ciudad et al., 2003) of the activator–inhibitor morphogen dynamics based on certain restrictive biological and mathematical assumptions (Alber et al., 2008) was used to explore some of its experimental, mutational and evolutionary properties (Zhu et al., 2010). It is unclear, however, to what extent this morphostatic assumption is justified in the developmental context. Moreover, the molecular identity of the putative LALI inhibitor in the TGF-β-fibronectin network has been elusive (Newman and Bhat, 2007).

Recently, in an attempt to clarify the identities of early acting determinants of precartilage condensations, Bhat et al. (2011) showed that two members of a class of glycan-binding proteins called galectins appear at the sites of prospective condensation in the developing chicken limb before any previously described condensation mediators such as fibronectin. These galectins are CG (chicken galectin)-1A and CG-8 (see also Lorda-Diez et al., 2011). Ectopic CG-1A induced supernumerary condensation formation in vitro and digit formation in vivo, both of which were inhibited by CG-8. What distinguishes the interaction of these gene products from other experimentally elucidated LALI networks is a mutually positive feedback loop exerted by the proteins on each other's gene expression with the inhibitory effect exerted at a different biological level, protein–protein interaction (Bhat et al., 2011). In addition, CG-1A induces the expression of a shared counterreceptor. (A more detailed review is given in Section 2.1.) A relevant question is whether the demonstrated interactions were sufficient to give rise to the characteristic condensation pattern or if additional components or interactions are required.

The purpose of this paper is to construct a mathematical model that incorporates the interactions of CG-1A and CG-8 multilevel regulatory network to explore their ability to form spatial patterns of condensations. We verify that this mathematical model does indeed reproduce the experimental findings, and in the process, gives rise to a condensation-like pattern. The model provides additional crucial insights into the pattern formation from a physical perspective: we show that the limb skeletal patterning is a morphodynamic process (as opposed to morphostatic one) and is thus dependent on mesenchymal cell motility. We also confirm the predictive potential of the model by verifying experimentally an important in silico finding: that abrogation in the interaction of both chicken galectins to their counterreceptors results in loss of pattern formation. A number of explicit predictions of the model for further experimental tests are listed in Section 5 at the end of this paper.

The mathematical model and its experimental validation described below establish a novel "dynamical patterning module" (Newman and Bhat, 2008), a LALI-type mechanism for which cell movement is an intrinsic component. This mechanism underlies spatial patterning of the avian limb skeleton, but may also be relevant to the generation of other spot-like or rod-like biological patterns in animal development.

2. Materials and methods

2.1. Developmental model

In this section we present the key biological findings that we implement in our mathematical model in order to test their ability to give rise to spatial patterns characteristic of precartilage condensations in culture.

Biological assumptions motivated by the experimental findings:

1. Limb mesenchymal cells move randomly with a constant diffusion rate unless their surface adhesive properties change.
2. All cells produce CG-1A, CG-8 and their respective counterreceptors.
3. CG-1A induces CG-8 gene expression; CG-8 induces CG-1A gene expression.
4. CG-1A induces enhanced binding activity of the shared counterreceptor, presumably via upregulation of protein expression of the shared counterreceptor.
5. CG-1A promotes cell–cell adhesion in a cellular suspension in the order of minutes.
6. Cell movement continues but becomes confined within condensations.

Detailed experimental justifications for these biological assumptions are presented by Bhat et al. (2011). The following assertions