Broad Impacts (3 pages)

Overview

Living cells can differentiate from one cellular state to another during growth, development, and regeneration. In higher organisms such as mammals, differentiation and proliferation create cells each with unique competence to either self-renew as stem/progenitor cells to maintain organismal/tissue homeostasis with desirable spatial patterns such as that in a stratified epidermis, or transiently proliferate and terminally differentiate to meet the needs of growth and regeneration of various cell types that carry out specialized biological functions at different spatial locations. How are proliferation and differentiation coordinately regulated? Are there regulatory themes that are common to all organisms in order to engineer desired cell differentiations and their spatial patterns?

This interdisciplinary proposal represents a joint effort between a computational mathematician with extensive collaborative experience in experimental biology, and an experimental biologist with prior collaborative experience in mathematical modeling. Specifically, we propose to use a systems biology approach integrating experiments with modeling/simulation to study the development of mammalian epidermal stem/progenitor cells during embryogenesis. The objective of the epidermal morphogenic process is to produce a stratified epidermis of a desired size composed of multiple cell types in proper proportion and spatial position from a single-layered primitive progenitor cells. A successful execution of the stratification process entails a precise and robust control of proliferation, differentiation, and migration, and must involve complex interactions between the extra-cellular environment and the intra-cellular regulatory networks. We will address how these interactions are integrated and disseminated at molecular and cellular levels to generate epidermis with a desirable size and spatial pattern.

One of our overall hypotheses is that a network of a small number of molecular and cellular components and regulations might be able to produce epidermis with correct size and patterns; however, the complexity of a network arises in order to achieve *robustness* (e.g. with respect to internal or environmental perturbations) of the development and growth process. Our work centers around Ovol1 and Ovol2, two homologous transcription factors with important roles in mammalian epidermal morphogenesis. We will develop new modeling and computational tools, delving into the known and proposed molecular networks encompassing Ovol1 and Ovol2, with the ultimate goal of rising above molecular details to unravel critical regulatory mechanisms that are likely to be generally applicable to multiple organisms and differentiation/developmental processes. We have two specific aims:

Aim 1: To investigate the role of Ovol1-Ovol2 mutual repression in controlling epidermal size and cell type proportion. Although expressed in different cellular compartments in developing epidermis, both Ovol1 and Ovol2 have a proliferation-suppressive role. They directly repress the expression of c-Myc, a proto-oncogene that promotes the proliferation of epidermal cells and has to be suppressed for terminal differentiation. Our preliminary modeling and experimental results suggest that a mutual repression between Ovol1 and Ovol2 produces bi-stability in c-Myc expression. We hypothesize that an Ovol1-Ovol2 mutual repression mechanism is required for robust epidermal morphogenesis by balancing proliferation with growth arrest, which is a prerequisite for terminal differentiation, and will perform modeling and experiments to test this hypothesis.

Aim 2: To investigate the complex molecular interactions that robustly control the spatial stratification of epidermis. Proliferation and differentiation are intimately linked to epidermal stratification. Although morphogen gradients may act globally through the proliferation module that contains the Ovol1-Ovol2 circuit to provide spatial information to cells for the stratification of epidermis, preliminary experimental data suggest that Notch signaling also plays an important role in epidermal stratification. We hypothesize that a cross-talk between the Ovol1-Ovol2-encompassing proliferation module network and a stratification module network is critical for the epidermal system to achieve several key performance objectives in spatial stratification, and will perform modeling and experiments to test this hypothesis.

Education, Outreach, and Diversity

For research at the interface between disciplines, education and outreach are particularly important and challenging. It is not just mathematics and biology that must be communicated to students, researchers, policy-makers and the public, it is also the range of possibilities that arise at the intersection of these fields that must be made known. Achieving these goals requires educational activities at all levels. Outreach must also be conducted to build the capacity of such training programs, and make effective use of the diversity in our nation's population. Indeed, the investigators are active participants of many existing education and outreach activities, and some of them are exactly at the interface of mathematics and biology.

Nie is currently the acting director and has been the associate director of an interdisciplinary Ph.D. program on "Mathematical and Computational Biology" (MCB) at UCI (http://mcsb.bio.uci.edu). He is also a regular instructor for one of the two core sequences of the MCB program. Dai is an actively participant of the MCB program, and has mentored mathematically trained MCB rotation students in their wet laboratory experience, as well as a biologically trained graduate student in her mathematic modeling collaboration. Recently, Nie gave a public lecture for 300 7^{th}–8^{th} graders and their parents in "Math Count", an annual Orange County mathematics contest, with a title of his presentation "Is Mathematics a New Microscope of Biology?". Dai presented scientific posters at Workshops during the Sally Ride Science Festival at UCI, designed to inspire and support 5^{th}-8^{th} grade girls' interests in science and math, and served as a judge at the Irvine Unified School District Science Fair. Both Nie and Dai have provided research opportunity for high school seniors and mentored undergraduate student research in their labs. Nie recently supervised an 11^{th} grader student, Brandon Sim, for a research project on modeling of cell lineages. The project won the Science Division of the 2010 *Southern California Science and Humanities Symposium* (http://www.cfep.uci.edu/jshs).
The unique combination of the researchers, students, and the research agenda in this proposal will present more and special opportunities for developing new activities in education and outreach at the interface of mathematics and biology.

Outreach to 4^{th}-6^{th} graders - Effective mathematics and science education has been recognized as a national need. A topic that combines mathematics, an abstract subject, and biology, a subject more easily accessible to students, may be particularly suitable and interesting for students. The PI and Co-PI have been frequent volunteers for classes of 4^{th}-6^{th} graders at a local public school, *Turtle Rock Elementary School*. We propose to give joint lectures to those 4^{th}-6^{th} graders, starting by helping to organize mathematics and science clubs at the school. We will also take the students to tour our wet and computing laboratories to further engage their interests in mathematics and science. We will transform these activities, if they show any initial success, into a more organized forum involving more researchers for a middle school and a high school in the same community.

COSMOS - The California State Summer School for Mathematics and Science (http://www.cosmos.uci.edu) is a month-long residential program for over 150 talented students in grades 8-12, taught by UCI faculty and scientists. The investigators will construct and implement a three-day Cluster on *Mathematical Modeling and Computation for Epidermis* for COSMOS. The graduate students and postdoctoral fellows participating in the proposed research projects will serve as tutors or instructors for this cluster.

The UCI Minority Science Programs and UCI Undergraduate Research Opportunities Program (UCOP) - The UCI Minority Science Programs, winner of the 2005 President's Award for Excellence in Science Math and Engineering Mentoring, provides an outstanding model for how to motivate and mentor undergraduate students from underrepresented groups. The UCOP is a longstanding program that provides opportunities for undergraduates to participate in research throughout the year, and it publishes The UCI Undergraduate Research Journal for undergraduate research results. The PI and co-PI are actively working with both programs to recruit and support minority students and undergraduate students in research. This grant will provide new research opportunities at the interface of mathematics and biology. The graduate students and postdoctoral fellows participating in the proposed research will assist the two investigators to mentor undergraduate students from both programs.

Graduate course - We propose to jointly teach one lecture on stem cells and epidermis for a core course of the MCB graduate program: *Critical thinking in systems biology (Development Cell 203,* http://lander-office.bio.uci.edu/CirticalThinking)**.** This special 10-week discussion course is structured to cultivate the ability of independent and critical assessment of published research, and to expose students to cross-disciplinary perspectives that will facilitate interdisciplinary collaborations. Each week, the students are assigned a pair of papers carefully selected from the Systems Biology literature. The papers are chosen primarily for their didactic value, and may be new or old. Prior to the two-and-half hour class meeting each week, the students are instructed to work in teams to decipher and critique the week's papers. The graduate students and postdoctoral fellows participating in the proposed research will also attend this lecture.

Journal club/seminar - We propose to develop a journal club on *Modeling and Computation of Stem Cells and Epidermal Systems***.** The journal club will be organized by two graduate students and postdoctoral fellows: one from Mathematics and one from Biology. In addition to the bi-weekly journal club activity, each year the club will invite three leading experts in the area of stem cells and epidermal systems to lecture at UCI.

Diversity - Nie supervised one female Ph.D. student, and currently is supervising three female postdoctoral fellows and two female Ph.D. students. Dai supervised two female Ph.D. students, three female M.S. students, and is currently supervising two female Ph.D. students and one female postdoctoral fellow. Both Dai lab and Nie lab have had minority rotation students. Dai has served multiple times as a faculty panel member at the UCI Minority Graduate Research Conference. The investigators are committed to diversity in their research groups, and will continue to be proactive in recruiting and supporting female and underrepresented minority students. The University of California at Irvine is a *Minority Institution* according to U.S. Department of Education criteria.

Data sharing - We will establish a database for new models and computational tools on stem cells, cell lineages and epidermal systems developed for the proposed research. We will assemble them with existing models sharing similar features, providing open access to the broader research community. The database for models and computational tools will be classified in terms of functions, scales, and critical features such as deterministic/stochastic, discrete/continuous, and spatial/non-spatial. Several software developed by PI Nie for studying spatial dynamics of development and growth has already been uploaded in a portal website for systems biology (http://systems-biology.org/resources/) organized by a group of laboratories at the Centers of Excellence for Systems Biology that include UCI Center for Complex Biological Systems.

Results from Prior NSF Support

Qing Nie (PI) was supported by NSF-DMS051169 (PI, 7/1/05-6/30/09). The major goal of the grant was to develop computational methods for interface dynamics. This grant resulted in seven publications (1-7). In (1-3), we developed a new class of efficient temporal schemes for stiff reaction-diffusion systems arising from modeling biological systems. This new method can be directly applied to models for diffusing molecules in this proposal. In (4-6), we developed accurate and fast front tracking methods for solving nonlinear PDEs with moving boundaries. The continuum models for epidermal growth proposed in this application involve moving boundaries, and our past experiences on computational tools for PDEs with moving boundaries will greatly benefit the proposed study in this application.

Xing Dai (Co-PI) has been support by NIH and other non-NSF funding sources during the past five years.

Intellectual Merit (12 Pages)

SIGNIFICANCE

Epidermal systems The skin epidermis is an excellent system to dissect the complex yet dynamic regulatory processes of tissue morphogenesis because: 1) it is easily accessible to experimental interrogations; 2) it has a well-characterized spatial layout, with different cellular stages expressing different but well-characterized protein markers and occupying specific locations (e.g. K14 marks the stem/early progenitor cells present in the basal layer; K1 marks the late progenitor/early differentiating cells in the spinous layers of the suprabasal compartment; loricrin marks the late differentiating cells present in the suprabasal granular layers) (Fig. 1).

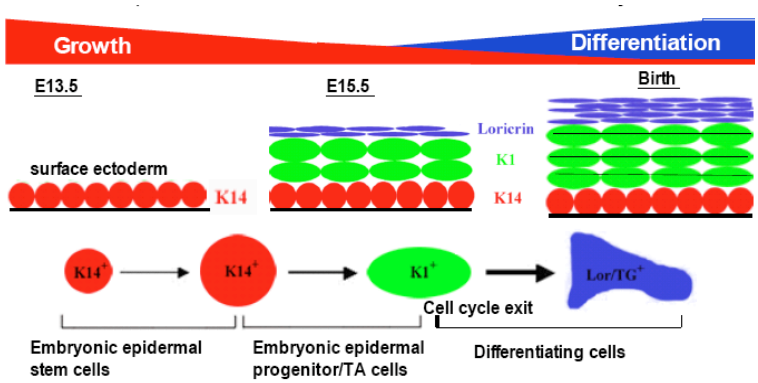


Figure 1. Temporal and spatial development of the embryonic epidermal systems of stems/progenitor cells (red), transient amplifying late progenitor (TA, green) cells, and terminally differentiated (TD, blue) cells.

The mature epidermis arises from a single-layer of basally attached (to basement membrane) primitive progenitor cells during mid-embryogenesis. During epidermal development, these progenitor cells choose between distinct cellular paths, namely proliferating symmetrically in a plane parallel to the basement membrane, dividing asymmetrically in a plane perpendicular to the basement membrane, cell cycle arrest, detachment from the basement membrane and migration upwards, and terminal differentiation (8). The epidermis regenerates itself throughout postnatal life due to the presence of stem cells capable of recapitulating what happens during embryonic development. Despite the identification of important signaling and transcriptional activities that control epidermal stem/progenitor cell proliferation and differentiation, important questions remain. How does an epidermal stem cell integrate different molecular activities to ensure a state of long-term self-renewal and to prevent premature entry into terminal differentiation? How then are various regulatory modules integrated to allow the otherwise proliferating progenitor cells to exit cell cycle and terminally differentiate? How are these molecular and cellular events orchestrated at a systems level to produce a stratified epidermis? While experimental approaches are continuously needed to clarify known regulations and to uncover additional control modules, a systems approach is imperative and timely to integrate multiple regulatory modules, derive systems-level behavioral principles, and predict novel regulations that function to enhance systems performance.

Ovol genes and epidermal development The Dai laboratory has a long-standing history of studying two related transcription factors that regulate epidermal development: Ovol1 and Ovol2. Ovol1 and Ovol2 are homologs that share a highly conserved zinc finger DNA-binding domain, and bind to nearly identical cognate DNA sequences (9). Both encode transcriptional repressors that are able to recruit histone deacetylase complexes to target genes and hence suppress transcription (10). Despite similar biochemical activities, the two proteins are expressed at distinct cellular locations in the developing epidermis – Ovol1 is predominantly expressed in the suprabasal spinous layers, and Ovol2 predominantly in the basal layer (11, 12). In vitro experiments using cultured keratinocytes (isolated epidermal cells) confirmed that Ovol1 is up-regulated as cells are induced to undergo cell cycle arrest and terminally differentiate, whereas Ovol2 expression is down-regulated upon differentiation (11, 13).

Studies using knockout mouse and cell culture models have elucidated a functional requirement for Ovol1 in the cell cycle exit of proliferating progenitor cells (10). Consequently, the developing epidermis in Ovol1-deficient mice is thicker than normal, and contains an expanded spinous compartment (Fig. 2). Interestingly, this function is downstream of a very important growth inhibitory signaling pathway, namely TGF-β. Ovol1 gene expression is induced upon TGF-β treatment, and Ovol1-deficient cells fail to undergo growth arrest in response to TGF-β (10, 14). While in vitro keratinocyte experiments have uncovered a critical function for

Ovol2 in maintaining the long-term proliferative potential of epidermal progenitor cells (see below), germline knockout of Ovol2 in mice results in early lethality before epidermal development (15) whereas epidermis-specific knockout of Ovol2 yielded no remarkable epidermal defects (data not shown). In striking contrast, the overexpression of Ovol2 in its "habitual" basal compartment leads to reduced basal proliferation and a significant thinning of the epidermal spinous layers (Fig. 3). Mechanistically, both Ovol proteins repress the transcription of c-Myc (10, 16), the expression level of which is proposed to govern whether a stem/progenitor cell should exit into a transient amplification (TA) mode, how well a TA cell proliferates, or whether it should terminally differentiate (17).

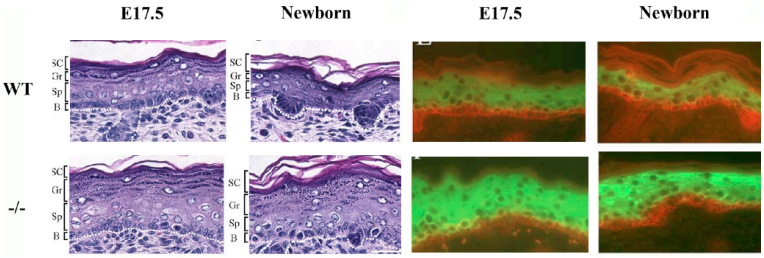


Figure 2. *Preliminary data*: Ovol1-defient (-/-) skin epidermis is thicker than wild-type (WT) control, with expanded K1-positive spinous layers. Age and genotype of samples are as indicated. White dotted line denotes the basement membrane. B, basal layer; SC, stratum cornea, Gr, granular layers, Sp, spinous layers. K1 antibody, green; K14 antibody, red.

An integrated experimental and computational approach While experimental studies portrait the details of isolated Ovol regulatory circuits, mathematical modeling and computation are needed to examine how Ovol proteins fit into the complex regulatory network involving multiple factors and how they serve to enhance the performance objectives of epidermal morphogenesis. The Ovol proteins are excellent candidates for a systems biology approach because they are nodal points that collect and interpret upstream signals (e.g., TGF-β signaling), and translate these signals into differential levels of critical downstream effectors (e.g., c-Myc) that execute the actual changes in growth and differentiation. Among many interesting questions concerning Ovol1/Ovol2, one wonders why two distinct homologs are needed in different cellular compartments to perform seemingly similar cellular/molecular functions. What is the significance of this in epidermal morphogenesis?

The Nie lab has joined force with the Dai lab using a systems biology approach to study Ovol1/Ovol2 function in epidermis, resulting in three joint publications (16, 18, 19). First, we established a mathematical framework for epidermis system using multi-scale and continuum models, and found that homeostasis can be achieved if the differentiation is regulated (18). Next, through an integrated experimental and theoretical study (16), we found that Ovol2 participates in the regulation of proliferation potential and proliferation rate via c-Myc. This work allows us to add Ovol2 to the list of known regulators of epidermal progenitor cell proliferation, including the c-Myc and TGF-α signaling pathway. Importantly, our experiments also demonstrate that Ovol2 suppresses terminal differentiation by repressing the expression of Notch1 (16), which is known to promote the stem/progenitor-TD switch, manifested as a switch from a basal fate to a suprabasal spinous fate (20). Motivated by these findings and the fact that Notch signaling occurs through adjacent cell-cell interactions, we recently developed a discrete cell model in 3D that allows the incorporation of Notch signaling for studying epidermal stratification (19).

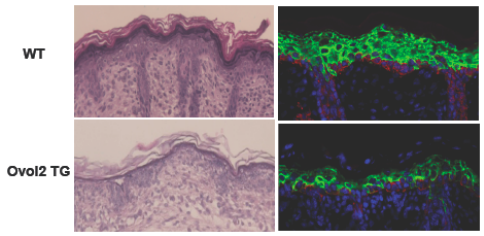


Figure 3. *Preliminary data*: Transgenic mice overexpressing Ovol2 (Ovol2 TG) in the basal layer produce a thinner epidermis with reduced K1-positive spinous layers. Samples were taken at newborn. K1 antibody, green; K14 antibody, red.

In this application, we propose to extend beyond a purely molecular modeling of the system to a multi-scale approach, incorporating the interactions within intracellular molecular networks while following the spatial and temporal dynamics of cells, such that the modeling outputs can be better related to experimental data. In a typical experiment for epidermal stem cell dynamics, one probes the intra-cellular molecular components, measures growth of different types of cells with best available methods and markers, correlates the observations, and finally infers the role of the molecular components in the whole system. Analysis of models consisting of experimentally measurable components as well as components that cannot be directly measured and manipulated using current methodology will lead to a better understanding of the overall behavior of the system.

INNOVATION

An important innovative aspect of the proposed research is a tight integration of mathematical modeling with experimentation. Also innovative, and we believe a strength of this proposal, is that the goal of modeling here is not just to test whether a particular mechanistic scheme is correct by fitting observations to models, but also to explore whether a given mechanistic scheme is capable—over any plausible range of parameters—of serving as a strategy for performing a certain type of job (e.g. achieving robust development or regeneration of the tissue in response to perturbations). In addition, the modeling and computational techniques proposed here combine complex intra-cellular interactions with extra-cellular environment and morphogens in both discrete and continuous set-up, and hence are challenging, innovative, and will likely provide an exemplary paradigm for studies of other biological systems. This proposal is also innovative in terms of the hypotheses that are put forth. For example, our hypothesis that a specific regulatory circuitry in epidermal development exists to control the correct size of the tissue and some other regulatory circuitries exist for the purpose of specifying stratification is novel. Likewise, our approach of dividing the complex network into one proliferation module and one stratification module, and our hypothesis that cross-talks between the two modules exist in order to achieve *robustness* are entirely novel. This hypothesis, if validated, has the potential to change the way biologists think about key signaling pathways and regulatory networks.

APPROACH

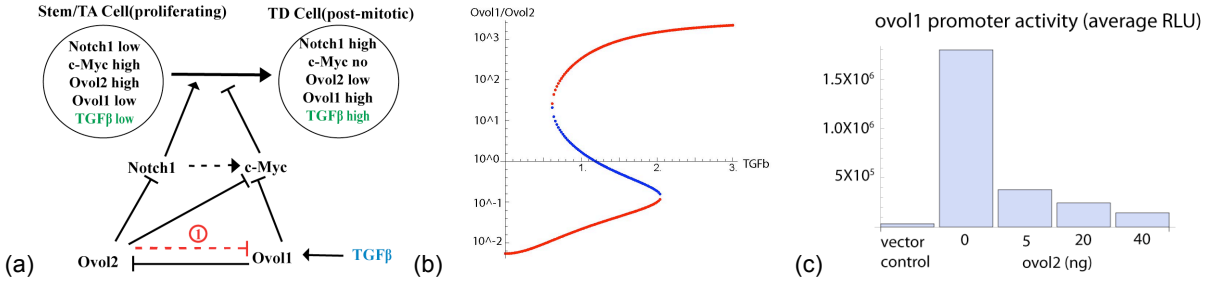


Figure 4. (a) A simple regulatory scheme of the stem/TA to TD switch. Solid: known regulations; black dashed: assumed regulation from existing literature; red dashed: proposed regulation to be tested by modeling and experiments. (b) Bi-stability due to mutual repression of Ovol1 and Ovol2. Stable steady states in red, unstable state in blue. (c) *Preliminary data*: The Ovol1 promoter activity inversely correlates with Ovol2 protein concentration.

Aim 1: Investigate the role of Ovol1-Ovol2 mutual repression in controlling epidermal size and cell type proportion

Rational Intuitively, during epidermal morphogenesis, both positive cross-talks and negative feedback mechanisms must exist between proliferation and differentiation to achieve tissue growth and maturation while put a brake in the system to prevent excessive or over-accelerated growth. Only so the tissue can reach, but does not exceed its desired final size. Under pathological conditions such as embryonic wound healing, positive cross-talks might be further enhanced whereas negative feedback diminished. While Ovol1 and Ovol2 both negatively regulate proliferation (via repressing c-Myc), Ovol2 also directly impinges on differentiation via repressing Notch1 and its downstream signaling. Hence, these two factors influence both short and long-term growth dynamics of a cell population, and their interactions may be exploited by the systems to achieve differential regulations of proliferation and differentiation under normal as well as perturbed situations.

We consider specifically a model of mutual repression between Ovol1 and Ovol2. Such a strategy can be particularly useful when it comes to two homologous genes as it provides a means for one homolog to sense the state of the other (in biological systems the loss of one often causes an up-regulation of another as part of a compensatory mechanism). More importantly, mutual repression between different genes can drive cellular differentiation where the strength of each repression dictates the final outcome of genes being up-regulated or down-regulated (21). We have previously shown that Ovol1 represses Ovol2 (12). We reason that Ovol2 might also repress Ovol1, and that Ovol1 represses c-Myc expression more efficiently than Ovol2.

This will enable Ovol2 to counteract Ovol1 repression of c-Myc, allowing c-Myc to be expressed at a low level unless there is a high level of extra-cellular stimuli (e.g., TGF-β). In essence, TGF-β levels govern the switch between Ovol1 and Ovol2 dominant states, and the interaction between them in turn governs c-Myc levels and consequently growth arrest (see Fig. 4a-b).

Overall hypothesis An Ovol1-Ovol2 mutual repression mechanism is required for cellular differentiation so that growth arrest occurs and the actual process of terminal differentiation begins, resulting in robust epidermal morphogenesis. The mutual repression mechanism allows the cell population to reach a desired, homeostatic final size relatively faster compared to a single regulatory element (e.g Ovol1 or Ovol2 only). While Ovol1 and Ovol2 have a similar function at molecular/cellular levels, their mutual repression together with different strengths in proliferation and differentiation regulation in cells allows the cell population to efficiently reach homeostasis during normal morphgenesis, or regeneration after perturbations.

Hypothesis 1 Ovol2 directly represses Ovol1 expression and Ovol1 represses the c-Myc promoter more efficiently than Ovol2.

Preliminary modeling and experimental data To explore the notion that Ovol2 represses Ovol1 expression, we experimentally depleted Ovol2 from cultured keratinocytes using the siRNA knockdown technology, and examined Ovol1 expression. A 1.6-fold increase (p value < 0.0001) in Ovol1 mRNA was observed in the knockdown cells. Moreover, we performed luciferase reporter assays, and found that exogenously added Ovol2 protein represses the activity of Ovol1 promoter (which drives luciferase activity) in a dosage-dependent manner (Fig. 4c). Together, our experimental data support an Ovol1-Ovol2 mutual repression.

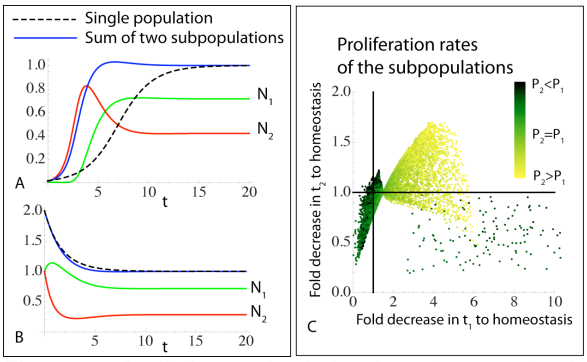


Figure 5. Preliminary simulation based on a simple population model shows the regeneration speed is faster for a two-population system than for a one-population system. P_1 and P_2 are the proliferation rates of N_1 and N_2 respectively. The faster proliferating N_2 population tend to occupy a smaller proportion of homeostasis (A) and (B) for example. A characteristic of increased speed to regeneration with 2 populations is that one of the sub-populations has greater proliferation rate than the homogeneous population, and is not preferentially biased by TGF-β (C).

To investigate the functional significance of this mutual repression, we used classical population dynamics models to compare the growth dynamics between 1) a cell population consisting of two reversible subpopulations (Ovol1 and Ovol2 dominant cells) and 2) a homogeneous population (Fig. 5). In both models we let the cell secreted TGF-β to feedback to repress cell growth. We found that, in some parameter regimes, the population with two subpopulations can obtain a faster speed to reach homeostasis than the homogeneous population (Fig. 5). This simple, preliminary model suggests that populational growth arrest is possible through TGF-β induction of Ovol1, leading to an Ovol1 dominant population, and that Ovol1 is likely to be a stronger repressor of c-Myc than Ovol2.

It has been hypothesized that differential proliferation potentials exist within the epidermis with slow but long-term proliferating stem cells and fast but transiently proliferating TA cells. Recent challenges to this paradigm is the model that the epidermis consists of a single progenitor pool of TA-like cells (22) in homeostatic conditions. Given our finding that regeneration time can be improved by differential proliferation between two interacting populations, *we propose that the epidermis consists of cell populations that can make reversible transitions (unlike the irreversible stem-TA transition) and that homeostasis consists mostly of a single dominant population*. Such reversible fate switches will help reconcile the seemingly contradictory experimental findings and might be an important novel aspect for regulating cell population growth. The effects of having two subpopulations are most striking in conditions away from homeostasis. In particular when cell density is much smaller than homeostasis, e.g. a wound, it gives rise to the transient growth that is dominated by the fast proliferating subpopulation (23).

Plan We will continue to study and expand the intra-cellular network model (Fig. 5) to uncover and analyze conditions for fast regeneration of a homeostatic state. We will perform similar analytical studies and

computational explorations as previously used for the study of olfactory epithelial regeneration (24) (25). In particular, we will compare the time scale of the reversible switching between Ovol1 and Ovol2 states, and the proliferation rates of Ovol1 or Ovol2 dominate cells and how they affect the total population size and the proportion between Ovol1 and Ovol2 subpopulations. Because of the existence of multiple steady-states of the models, we will explore parameter regimes to search for insight into how the relative proportions of Ovol1 and Ovol2 dominant populations are regulated to achieve a unique homeostatic state consistent with experimental observations.

Experimentally, 1) we will test whether the repression of Ovol1 promoter by Ovol2 is a direct effect. We will repeat the luciferase reporter assays, but this time using deletion fragments of, or point mutations in, the Ovol1 promoter to delineate the Ovol2-reponsive element. Moreover, we will perform chromatin immunoprecipitation (ChIP) experiments, a method to detect occupancy of a transcription factor at its target promoter, to test if Ovol2 physically binds to the Ovol1 promoter at the predicted site(s). ChIP experiments will be performed using primary keratinocytes isolated from wild-type as well as Ovol2-deficient newborn mice, as the latter serves as a useful specificity control. 2) We will compare the repression and binding strengths of Ovol1 and Ovol2 on c-Myc promoter using luciferase reporter and ChIP assays, respectively. Since Ovol1 and Ovol2 antibodies differ in their affinities for their cognate proteins, we will generate constructs expressing Ovol1 or Ovol2 but each fused to a common antigen tag (e.g., Flag). These constructs will be transiently transfected into cultured keratinocytes for reporter assays, and stably transfected into these cells for ChIP assays. Relative repression strength will be calculated as fold repression per microgram of protein expressed (detected using an anti-Flag antibody). Relative binding strength will be calculated as ChIP signal per microgram of protein expressed (both detected using the anti-Flag antibody). Using this method, we will be able to directly compare the repression or binding strength of Ovol1 or Ovol2 on c-Myc promoter.

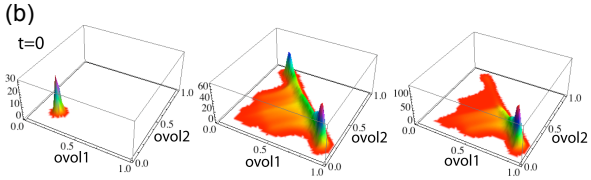
(a)

$$\frac{\partial N}{\partial t} + \nabla_{\bar{X}} \bullet (\bar{X}_t N) = 2\int_{\Omega} \Gamma_{div}(\bar{X}')p(\bar{X} \mid \bar{X}')N(\bar{X}',t)\, d\bar{X}' - \Gamma_{div}N - d_{removal}N$$

$$\frac{dO_1}{dt} = f_1(\bar{X},T_{GF},E_{GF}), \frac{dO_2}{dt} = f_2(\bar{X},T_{GF},E_{GF}),\ T_{GF} = f_{\text{population}}(N),\ E_{GF} = g_{\text{population}}(N),$$

$$\Gamma_{div} = h_{\text{multiscale}}(\bar{X},T_{GF},E_{GF}), \bar{X} = \{O_1,O_2\}$$

(b)



(c)

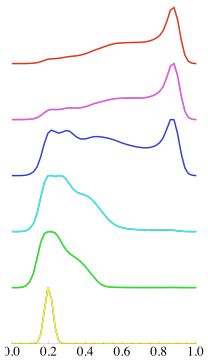


Figure 6. (a) A multiscale model linking the molecular components represented by the vector *X* that contains ovol1, ovol2, TGF, EGF and cMyc to the dynamics of the cell population, *N*. *p* is the partition function of intracellular products after each cell division. (b) Preliminary simulations for a model of two genes showing the population *N* in distribution of the genes at different times converging towards homeostasis; (c) model results that show cell numbers in the Ovlo1 direction (bottom at early time, top at later time) resembles typical FACS data in experiment.

Hypothesis 2 The Ovol1-Ovol2 mutual repression is an important strategy for efficient reversible fate switches. Specifically, the Ovol1-Ovol2 mutual repression may form a bi-stable switch, and TGF-β can induce a unidirectional switch from Ovol2-dominant to Ovol1-dominant expression in cells. How cells might achieve reversible expression between Ovol1 and Ovol2 is unclear. Stochastic gene expression can induce reversible switching (26). Our preliminary data suggest that the interaction between cell division and a mutual inhibition motif also allow for bi-directional switching (27). We hypothesize that a synergy among stochasticity in gene expression, mutual inhibition, and cell division is critical for achieving the correct portions of different cell types (e.g. a bimodal population distribution in Ovol1 and Ovol2) and for maintaining robust homeostasis.

Preliminary modeling data We have developed multi-scale models that couple cell population with gene expressions based on our previous work (18). Such type of model allows straightforward inclusion of stochastic effects (e.g. stochastic participation of intra-cellular components during cell division) and direct comparison with experimental data (e.g. FACS data which display cell number as a distribution of gene expression level). Our preliminary simulations suggest that the feedback through TGF-β repression of c-Myc and its induction of Ovol1 is critical for reaching homeostasis (Fig. 6). The bidirectional switching between Ovol1 and Ovol2 dominant states inside cells are due to stochastic partition of molecules to daughter cells after division.

Plan First, we will explore the conditions of the multi-scale model that allow for a robust bimodal cell distribution in Ovol1- and Ovol2-expressing states as observed in experiments. Next, we will study and uncover the conditions in which the system can reach homeostasis after wounding. Clearly, the capability of bidirectional switching in a bi-stable system is important in this regard. We will incorporate the stochastic dynamics of molecules (28) (29) into our current multi-scale model to study effect of noise in maintaining homeostasis. The analysis and simulation from the model will show whether the epidermal development system is constrained by the stochasticities, or instead exploits them to reach performance objectives such as fast regeneration to a unique homeostatic state.

Experimentally, we will examine whether Ovol1 and Ovol2 are required for a speedy recovery after wounding. Specifically, we will use dermal punches to create wounds in the backskin of newborn Ovol1-/- and Ovol2 skin-specific knockout mice. One wound will be generated per mouse, and three mice will be used per genotype. The wound front will be excised at different time points after wounding (up to 14 days, when wound is normally closed). Hematoxylin/Eosin staining will be performed on skin sections to compare the kinetics of wound closure in wild-type and Ovol1 or Ovol2 mutant skin. The final size and morphology of the regenerated skin will also be assessed. Moreover, the expression of proliferation and differentiation (different layers) markers will be examined to monitor the proliferative activity as well as distribution of different cell types in the regenerated skin. All reagents and methods have been established in the Dai laboratory. If time permits, we will perform similar experiments on skin-specific knockout mice of Smad4, a common effector of TGF-β signaling the loss or reduction of which will result in compromised TGF-β signaling (30). Although previous studies have demonstrated the involvement of Smad4 in wound healing (31), our focus will be on analyzing the healing kinetics and the relative presence of Ovol1- and Ovol2-expressing epidermal cells in the healing wound.

Modeling and computational challenges Our proposed multi-scale model is based on population balance framework which uses conservation of mass to describe the cell distributions in terms of the intra-cellular components (32). In this framework, the model couples intra-cellular regulations described in a system of ODEs with cell population and cell division in terms of hyperbolic PDEs (Fig. 6a). Previously, we applied such framework to c-Myc-dependent population growth and differentiation (18), and the modeling study provided many insights on population distributions in terms of c-Myc and regulations needed to maintain homeostasis. The output of such modeling approach is directly related to experimental observations, and it can be easily used for testing a specific hypothesis. This approach has been less explored for systems involving more than one intra-cellular component partly because it requires solving hyperbolic PDEs in high dimensions. We propose to use higher order finite difference Weighted Essentially Non-oscillatory (WENO) schemes (33) to solve these types of models. The WENO method, which evaluates the numerical flux dimension by dimension in rectangular domains, and has computational complexity proportional to the total number of grid points, is particularly suitable for the multi-scale models based on population balance.

Aim 2: Investigate the complex molecular interactions that robustly control the spatial stratification of epidermis

Rational Control of proliferation and differentiation is intimately linked to epidermal stratification. Intuitively, the ultimate spatial pattern should be jointly governed by cell division rates and differentiation probability, size and shape of the cell populations, and their interactions with the environment and other cells to provide the spatial information that controls cellular behavior. The objective of this Aim is to investigate and to experimentally validate additional molecular interactions and control strategies that are used in combination with the molecular circuitry described in Aim 1 to robustly form the stratified layers of different cell types in the mature epidermis.

Some of the key performance objectives that we know about epidermal stratification are: 1) there is a short patterning range as the mature epidermis is a very thin structure of only 6-8 live cell diameters (excluding cornified layers); 2) there are sharp boundaries of gene expression (K1, K14, etc.) between layers; 3) the basal layer is only a single cell thickness; 4) proliferation occurs almost exclusively in the basal layer; 5) patterning should be robust despite of the likely presence of gene expression noise, cell variability, extracellular “noise”, and variation in environment to a population of heterogeneous cells; and 6) the original tissue structure needs to be regenerated after severe perturbations such as one or more layers being

removed. These objectives are quite strict and have to be satisfied by the molecular circuitry, which, as indicated above, also has to satisfy the proliferation and differentiation performance objectives from Aim 1.

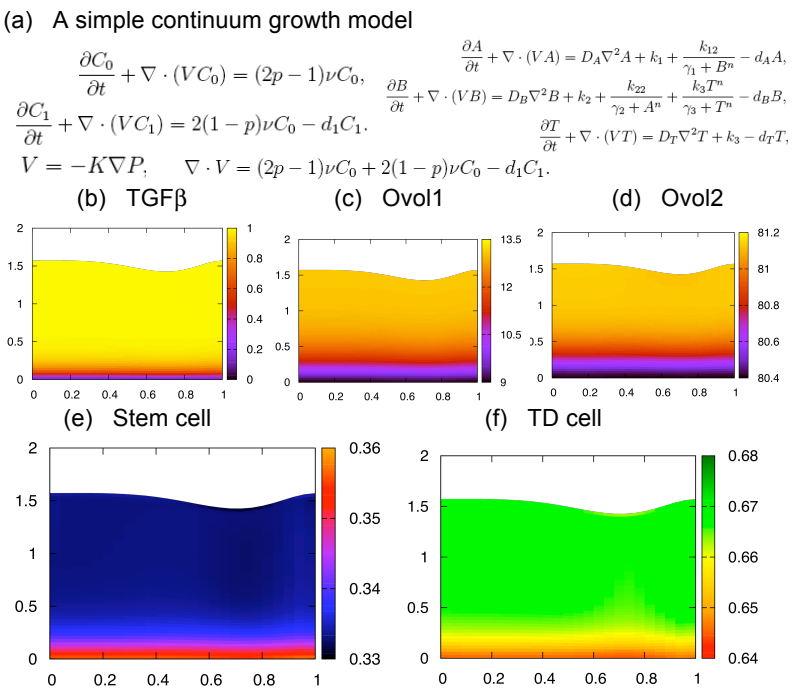


Figure 7. (a) In the simple two-dimensional model, T: TGFβ, A: Ovol1, B: Ovol2, C_0: stem cell, C_1: TD cell. (b-f): preliminary simulations of (a).

In Aim 2a, we will start with the basic core Ovol1/Ovol2 module from Aim 1 and introduce extracellular morphogen gradients of TGF-α and TGF-β as the mechanism to achieve stratification (Fig. 8; proliferation module in Fig. 11). As one adds more performance objectives to a system, in this case requiring the Ovol1/Ovol2 proliferation module to also control spatial stratification, then new constraints are created which may require tradeoffs in the ability of the system to satisfy its other proliferation objectives. One of our goals is to uncover these new constraints and the corresponding tradeoffs, thus providing us with knowledge about the effectiveness and limitations of morphogen gradient systems in achieving stratification.

Our expectation is that the morphogen gradients will be sufficient to specify stratification of the epidermal layers, but they may not be robust enough to jointly satisfy all performance objectives for proliferation, differentiation and stratification. For example, the basal layer is just a single cell layer thick, so the gradients must have a very short length scale over a couple cell diameters thus suggesting either an extreme value for the decay rate of the morphogens, an upper limit on the diffusion rate of the morphogens, or both. Furthermore, our preliminary experimental findings (Figs. 2, 3) that loss of Ovol1 and overexpression of Ovol2 result in overall a thicker and thinner epidermis with more or fewer spinous layers, respectively, but do not destroy stratification per se, suggest that the Ovol1/Ovol2 module does have some input into the stratification process but additional control circuit(s) is used to initiate stratification.

In Aim 2b, we study the role of cell-cell contact-mediated communication via the Notch signaling pathway. We postulate that Notch signaling constitutes a separate molecular circuit, independent from the Ovol1/Ovol2 circuit for proliferation and differentiation, to achieve epidermal stratification (Fig. 9; stratification module in Fig. 11). Indeed, experimental evidence indicates that Notch signaling is a key component in epidermal stratification where it functions as a differentiation switch for basal cells as they move into the spinous layer (34, 35). Interestingly, Ovol2 has been experimentally shown to inhibit Notch1 gene expression, which can be taken as evidence to suggest that there are direct interactions between the proliferation and stratification modules. Is this stratification module more capable of achieving the performance objectives of stratification than morphogen gradients? Which objectives can be better achieved with mechanisms utilizing local (Notch signaling) versus global (TGF-α and TGF–β gradients) spatial information? We will study these questions both computationally and experimentally.

In Aim 2c, we include p63 - a known master regulator controlling both stratification and proliferation (36), as a control module that “talks” to both the proliferation and stratification modules (Fig. 11, control module). The Notch signaling pathway performs its function as a differentiation switch through cross-regulation with p63 (37). Cross regulation between TGF-α and p63 also occurs, as TGF-α acts positively on p63 through inhibition of miR-203 which then inhibits p63 (38, 39). We will investigate putative cross-talk regulations

between the proliferation and stratification modules to understand what information these interactions transmit and how they control the functions of the individual modules. We will also investigate putative cross-talks between the control module and the proliferation module. The results of these studies will illustrate the capabilities and tradeoffs of different control structures including a centralized control circuit, direct cross-regulations between modules, and a combination of both. Collectively, they will make novel predictions about possible cross-regulations that will then be experimentally validated in Aim 2d.

2a: Test the hypothesis that TGF-α and TGF-β morphogen gradients empower the Ovol1/Ovol2 proliferation module to specify stratification

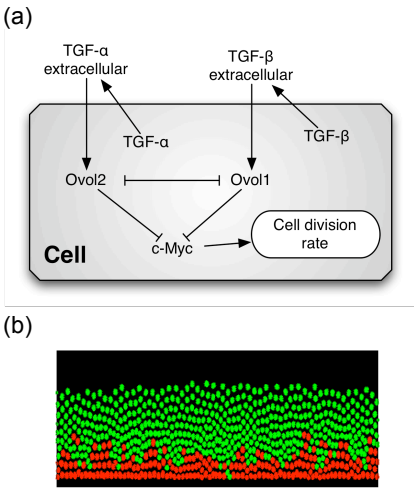


Figure 8. (a) A intracellular gene network with Ovol1/Ovol2 proliferation circuit and cell-produced TGF-alpha and TGF-ß extracellular morphogen gradients for stratification; (b) Simulations of a 2-D discrete cell model with two cell types using the network in (a).

TGF-α belongs to the EGF family of growth factors and is a well-known growth promoting pathway in skin epidermis both during normal development, upon wound healing, and under pathological situations such as psoriasis and skin cancer (40). In addition to enhancing epidermal cell proliferation, this pathway also stimulates cell migration, which we will not consider in this proposal. Consistent with its role in proliferation, TGF-α expression occurs primarily in the basal, proliferative layer of the skin epidermis (41).

TGF-β signaling is known for its growth-inhibitory function. Mouse epidermal cells cultured under non-differentiating basal conditions express no TGF-β1 and low levels of TGF-β2, whereas calcium treatment, which induces growth arrest and terminal differentiation, leads to increased expression of these growth factors (42). In both normal and pathological skin epidermis, TGF-β production seems to be confined to the suprabasal, differentiating layers (43, 44). Overexpression of TGF-β1 in basal epidermal cells results in hypoproliferation and a thinner epidermis (45). TGF-β homologs in lower organisms, such as dpp in *Drosophila*, act as morphogens, which are produced from a localized source but spread to form a concentration gradient across a developing tissue (46). Although there is no direct evidence for a TGF-β gradient across the mammalian epidermal layers, existing data are consistent with the possibility that TGF-β are produced from differentiating cells and constitutes a feedback mechanism to inhibit stem/progenitor cell proliferation, hence leading to stratification and homeostasis. We hypothesize that TGF-α and TGF-β morphogen gradients empower the Ovol1/Ovol2 proliferation module to specify stratification. However, we also envision that the utilization of these morphogen gradients alone is not sufficient to achieve stratification that is efficient and robust.

Preliminary data We have developed a preliminary two-dimensional continuum model with moving boundaries to investigate this hypothesis. The simulations of the model, which is based on our previous one-dimensional model on olfactory epithelium(47), show that the proliferation module shown in Fig. 8a is capable of producing stratification (Fig. 7) . We have also employed a preliminary hybrid multi-scale model that incorporates discrete cells containing a gene network and proliferation coupled with continuum models for extracellular signaling molecules of TGF-ß (see Aim 2e for more discussions) to investigate stratification and role of cell-to-cell interactions in stratifications. Preliminary simulations (Fig. 8b) that are consistent with the continuum models show qualitative features of a typical experimental observation for wide-type animals.

Plan We will perform three types of computational studies by utilizing strengths of each of the continuum and the discrete models. The first will determine the range of parameter values for the morphogen gradients that are sufficient to produce the stratification phenotype. The second will assess the extent of alteration of Ovol1/Ovol2 module's ability to robustly achieve its proliferation objectives from Aim 1 by the additional stratification objectives. Lastly, we will evaluate the model's robustness to noise by introducing stochasticity and heterogeneity in the intracellular gene network of each cell, the secretion and reception of extracellular factors by cells, and the dynamics of extracellular signaling molecules. These studies will yield knowledge

about the ability of a single specialized molecular circuit to concurrently perform two major tissue functions, namely proliferation and stratification.

2b: Test the hypothesis that a regulatory circuit composed of Notch signaling (stratification module) can specify stratification independently from the Ovol1/Ovol2 proliferation circuit

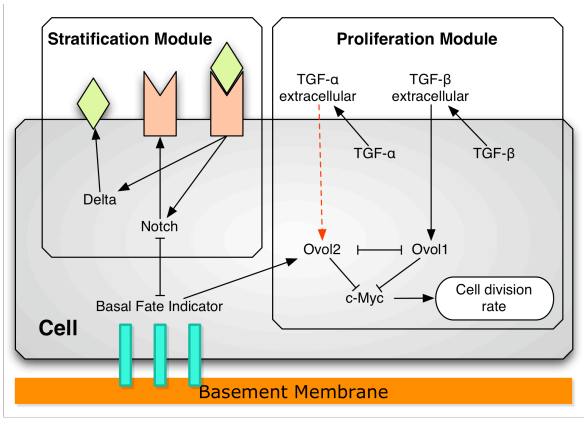


Figure 9. Intracellular gene network with Ovol1/Ovol2 proliferation module and Notch signaling stratification module.

We consider the Ovol1/Ovol2 proliferation module from Aim 1 but include a separate Notch signaling circuit to control stratification (Fig. 9). We introduce a "basal fate indicator" that acts as an observable signal of basal cells and causes these cells to attach to the basement membrane; cells which have their "basal fate indicator" down-regulated through Notch signaling would detach and move upward into the suprabasal layers. The modeling methodology described in Aim 2e will allow us to directly implement Notch signaling with ligands (collectively considered as Delta in this application), receptors (Notch) and bound receptor complexes derived through explicit cell-cell contact. This model still utilizes TGF-α and TGF-β extracellular signaling but these factors do not construct gradients, and instead they act as population level signals as in Aim 1. This said, we will also explore an alternative model where TGF-α and TGF-β form gradients as in Aim 2a, which do not directly control stratification but may be beneficial for proliferation. Our expectation is that the independent Notch signaling module will more robustly meet many of the stratification performance objectives than the morphogen gradient circuit of Aim 2a. However, we also anticipate that this model will be fragile under certain types of perturbations. Specifically, if there is no interaction between the two modules, perturbations to one module may not be compensated within the other module, leading to abnormal phenotypes.

Three types of computational studies will be performed. The first will determine the range of parameter values for Notch signaling that are sufficient to produce the stratification phenotype. The second will assess the model's robustness to noise by introducing stochasticity and heterogeneity in the Notch signaling pathway. Lastly, we will perform perturbations in the proliferation module, such as altering the cell division rate, increasing cell variability in network parameter values, and altering extracellular signaling dynamics, then evaluate the stratification module's ability to continue functioning in a robust fashion. Perturbations to the stratification network will also be performed to evaluate changes in the function of the proliferation module. These experiments will characterize the extent to which modularity in biological systems can satisfy multiple objectives in comparison to a single specialized circuit.

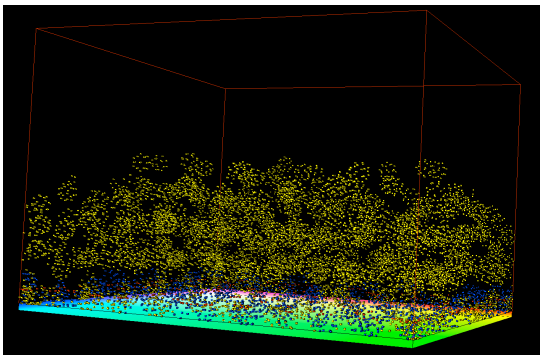


Figure 10. Preliminary simulation of layers of cells based on a 3D hybrid discrete cell model

Preliminary data We have performed preliminary simulations, and the result shows that this 3D model can produce stratification, mostly notably meeting the performance objective of forming a basal layer of single cell thickness (Fig. 10). Therefore, continued modeling using this methodology is likely to be feasible and highly informative.

2c: Investigate the role of putative cross-regulations between the Ovol1/Ovol2 proliferation module and the Notch signaling stratification module, as well as with the p63 control circuit to coordinate module functions

This subaim investigates interactions between the aforementioned regulatory modules and the required circuitry to coordinate their actions. Input from the proliferation and control modules into the Notch stratification module has already been demonstrated by work from others and us. As discussed above, Ovol2 inhibits Notch1 thus suggesting the transmission of proliferation status for an individual cell. What about global information like tissue size? We propose that TGF-α or TGF-β may regulate Notch signaling, and thus provide that information. There has been no direct evidence suggesting any input from the Notch stratification module into the Ovol1/Ovol2 proliferation module during epidermal morphogenesis. However in other non-epidermal systems, there is strong evidence that c-Myc is a direct target of Notch1 (48). We therefore propose that Notch signaling positively regulates c-Myc expression in the developing epidermis, and that this is important to ensure the correct size of the spinous layers. Furthermore, given the essential role for p63 in epidermal stratification and progenitor cell proliferation, we hypothesize that p63 acts as a separate, master control module. Curiously, the only demonstrated output from p63 into the Ovol1/Ovol2 proliferation and Notch signaling stratification modules is a negative regulation of Notch signaling (49). Moreover, the only demonstrated input into p63 from these modules is that TGF-α acts positively on p63 through miR-203 (38, 39). We therefore propose cross-regulations between p63 and c-Myc, p63 and Ovol2, as well as Ovol2 and TGF-α as ways to coordinate positive and negative effects on proliferation, as well as proliferation and stratification.

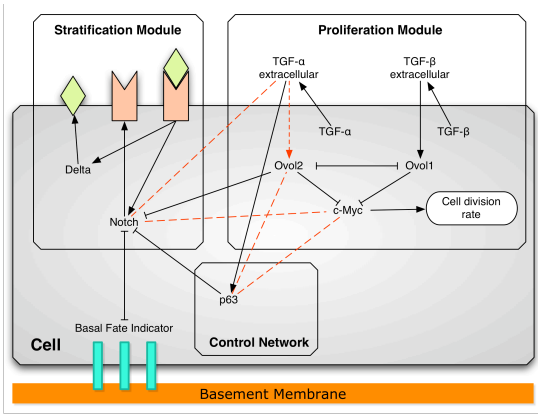


Figure 11. Model with cross-regulations between stratification and proliferation modules, and a separate p63 control circuit. Red dashed lines are postulated regulations.

We will perform two types of computational experiments. We will initially investigate the ability of direct cross-regulations, both known and postulated, between the stratification and proliferation modules to robustly satisfy the performance objectives for both modules. Specifically, we will characterize what information is actually being transmitted and why it is important. In other experiments, we will consider the role of a separate p63 control circuit to coordinate the proliferation and stratification modules, and the impact of putative novel regulations including p63 acting on Ovol2 and c-Myc. The results of these experiments will yield knowledge about the strategies that are useful in integrating information from various cellular processes and molecular circuits to control cell behavior and tissue outcome. They will also elucidate the tradeoffs and constraints involved in such control circuitry, and most importantly characterize the solutions used by biology to robustly satisfy multiple objectives in the context of complex developmental processes.

2d**: **Experimentally validate the important cross-regulations identified in modeling from 2a-2c

Our proposed modeling work will yield at least two types of information. First, we might learn that two different regulatory circuits (e.g., Ovol1-Ovol2 and Notch signaling) show synergy in best achieving the aforementioned performance objectives. Second, we might learn that a particular molecular interaction, e.g., proposed regulation of Ovol2 or c-Myc by p63, is critical for the systems behavior. We will design experiments to test both lines of predictions. We will use the putative p63–Ovol regulation as an example but the same principle holds for other potential regulations proposed in Fig. 11.

Preliminary data In characterizing the molecular alterations caused by Ovol2 overexpression, we observed a striking resemblance between Ovol2-overexpressing and p63 knockout skin phenotypes. Specifically, the expression of K14 and AP2γ, the activation of which requires p63, is significantly reduced upon Ovol2 overexpression (Fig. 12). Moreover, the expression of K8 is significantly increased in Ovol2-overexpressing epidermis, reminiscent of that seen in p63 knockout mice. These data suggest that Ovol2 and p63 converge on regulating common molecular events, but in opposing directions. Interestingly, p63 expression is not affected by Ovol2 overexpression (Fig. 12F-G, leaving open the possibility that p63 might regulate Ovol2 instead.

Plan If our modeling results suggest that a regulation of Ovol2 by p63 indeed affects the performance objectives of epidermal proliferation and stratification, we will perform mouse genetic experiments to test if double mutation of the two genes will show rescued or synthetic phenotypes. For example, we will cross Ovol2-/- or Ovol2 TG mice with p63-/- or p63-overexpressing mice to generate Ovol1/p63 double mutant mice. We will then perform histological and marker expression analyses of the developing epidermis at different stages using established methods to examine the impact of compound mutations on the speed and outcome of epidermal morphogenesis. We will also perform BrdU and Ki67 staining assays to examine the effect of such genetic manipulations specifically on cellular proliferation. We anticipate that if p63 represses Ovol2 expression and this is a lineage pathway, then the loss or reduction in Ovol2 might rescue the epidermal phenotypes of p63-/- embryos, but instead aggravate the skin phenotypes of p63-overexpressing mice. The Dai laboratory is proficient in such mouse genetic experiments, and the proposed mutants are either currently available in the lab or can be readily obtained from others.

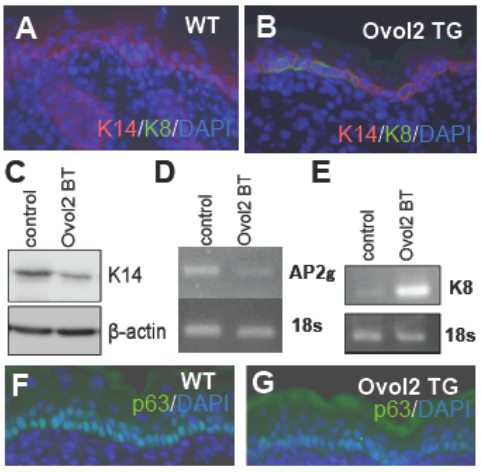


Figure 12. Preliminary evidence suggesting a functional convergence of Ovol2 and p63. (A-B) Immunofluorescence results revealing increased K8 expression in Ovol2-overexpressing (TG) skin. (C-E) Quantitative analysis showing decreased K14 and AP2g expression, and increased K8 expression in TG skin. (F-G) p63 expression is not affected by Ovol2 overexpression.

We will also use biochemical/molecular approaches to directly test the individual regulation(s) identified by modeling as being important. For example, if the model predicts that a negative regulation of Ovol2 by p63 is essential for robust epidermal stratification and for the epidermis to reach its optimal size, we will then 1) test if Ovol2 expression is increased in p63-deficient epidermal cells; 2) test if overexpression of p63 represses the Ovol2 promoter in epidermal keratinocytes using luciferase reporter assays; 3) test if p63 binds to the Ovol2 promoter in epidermal keratinocytes using the aforementioned ChIP assay. All these methods are well-established in the Dai laboratory.

2e: Develop and improve multi-scale and hybrid models coupling intracellular networks, with cellular interactions and extracellular morphogens in both discrete and continuum descriptions

We will use continuum models, discrete cell models and hybrid models to study the proposed biological hypotheses. The choice of models depends on the hypothesis that we aim to test and strengths and weakness of each type of model.

	Matlab	C code	GPU code
ODE	80+ (h)	10+ (h)	25 (m)
PDE	2+ (h)	15 (m)	15 (s)
SEM	Unknown	180 (s)	10 (s)

Table 1. GPU algorithms have significant speedup compared to C code and Matlab. Times are approximate for solving 10^6 ODEs, a reaction-diffusion PDE on 100x100 grid, and subcellular element method (SEM) for 128 cells.

Spatial cell lineage models In this model, different types of populations and the transitions through differentiation and proliferation are explicitly modeled (50-53) with regulations typically modeled through the total cell numbers of one population. This type of model usually results in moving boundary systems for a growing tissue. Because the epidermal boundaries usually do not deform much from a flat surface, most of the standard interface tracking methods, such as front tracking (54-57), level set methods (58, 59) and phase field methods (60, 61) may be applied directly. Previously, we have successfully applied it to studying multi-stage cell lineages and tissue stratification in olfactory epithelial growth (47).

Discrete cell and hybrid models One of the major disadvantages of the continuum models in Aim 1 is that cell-cell interactions, such as Notch-Delta signaling, cannot be modeled directly. Instead, some sort of local averaging has to be used, as demonstrated for cell-cell adhesion in a recent advance of continuum modeling (62). An alternative *cell ensemble* (63, 64) or *agent-based model* (65-67) can describe such cell-cell interactions more directly. In addition, an intracellular gene network driving individual cell behavior can

be incorporated within each cell in a straightforward fashion. These modeling frameworks that couple intracellular networks with populations of spatially arranged cells can be extended to incorporate continuum models for spatial dynamics of extracellular secreted molecules (e.g. TGF-α and TGF-β). Here we will address the primary challenges for these hybrid multi-scale models including proper coupling between discrete and continuum descriptions, coupling between multiple temporal and spatial scales, and efficient computational algorithms.

GPU Algorithms We have developed the computational tools necessary to simulate sophisticated 3D biological models that integrate numerous biological phenomena such as spatially-explicit heterogeneous cells, intracellular gene networks driving individual cell behavior with the network coupled to cell-cell and cell-environment interactions, and extracellular spatial dynamics of diffusing molecules (19). Our tools are based upon graphical processing unit (GPU) algorithms which are tightly-coupled data-parallel processors found in inexpensive video cards. With careful programming they can be significantly faster than traditional programming environments. Table 1 compares the execution times between GPU, C and Matlab for a few mathematical models. Our expertise with this exciting new hardware architecture will enable us to evaluate millions of spatial models over the course of this project.

Cell movement and Mechanics A major challenge for modeling cell proliferation and growth of tissue is to provide rules for cell movement and the mechanics effect. The epidermis system involves most likely passive cell movement caused by division, differentiation, and mechanical effect in the tissue. Our preliminary model of epidermal development (19) utilizes the sub-cellular element method (68), which provides a continuum description of growth and movement for discrete cells. This approach allows coupling to external fields such as assuming that cells move along their "pressure" direction (69) using so-called multi-phase models adapted from studies of flows in porous media [e.g. (70) for application in tissue engineering and (71) for a review of applications to tumor modeling]. We will also explore agent-based models with standard discrete rules for cell movement and growth (66, 72-74).

Extracellular spatial dynamics of molecules The reaction-diffusion systems for modeling secreted extra-cellular molecules are usually very stiff due to drastic differences in reaction time scales. The stiffness puts great constraint on time step size for explicit temporal methods; on the other hand, implicit methods requires extra and large computational costs for solving nonlinear systems at every time. We plan to use our recently developed fast, efficient, and semi-implicit temporal methods (1, 2, 75) to circumvent these issues.

Intracellular gene network Computational techniques to simulate gene networks include deterministic approaches as represented by a system of coupled ordinary differential equations (ODE) (76) or stochastic approaches (77). We will initially focus on translating the network structure into ODEs and use standard numerical methods to simulate their time evolution, but we will utilize stochastic differential equations (SDE) and stochastic simulations to study how small population stochastic effects play a role in the dynamics. We will consider nonlinear Hill-type functions (76, 78) for our gene regulatory networks as they have the ability to express the more sophisticated behaviors of cooperativity, thresholds and saturation. Cells can be given identical parameter values or varying values to introduce intrinsic cell heterogeneity. Our GPU algorithms can simulate a large number of ODEs in parallel, and if the number of equations in the ODE is large then we can simulate each equation in parallel as well.

Cell-cell signaling Genes annotated as being part of a signal transduction pathway will automatically generate any additional intracellular model components necessary to complete the pathway. For example, we are not measuring receptor expression so a dummy variable will be created in the model with the assumption of sufficient receptors to transmit the signal. **Preliminary data**: our GPU algorithms implement multiple signal transduction pathways, such as the TGF-b signaling pathway, in a 3D spatial environment where they are coupled with each cell's internal gene network. *Secretion and reception* Genes annotated as secreted factors will automatically generate the additional model components to represent the population of gene products separate from the gene's expression. Likewise, the local concentration of extracellular molecules can be calculated to provide reception of those signals by a cell and incorporation into its internal gene network. **Preliminary data**: our GPU algorithms implement the secretion and reception of extracellular factors by discrete cells in a 3D spatial environment.