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Network Modeling of *Drosophila* External Sensory Organ Precursor Formation: The Role of Recently Studied Genes

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In order to find out how computational modeling in dynamical simulation of molecular biological network may help researches in genetics and molecular biology, we have performed computer simulation on cell fate determination in development. For sensory organ precursor (SOP) formation in Drosophila development, we have investigated a number of models involving lateral inhibition through cell-cell interaction. For genes that were recently identified to be associated with formation of SOP, senseless and phyllopod, we test the models' robustness through the parameter space of the models. We conclude that the control of the lateral inhibition network is further enhanced by the new reactions.

Key words: lateral inhibition, computer modeling, genetic network, sensory organ precursor, Drosophila, senseless, phyllapod.

Introduction

A living organism is a very complex system of biological chemistry that has many special properties: it has highly precise control capability when necessary; it is able to function normally over a range of fluctuation of environmental factors or even individual differences; it can adapt to changes in the environment[4, for example], and it evolves and mutates over generations. A static description over the elements and pathway properties of the network provides desirable information to understand their roles and some of their control properties. However, without a dynamical description, we would probably never be able to completely understand the whole picture. For example, as shown previously, nonlinear dynamics has offered the capability to generate a desirable pattern from natural occurring noises[10, 34, 35]. Such a property is often not obvious from our intuition. As another example, the role of redundant elements or even redundant pathways is not quite clear until a modeling study and detailed analysis[16, for example]. In principle, it is clear that a dynamical

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description of biological networks is necessary in order to understand how living organisms live and what may be the factors that contribute to their living.

With today's advanced computer hardware and software technologies, dynamical simulation of biological networks can be performed rather easily. However, it is not totally clear how such studies may directly help molecular biologists understand their experimental results. Compared with studies in other disciplines such as chemistry and physics, in the field of molecular biology, detailed theoretical modeling is not yet one of the routine tools in most laboratories, though a few research groups have already started testing hypotheses with numerical modeling and comparing the computational results with experimental ones[5, 6, 12]. At this point, we believe that it is necessary to explore the general aspects of modeling simulation *for molecular biology studies*: how one may build a reliable network and what kind of information network modeling can provide. In the present work we present the results and conclusions from such explorations.

In order to perform a dynamical simulation for biological networks, the *quantitative* aspect of the model needs to be constructed. It is now readily possible to obtain a *qualitative* model from molecular biological experimental works (i.e. the elements and connection among elements - activation, binding, repression, etc. - in the network), but *quantitative* information is rarely provided. For example, the amount of a particular protein involved and the reaction rate constants are not available from typical molecular biological or genetical experiments. Time-dependent and quantitative measurements are necessary for such information, but such measurements are not routinely performed in many areas of study. (The study of signals transduction in neurons is one exception, as shown in ref. 5)

The robustness of biological networks is a remarkable property that differs biological dynamics from most of other nonlinear dynamical systems. For example, sensitivity to initial conditions is known to be one special property of nonlinear dynamic systems when "chaos" occurs. Such sensitivity can be seen in the analysis of economical changes or weather forecast modelings. However in biological systems, such sensitivity does not usually exist except when the biological function of the system is to sense certain changes internally or externally. For example, a living organism is able to maintain its living condition over a range of temperature or different levels of food supply. Another observation comes from having seen similar biochemical systems over a range of species. Homological enzymes perform their function almost equally well despite of mutations and differences in the *in vivo* conditions. In other words, we can probably assert that biological systems are robust machineries that are highly specialized in their own functions.

In this article, we explore the robustness property of the biological systems. In ref 36, it has been demonstrated that plausible and implausible qualitative models can be differentiated by probing the space of possible parameters for the reaction kinetics. A set of parameters is determined to be "possible" when the corresponding network can generate the desired output (in ref 36, it means having the segment polarity pattern formed among a linear cluster of cells). It is found that when a model is plausible, the possible parameter space is very much larger than that of an implausible network model, as indicated by the quantity "hit rate" when a random search is performed by a computer program. It was further commented later, that the robustness may serve as a measure of plausibility [23]. As will be discussed below, it is possible to construct a biologically plausible model at both qualitative and quantitative levels. The topology of models is determined by experimental results of

molecular biology. Such topology can be tested against its robustness over the unknown parameter space. For models that are highly robust, we plan to further refine the quantitative range parameters by requiring the model to perform similarly to experimental observations.

In development, cells of the same or similar nature later differentiate into different precursors of tissues or organs. It is now known that through cell-cell interaction the activity of genes in each cell can be controlled and thus cell fate can be determined. Lateral inhibition is one such process where one or a few cells are slightly favored through random fluctuation or certain pre-patterning mechanism, then the favored cell(s) suppresses its (their) neighbors from assuming the same fate, presumably through cell-cell interaction. One classic example of lateral inhibition is in the neurogenesis. In *Drosophila*, the formation of sensory organ precursors (SOP) is one of such well-studied examples where lateral inhibition is performed through a number of genes that control cell fates. Modeling study of lateral inhibition offers an ideal test bed of how computational works may help understand the system behavior and its dynamics, especially in testing the role of new genes associated with the system.

In this article we describe how one can construct a quantitative model for the lateral inhibition in the formation of SOP as an example, from qualitative information in the literature.

Methods

We have simulated the dynamic behaviors of all nodes (namely mRNAs and proteins) listed in Fig. 1 by using the network simulator Ingeneue v.0.8[20], performed on IBM compatible personal computers running Linux. In the simulation, the concentration of each component in each cell is calculated through a set of ordinary differential equations (ODEs) including terms that represent transcription, translation, degradation, dimerization, recycle, and diffusion over the cell membrane. Detailed mathematical representation of those processes can be found in the supplemental material provided for ref.21. A group of 5×5 cells are modeled in the present study. Intercellular interaction only occurs for Notch-Delta binding across neighboring cells. Each cell is modeled to has six neighbors. Inside each cell it is modeled as a constantly stirred tank reactor (CSTR), i.e., with the concentration of each component being a real number across the whole cell.

As in an earlier work,[21] the initial conditions were set for Achaete (Ac), Scute (Sc) and their mRNAs at normalized concentration of 0.05 in the central cell of the proneural cluster, surrounded by cells with these four components at normalized concentration of 0.025. For proper boundary effect, we have added a third layer of cells with Ac, Sc and their mRNAs at zero concentration. Ubiquitously expressed genes such as *Notch(N)*, *daughterless (da)*, *Hairless (H)*, *seven in absentia (sina)* and *tramtrack (ttk)*, are set to be initially 1.0 in its relative concentration, with a constant activator at concentration of 0.25, as used in the previous study by Meir et al[21]. The simulation starts at 8 hours after puparium formation (APF) and we propagate the ODEs for 4 hours. At 12 hours APF, the SOP formation is judged by the condition where the central cell has Ac and Sc greater than concentration of 0.20 and the surrounding cells less than concentration of 0.02.

At the beginning of our simulation, every set of the parameters in all of the models were randomly assigned. If an SOP cell is successfully formed at the end of simulation (12 hours APF), the parameter set are recorded as a valid set. We acquire the "hit rate"*(R)* by dividing the number of valid parameter sets by the number of all random sets tested. The averaged probability of

obtaining a valid value for each parameter is the *n*th root of the hit rate, where *n* being the total number of free parameters. With such averaged hit rate, we are able to understand how well a particular network model is in resisting parameter variation, as a measure of its robustness and possibly a measure of plausibility.

Results

The body of an adult fruit fly is covered with external sensory (es) organ which contains a bristle and associate cells. The formation of an es organ involves expression of proneural genes *achaete (ac)* and *scute (sc)*, leading to the formation of the SOP in imaginal disks (reviewed in ref.8, 13). Initially each cell in the "proneural clusters" is competent to form a neural precursor, but only one single cell in a cluster actually forms an SOP later. It has been shown [15, 25, 31] and computationally demonstrated[10, 21] that the Notch (N)-Delta signaling pathway offers the competition mechanism among neighboring cells through lateral inhibition. Such cell-cell interaction pathways has been reviewed in ref.1, 14.

Genes controlling Drosophila SOP formation

Recently a few more genes were found to be associated with the SOP formation. We briefly outline these studies below. Four different network models are constructed from these studies as shown in Fig. 1.

Sens is a nuclear protein with four Zn fingers, whose expression is dependent on proneural genes *(ac, sc)* and *daughterless (da)* [24]. Sens is expressed and required in SOPs of imaginal discs, and Sens is suffcient to induce the development of external sensory organs . On the other hand, it has been suggested that *sens* could be inhibited by Enhancer of split (E(spl)), as a similar regulation seen in the genes *ac* and *sc*. In addition, Sens indirectly promotes Ac, Sc and Sens itself, possibly through binding to E(spl) and blocking the inhibition activity of E(spl)[17].

Hairless (H) is an ubiquitously expressed gene required for a relatively late step in the development of the proneural clusters. Mutation in *H* almost always leads to failure in SOP formation, even for a single cell with high levels of *ac* and *scabrous (sca)* expression that is a characteristic of SOPs[3]. Furthermore, the activity of the genes of the *E(spl)* complex is required for this failure, which was demonstrated by epistasis experiments [3]. Another observation related to *H* is as follows: when an active form of *N* receptor is over expressed, the SOP formation fails and a loss of bristles is observed. Simultaneous overexpression of *H* has the capability of suppressing such effect, recovering the SOP formation[3].

The gene *phyllopod (phyl)* is required for the cell fate determination of photoreceptor cells[9, 11]. Recently it is shown to be required both in the formation of SOP cells and in a later stage of es organ development[27]. The gene *phyl* is activated by Ac/Da and Sc/Da and inhibited by E(spl)[26]. Further observation suggests that Phyl and H form a complex with Suppressor of Hairless (Su(H))[26]. It is believed that the complex H/Su(H)/Phyl has an ability to antagonize the Notch signaling from neighboring cells.[26]

Asense (ase) is a member of the achaete-*scute complex* (AS-C) genes which encodes a bHLH protein. Unlike other AS-C members, *ase* is identified to be a *Drosophila* neural precursor gene expressed specifically in SOP and is involved in SOP differentiation [7, 18]. *ase* is found to act downstream of proneural genes. Misexpression of *ase* leads to a gain-of-function phenotype that is characteristic of misexpression of proneural genes. Therefore *ase* has the capability to initiate the sensory organ fate in cells [7].

Tramtrack (Ttk) is a Zn finger DNA-binding protein that suppresses *ac*, *sc* activity

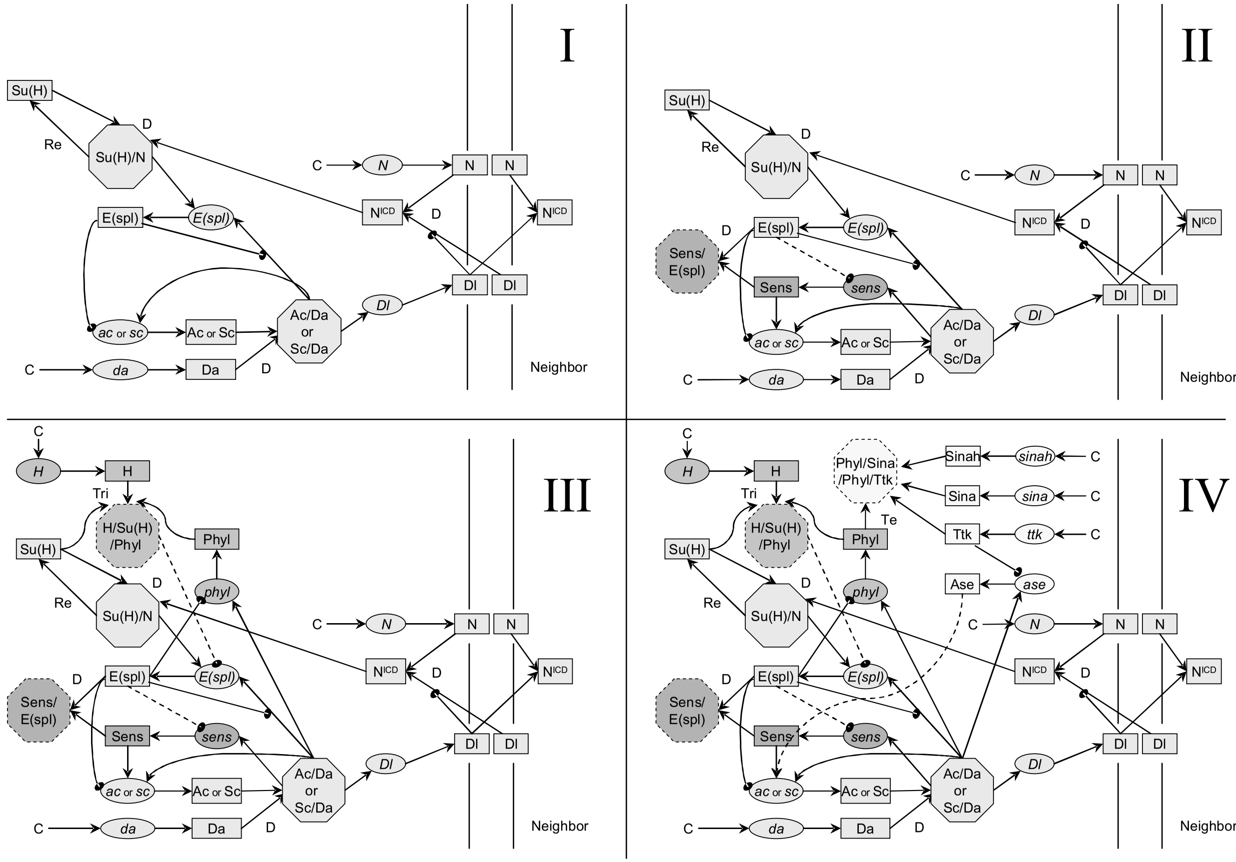

Fig 1. The neurogenetic network in *Drosophila*: The networks studied in the present work. Ovals stand for mRNAs, rectangles for proteins, and octagons for protein complexes, including dimer, trimer, and tetramer. If not indicated, arrows represent activation of transcription (from protein or protein complex to mRNA in this case) or translation (from mRNA to protein), while lines with a circular dot represent inhibition. If the entire combined multi-regulation of certain mRNA is inhibited, the circular dot of the inhibition line is placed on the oval symbol of this mRNA. On the other hand, if only a specific reaction is inhibited the dot end is placed on the reaction arrow. Interactions and complexes under our speculation are shown by dashed lines. Though not shown, each node in this network has its own degradation, characterized by half life time, except Su(H) (whose amount is affected only by "recycle", see text for details). In order to mimic the effect of the non-existing Sina/Phyl/Phyl/Ttk complex formation, in model III we have added a dimerization reaction for Phyl (not shown).

Abbreviations: "C" = constant transcriptional factor, "D"= dimerization of proteins, "Tri" = trimerization of proteins, "Te" = tetramerization of proteins, "Re" = recycle.

and inhibits *ase* expression. In the *ase* promoter region, there are many clustered consensus Ttk69-binding sites, which indicates that Ttk can inhibit the expression of *ase* by directly repressing the proximal promoter [2]. Since Ttk also suppresses *ac* and *sc* expression, we speculate that *ase* positively regulates *ac* and *sc* as a mediator for this suppression. On the other hand, it is observed that AS-C/Da-binding sites exist in the *ase* regulatory region. Deletion of these binding sites leads to a reduction in *ase* expression level[18]. Therefore, in our model *ase* is positively regulated by Ac/Da and Sc/Da.

Phyl was found to function together with Seven in absentia (Sina) to antagonize the activity of *ttk*[33], possibly by degrading the Ttk protein, to promote fate specification of SOP[2]. However, the heterodimer interaction of Sina/Ttk are quite weak in recent observations[19]. On the other hand, the dimer interactions for Sina /Phyl and Ttk/Phyl, as well as the homodimer Phyl/Phyl seem to be quite strong[19, 26]. It is believed that either the trimer (Sina/Phyl/Ttk) or the tetramer (Sina/Phyl/Phyl/Ttk) based protein complex is responsible for such degradation. In the current study, we simulated this part of reaction on a tetramer basis.

When a cell receives a weaker N signaling from neighboring cells than the signal it sends out, Phyl in this cell has a chance to degrade Ttk with ubiquitously expressed Sina. Meanwhile, the neighboring cells receive more Delta ligand (Dl), enhancing N signaling in them, antagonizing *phyl* and therefore SOP formation is inhibited through E(spl).

Model construction

In order to study the influences of various cluster of components, four different models are adopted in the present work (as shown in Fig. 1). The “augmented” model from Meir et al. [21] is modified here as our first model (model I). Since there is a high degree of homology exists between proneural genes *achaete (ac)/scute (sc)* and their products, the corresponding reaction parameters utilized in their regulatory actions were set to be of the same value, subject to random search of a computer program. This is to reduce the number of independent parameters in the exploration of parameter space, while keeping the model close to reality, so that the search of possible parameter sets is easier. In a later stage one can always introduce fluctuations for minute variation that may exist between these two genes.

Model II includes model I and the gene *senseless (sens)* with related interaction paths. In this model, *sens* is activated simultaneously by both Ac/Da and Sc/Da dimers. The protein Sens activates *ac* and *sc*, thus forms a positive feedback loop [17, 24]. Inhibition by E(spl) on *sens* transcription and binding of Sens with E(spl) are also included as discussed above.

Model III includes model II plus several reactions involving Phyl and H. This part includes the regulation of the gene *phyl* and formation of the complex H/Su(H)/Phyl as mentioned above. To provide a ground for the requirement of *E(spl)* in the effect of *H* mutants, a suppression effect on *E(spl)* transcription by the complex H/Su(H)/Phyl is included in this model[26].

Model IV includes model III as well as

Table I: Results of probing the possible parameter space for the models studied

Models	n[a]	R[b]	$R^{1/n}$ [c]
I	46	1/191	0.8921
II	60	1/142	0.9207
III	81	1/128	0.9419
IV	105	1/139	0.9541

[a] Number of independent parameters of the model

[b] Hit rate

[c] Averaged hit rate (per parameter)

the interactions involving Ase and the tetramer Sina/Phyl/Phyl/Ttk. Ase forms a positive regulatory loop with AS-C/Da complexes by (postulated) positive regulation to *ac* and *sc* genes, and with *ase* being positively regulated by AS-C/Da. In *Drosophila* adult flies, the bristle phenotypes of *sina* mutants are weaker than those of *phyl* mutants. It is possible that maternal transcripts of *sina* could explain this phenomenon. On the other hand, a *sina*-independent pathway is another possible cause[27]. A homolog of *sina (sinah)* is therefore introduced as a redundant pathway to conform to this experimental observation.

Computer simulation

As the major result in this report, in Table I we demonstrate that the possible parameter space probed increases as we include more elements and pathways from recent experiments. Hit rate R measures the probability of obtaining a set of parameters that can generate an SOP out of a cluster of cells with the condition described above. The *averaged* hit rate, $R^{1/n}$, represents the averaged probability of each parameter being in the SOP formation range. As shown in Table I, the overall hit rate increases as the model grows from I to III. From model III to IV the hit rate decreases by a small fraction, while the *averaged* hit rate increases significantly. Such result indicates that the possible parameter space increases with our new models.

Discussion

The results of increasing possible parameter space with *sens* and *phyl* pathways may be understood as a natural consequences when the network model has more "control power" in generating the desired output (i.e. SOP formation). It can be seen that both *sens* and *phyl* offer redundant control pathways to enhance *ac* and *sc* levels in SOP cells. A similar observation has been made in ref. 27 where *phyl* mutants are shown to have clusters of *ac-lacZ* expression remaining while in normal wild type fruit flies such expression has been confined to single cells. Even though a network without *phyl* would still contain many of the essential elements for lateral inhibition, the result of having much fewer bristle formation in the *phyl* mutant can be understood as not having the enough controlling capability over the natural fluctuation in different clusters of cells. Here our results of increased possible parameter space are consistent with experimental results in a different measure of robustness in biological networks.

There are a few additional remarks regarding the details of our simulation work. First, following the previous work[21], there is one reaction called "recycle" in our network, appearing at the connection between Su(H)/N and Su(H). It is a simplified way to simulate a constant production rate and a equal degradation rate for mRNA of *Su(H)*. The total degradation rate of Su(H)/N and Su(H) is assumed to be the same as the production rate of Su(H). Therefore, there is no need to write (and solve) a separate ODE for *Su(H)* mRNA. In other words, the total amount of Su(H)/N plus Su(H) is conservative, and thereby, a recycle between these two is suffcient for this part of our first and second models. Second, the term N^{ICD} (Notch intracellular domain) is used to stand for the active fragment produced through a mechanism involving proteolytic cleavage in response to ligand binding [30, 32]. In our present work, N^{ICD} was simulated as equivalent to the amount of the N/Dl dimer. After the proteolytic cleavage, the extracellular part of N and the Dl ligand will degrade with no activity. Therefore the active N^{ICD} concentration is equivalent to the amount of the N/Dl dimer.

Actually there are some other aspects of SOP formation that are not included in the current study. For example, the possible

"long range" interaction involving the gene *scabrous*. At the time of selecting SOP, there are 4-5 layers of epidermal cells between SOP cells. Not all of them are in direct contact with an SOP cell. Therefore it is interesting to find out whether a model with only neighboring cell-cell interaction would be suffcient to laterally inhibit the next-nearest neighbor cells from becoming an SOP cell. It is not yet confirmed either, that whether the N-Dl pathway along can give rise to the evenly spaced bristles (eventually bristles are about 5 epidermal cells in distance). The gene *scabrous*[22] is studied under this context. It is concluded that *scabrous* is required for inhibition of cells not adjacent to the precursor[28]. Moreover, the role of *scabrous* on forming the orderly spaced bristles has also been explored[29]. While it would be very interesting to model and observe the consequences of such long-range signaling and the pattern formation among bristles, in the present study we focus on building a correct and realistic model for short-range lateral inhibition. Such model can then be extended to include the long-range inhibition function of *scabrous* to study its role on the overall bristle array patterns in the future.

In summary, we have reported the results of randomly exploring the parameter space for the lateral inhibition network in *Drosophila* SOP formation. The genes *sens*, *phyl* and their related partners have formed regulatory pathways that enhances the SOP formation conditions. We have shown that, in terms of possible parameter space (hit rates), those pathways have indeed enhanced the robustness of the overall SOP formation model.

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