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3D Multi-scale Modeling Of Early Stage Chick Limb Development

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Limb development has been one of the focus of research in the US since the Thalidomide disaster of the 1950s and 60s, in which children of mothers who took the drug for morning sickness, were born with truncated limbs. This was a major spur to modern developmental toxicity regulation. First step in addressing the toxicity is to thoroughly understand the regulatory processes involved in the limb development. Experimental evidence from both the mouse and chick model systems has contributed greatly to our understanding of the developmental processes involved. In embryonic chicks, the wing buds begin to form as a slight swelling in the lateral body wall, adjacent to the 15th through the 20th somites, at Hamburger-Hamilton (HH) stage 16 [1]. The growth results from an increase in the proliferation of limb bud mesenchyme relative to the surrounding flank mesenchyme[2] (Figure 1A). Recent evidence suggests that fibroblast growth factors (FGFs) secreted by a specialized ectodermal tissue called "Apical Ectodermal Ridge" (AER), play a major role in promoting cell proliferation of mesenchyme [3]. AER is a pseudo-stratified epithelial tissue that is formed at the interface of the *fringe* expressing dorsal ectoderm and *fringe* non-expressing ventral ectoderm via Notch-Delta ligand formation[4]. From HH stages 23-24, the limbs grow into asymmetrical "paddles".

In this paper we present a 3D multi-scale model of the early stage (HH16 to HH23) limb development showing the limb outgrowth using a multi-cell modeling platform Compucell3D (CC3D) (www.compucell3d.org)[5]. CC3D allows us to virtually represent cells using lattice points in space, for which cell-like properties can be defined. Our model is built upon three major components integrated together to give rise to overall limb outgrowth: (a) First, at stage HH16, the AER is formed through inter- and intra-cellular Notch-Delta signaling – modeled using ordinary differential equations (ODEs), (b) Growth and proliferation of the limb mesenchyme promoted by the FGF8, secreted by the newly formed AER – modeled using CC3D, and (f) Growth of ectoderm over limb mesenchyme – modeled with a specifically developed contact inhibition mechanism using CC3D.

(a) AER formation through Notch-Delta Signaling: Based on the literature, we have developed a mechanism (Figure 1B) and an ODE model through which inter-cellular Notch-Delta signaling gives rises to high Notch activity only at the boundary, transforming the normal ectodermal cells in to Apical Ectodermal cells. The ODE model is integrated with CC3D platform using BionetSolver [6].
(b) Growth and proliferation of limb mesenchyme: The formed AER secretes the morphogen FGF8. The FGF8 diffuses through the limb mesenchyme promoting proliferation. We simulate the diffusion of FGF8 using partial differential equations (PDEs). The mesenchyme is set to grow and multiply at a constant rate in response to an FGF8 gradient threshold.
(c)Growth of Ectoderm over mesenchyme: As the limb outgrows, the ectoderm also expands along with the growing mesenchyme. The ectoderm grows in an "as needed" basis when stretched by the underlying limb mesenchyme. We have developed a specific approach where the ectodermal cells grow based on the loss cell-cell contact area. The rate of growth of the ectodermal cell volume is set as in equation (1), which allows the ectoderm to grow over the mesenchyme without any tears, thereby covering the limb outgrowth.

$$\frac{dV_{cell}}{dt} = k_m \times \left(\frac{\alpha_c^{\,n}(1-\alpha^n)}{\alpha_c^{\,n}+\alpha^n} \right) \times \left(\frac{\beta^m}{\beta_c^{\,m}+\beta^m} \right) \left(\frac{\beta_c^{\,m}+0.5^m}{0.5^m} \right) \quad (1)$$

$$\alpha = \frac{\text{Ectoderm-Ectoderm contact area}}{\text{Ectoderm cell surface area}}, \beta = \frac{\text{Ectoderm-Mesenchyme contact area}}{\text{Ectoderm cell surface area}}$$

Results

By successfully integrating methods (a) through (c), we have computationally simulated the early development of chicklimb in CC3D platform (Figure 1C and D). We show the limb bud outgrowth along with the AER in two different views. We have used mesenchymal cell diffusion data as well as real cell cycle times to scale our simulations to the experiments in the literature. At stage HH23, the length of the limb bud in the proximodistal (PD) direction is equal to the length in the Anterior-Posterior (AP) direction. However, our simulations produce a limb outgrowth whose PD length is smaller than observed limb PD length, showing that there are mechanisms other than simple proliferation are involved. We then include cell-intercalation in the mesenchyme in addition to proliferation and show that the PD length of the limb increases compared to growth obtained using proliferation alone.

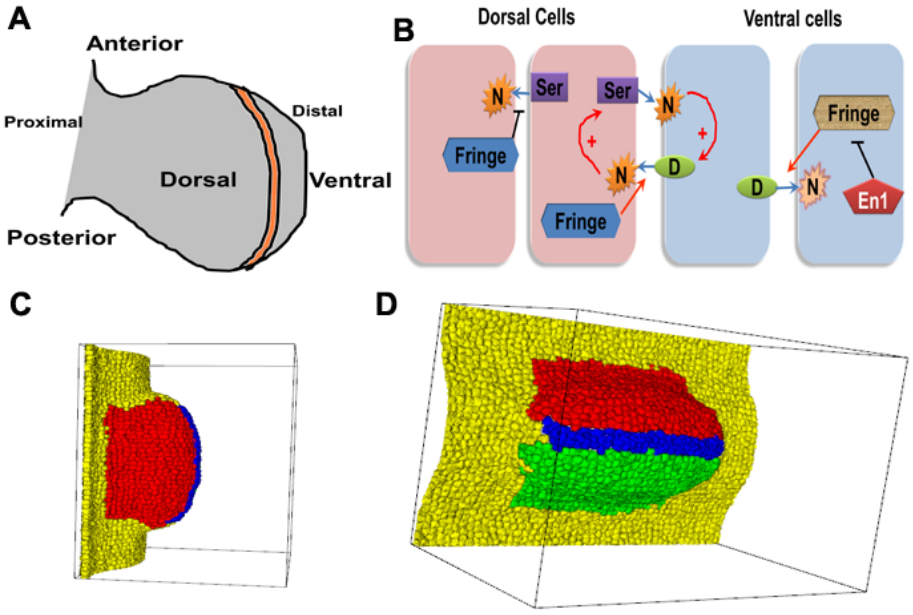

Figure 1 A. Illustration of limb. B. Mechanism for AER formation involving Fringe, Notch(N), Serrate (Ser) and Delta(D), C. Simulation showing proximo distal outgrowth of the limb. D. Alternate view of the simulation of limb bud showing AER (blue), dorsal ectoderm (red) and ventral ectoderm (green), and limb ectoderm (yellow).

Our model is the first multi-scale computational effort that depicts the complex fundamental processes involved in the early limb development including the inter- and intra-cellular signaling for the AER formation. Our model can be extended to contain complex signaling mechanisms and has the potential to be developed into a complex predictive model for toxicological studies.

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