A stochastic model of melanoblast colonisation

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Introduction

In mice melanoblast precursors delaminate from the neural crest at embryonic day 9.5 (E9.5) and upregulate melanoblast specific markers by E10.5. They migrate along the dorsolateral pathway between the somites and the developing epidermis and begin to localise to embryonic hair follicles around E15. The failure of melanoblasts to properly colonise the developing epidermis is associated with belly spotting phenotypes. In adults melanoblasts give rise to a population of melanocytes in the hair matrix and basal epidermis and melanocyte stem cells (MSCs) that reside in the bulge region of the follicle. We are integrating live-imaging and mathematical modeling to try and better understand the behavior of embryonic melanoblasts and explain the pigmentation defects seen in mouse mutants.

Methods

In order to fluorescently label the melanoblast lineage we combined *Tyr::Cre* mice with *R26YFPR* reporter animals. Embryonic skin from *Tyr::Cre/R26YFPR* embryos (E13.5-E15.5) is dissected and mounted in a custom 6-well culture apparatus. The skin is clamped epidermal side down against a gas permeable Lummox membrane, allowing the maintenance of an air-liquid interface (ALI) across the surface of the skin (**Fig. 1**). The chamber is then mounted on the stage of a Nikon A1R confocal microscope allowing live confocal imaging of melanoblast migration.

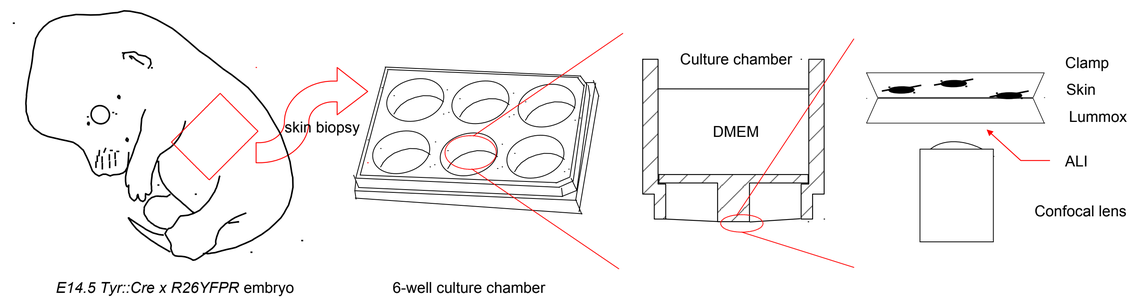
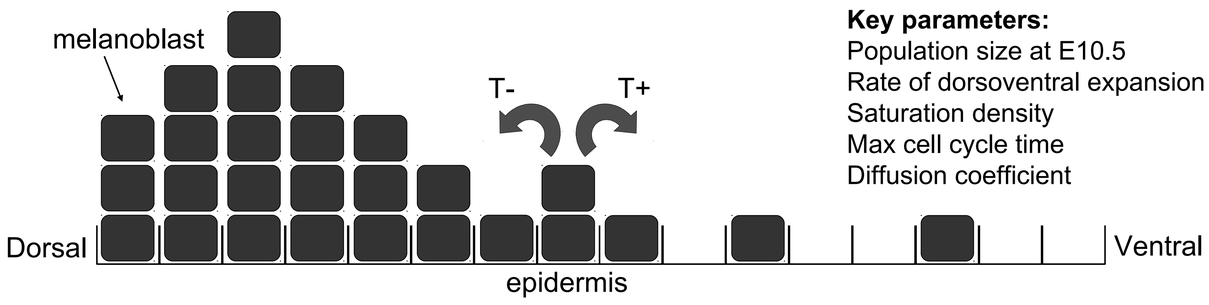


Figure 1: *Ex vivo* culture system for the live cell imaging of the melanoblast lineage.

Stochastic model of melanoblast migration

To better understand the behavior of he migrating melanoblast population we have developed a stochastic position jump model. In the model melanoblasts are assigned probabilities to move between boxes, proliferate and die. **(Fig. 2)**.



Key parameters:
Population size at E10.5
Rate of dorsoventral expansion
Saturation density
Max cell cycle time
Diffusion coefficient

Figure 2: Stochastic model of melanoblast colonisation. The domain is divided into boxes, to increase its length the number of boxes is increased.

Tissue expansion, cell density and number of progenitors

Analysis of whole embryos scanned using optical projection tomography allowed us to define a rate of dorsoventral expansion of **58 um/hour**. We used *Dct::LacZ* embryos to count the number of founder melanoblasts at E10.5 (**Fig. 3A-B**) and *Tyr::Cre/R26YFPR* embryos to calculate melanoblast densities between E12.5 and E15.5 and define a saturation density (**Fig. 3C**).

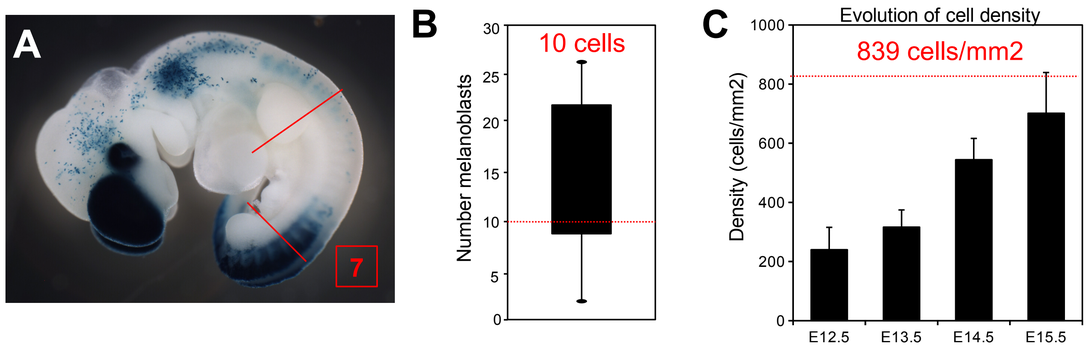


Figure 3: A: Example of a *Dct::LacZ* embryo at E10.5 showing 7 melanoblasts. B: Quantification of melanoblasts in the trunk at E10.5. C: Melanoblast density increases between E12.5 and E15.5.

The melanoblast population is diffusing

Using custom macros written for ImageJ we are able to automatically track the trajectories of the majority of cells in a time lapse movie allowing a detailed analysis of their behavior. From this data we are able to calculate velocities, trajectories and population based stats (**Fig. 4**).

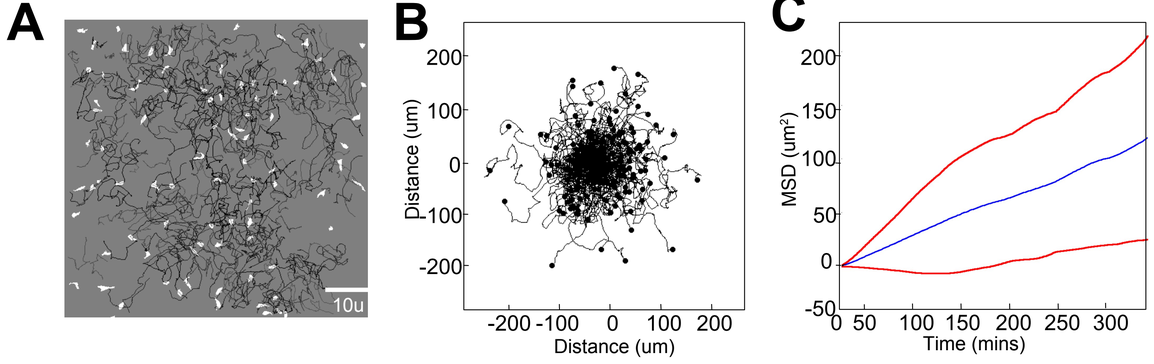


Figure 4: A: All the tracks generated over an 18-hour time lapse. B: A vector plot of the same data. C: Mean squared displacement (MSD) plotted against time. The slope of the graph is equal to the diffusion coefficient (D) of the population.

Velocity, diffusion and cell cycle are density dependent

Detailed investigation of time-lapse movies revealed that velocity, cell cycle time and the diffusion coefficient (D) are all density dependent (**Fig 5. A-C**). From this data we derived a diffusion coefficient that varied with density from 8 um^2 min^{-1} to 32 um^2 min^{-1}. We also defined a maximum possible cell cycle time of 6 hours.

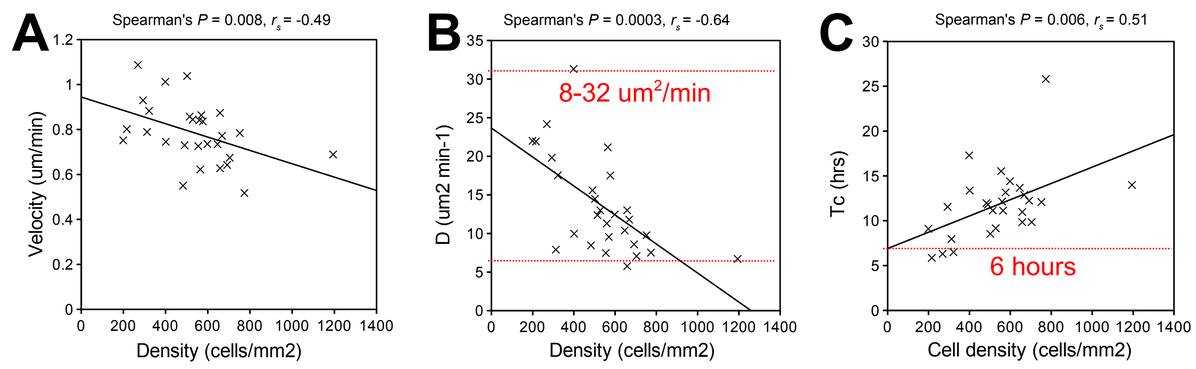


Figure 5: A: Mean cell velocity plotted against cell density per movie, there is a strong negative correlation B: D plotted against cell density, there is a strong negative correlation. C : Cell cycle time (Tc) plotted against cell density, there is a strong positive correlation.

Colonisation using a stochastic model

Figure 5 summarises the results using the following parameters:
Population size at E10.5 = **10 cells**
Rate of dorsoventral expansion = Linear **58 um/hr** (increase in number of boxes)
Saturation density = **839 cells/mm^2** (proliferation ceases)
Max cell cycle time = **6 hours** (but varies with density)
Diffusion coefficient = **8-32 um^2/min** (varying with density)

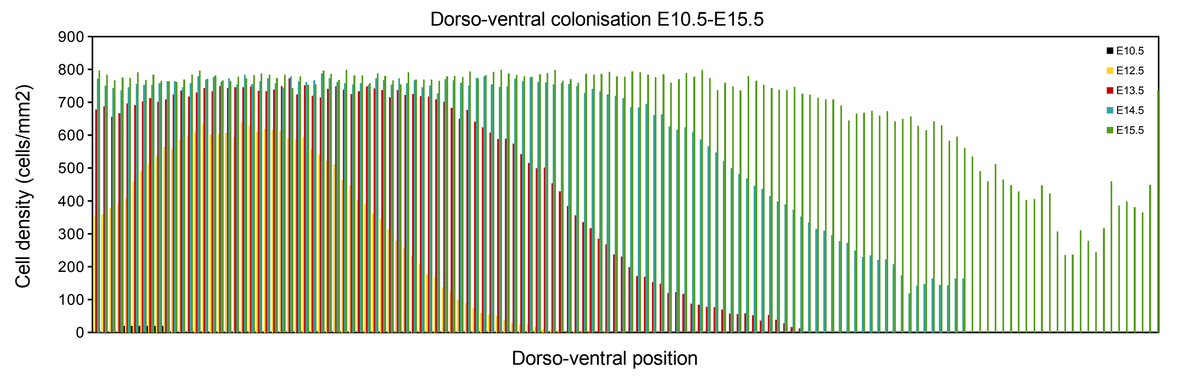


Figure 6: Modelling results averaged from 100 realisations at each time point. The 10 melanoblasts specified at E10.5 have expanded and the population has grown to fill the entire dorsoventral axis by E15.5.

Validation of stochastic model

We compared the data from our stochastic model with data measured from our biological system. The cell density profile and cell cycle times the model predicts agree closely with the measured data.

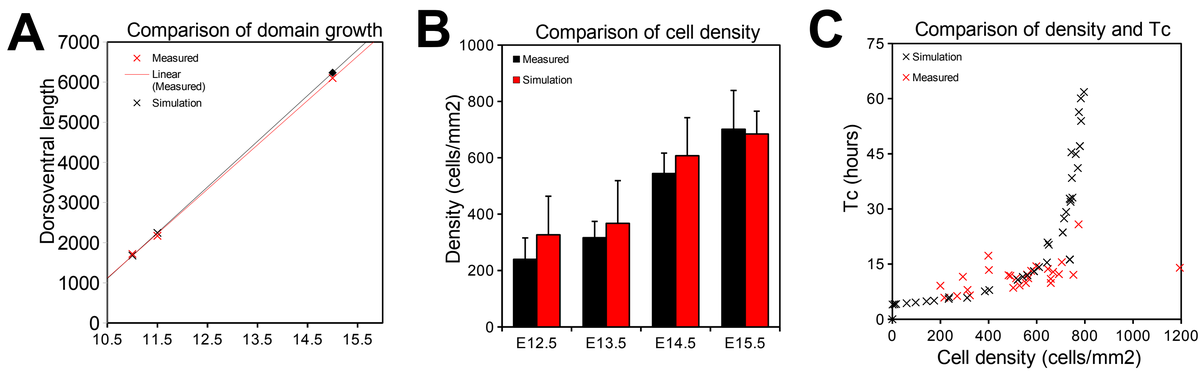


Figure 7: Validation of our stochastic model showing similar trends in tissue expansion, cell density profile and cell cycle time between the measured and simulated data.

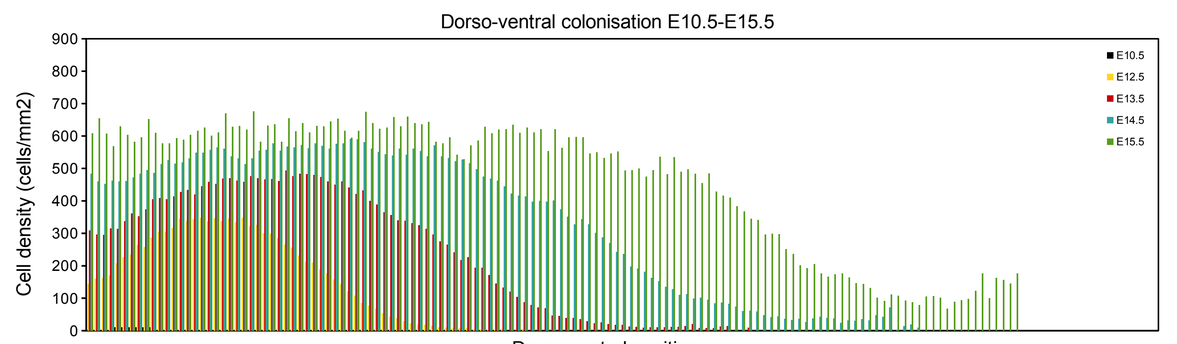


Figure 6: Modelling results averaged from 100 realisations at each time point. Colonisation fails if the maximum cell cycle time is increased from 6 to 10 hours.

Conclusions

We describe a stochastic model which allows us to integrate our measurements of the development of the melanoblast population. Using the parameters we have measured our model shows good colonisation of the dorsoventral domain. We show that by reducing proliferation in our model we can prevent full colonisation suggesting a possible mechanism for belly spot formation in mice.

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