SIMFLY2: Simulation of a Fly Embryo

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Abstract

Biological analysis of segment formation in Drosophila embryogenesis provides a good ground for modelling interaction of DNA-binding proteins. In this paper, we propose a threshold model for qualitative simulation of the interaction, and introduce SIMFLY2. This revised version of SIMFLY, a simulator for protein interaction, integrates genetic algorithm for the search of optimal relations among proteins. We confirmed that SIMFLY2 did find the relation we had found last time in SIMFLY by exhaustive search. SIMFLY2 also found interaction models between two pair-rule proteins and gap proteins.

1 Introduction

Many DNA-binding protein molecules are said to have a symbolic function resembling a digital switch; they can turn on or off the activation of other protein molecules. In literature, the function of this genetic switch (GS) is characterized as activation, repression, or squelching [4]. This intuitive classification shows that GS works with crisp response, not with gradual or differential reaction. With this perspective exaggerated to the extreme, the function can be modelled with Boolean logic. However, quantitative features such as the concentration of regulatory molecules and their kinetic parameters let them act not the simple switch with on and off, but the multi-leveled volume switch. Then, what model instead of Boolean logic would be appropriate for this volume switch? So far, some enzyme reactions have been successfully described with differential equations. In most reactions, however, only qualitative relation is known and quantitative parameters are totally unknown. So we need more qualitative, intuitive model than the model with differential equations. Simple, yet eloquent model is the one we desire. In this paper we introduce “threshold model” for the qualitative simulation of this volume GS.
The crispness of the GS, often understandably denoted using a sigmoidal curve, depends on the concentration of the proteins and the sensitivity of DNA sites. As the primary approximation instead of complex interaction at the molecular level, we consider only proteins, and no DNAs or RNAs. More specifically, only amounts of proteins are considered. Following is the basic interaction scheme in our model.

\[
\text{if } \text{Threshold } \alpha > \text{amount}(A) > \text{Threshold } \beta \text{ then create}(B)
\]

\[
A, B \quad \text{proteins}
\]

\[
\alpha, \beta \quad \text{threshold-amounts of proteins}
\]

With these thresholds, we can describe following regulations.

**Activation**

\[
\text{if } \text{amount}(A) > \text{Threshold } \beta \text{ then create}(B)
\]

**Repression**

\[
\text{if } \text{Threshold } \alpha > \text{amount}(A) \text{ then create}(B)
\]

**Competition and Cooperation**

\[
\text{if } \text{amount}(A) > (\leq) \text{Threshold } \alpha \text{ or } (\leq) \text{Threshold } \beta \text{ then create}(C)
\]

We applied our threshold model to an embryo of a fruit-fly. The embryonic segmentation of *Drosophila melanogaster* is famous for being regulated by complex genetic network (interaction among proteins), but its mechanism of interaction is little known, leaving most part to be elucidated in molecular biology. The prediction of this relationship would be a great help in determining the direction of biological experiments.

Last year we built Simply, a simulator for checking and predicting relation among proteins, as well as for the grasp of expressive power of our threshold model. We simulated the relation among gap proteins using exhaustive search, and found the following result.

- The repressive power of *Kr* and *kni* proteins against *gt* are greater than that of *gt* protein against these.

This result assumes that these three gap proteins only repress with one another, an assumption which is hardly true in a real embryo. We had to consider other cases in which each protein has its activation site. We also wanted to further simulate pair-rule protein stage, formation of 7 stripes after gap protein stage. But, there was a problem: immensely vast search space. This time, we employed genetic algorithm (GA) for the search and found the seemingly good relation among pair-rule proteins and gap proteins.

In the next section how the embryo of *Drosophila* develops is briefly described. In Section 3, a related simulation of this embryogenesis is introduced and compared with our model. In section 4, our Simply2 system – what threshold model is, how GA is used, and what result is obtained – is explained. The last section is allocated for discussion, current problems, and future work. After Conclusion, some rule patterns obtained by our system are described in Appendix.
2 Pair–rule Proteins

The process of segmentation in *Drosophila* blastoderm proceeds in a hierarchical manner in which the middle part of an embryo is stepwise divided into 14 segments. Segmentation starts with localized activities of maternal determinants: *bicoid* (*bcd*) in the anterior pole, and *nanos* (*nos*), *oskar*, and *caudal* in the posterior. Anterior zygotic proteins including *hunchback* (*hb*) and *Krüppel* (*Kr*) are activated by *bcd*, while posterior *knirps* (*kni*) and *giant* (*gt*) are activated by *caudal* [7]. These four zygotic proteins as well as *tailless*, *hairy* and three others are called *gap* proteins and are regulated by one another. The relation among these proteins are not completely clarified; *hb*, *Kr*, *kni*, *gt* are known to repress with one another in high concentration, but *Kr* and *hb* activate *kni* and *Kr* respectively in low concentration [3, 9, 10]. These fine *gap* tunings are the result of cooperative regulation of proteins.

![Pair-rule Diagram](image)

Figure 1: Segmentation

There are two steps in making *pair–rule* pattern. Primary genes including *even-skipped* (*eve*), *hairy*, *runt* are regulated by *gap* proteins, and are responsible for the secondary genes including *fushi-tarazu*. Here, two protein pairs, *hairy* against *runt* and *eve* against *fushi-tarazu*, complementarily emerge and form 7 stripes each. These pairs are expected to repress mutually.

The regulation mechanism is little clear [2, 6, 8], but these observations show primary *pair-rule* genes are cooperatively activated or repressed by neighboring *gap* proteins. What we want to know is following aspects.

- Are gradients of only *gap* proteins enough for the primary *pair–rule* activation?
- Does each stripe has its own enhancing region?

3 Related Work

Reinitz and Sharp [5] have done a good work on the same subject; they used differential equations and determined the parameters among *gap* and *pair–rule* proteins by simulated annealing.
Their prediction contains repressing and activating power, as well as diffusing, decaying, and producing rate of proteins. After having computed quantitative biological data, they drew qualitative relationship among proteins. For example, they found that even-skipped (eve) protein hardly diffuses.

Our approach is different though our aim is the same. We assume qualitative result can be drawn from qualitative input. As for the previous example, the fact eve protein hardly diffuses is clear from the sharpness of 7 stripes. (If it should diffuse, the borders of stripes must blur.) We uses only qualitative data; spatial relationship among proteins. With this abstraction, however, we have to give up simulating temporal order of stripe formation and the width of stripes. Though there are drawbacks that diffusing and producing rate are ignored, we believe that still much to see remain in the qualitative result.

4 Simfly2

In Simfly [1], relation among proteins, activation or repression, were specified in advance, and the simulator searched intensities of regulators (threshold values) only. The vast search space hindered us to check every regulatory relation. To overcome this problem, Simfly2 employs GA. Abstraction of proteins is almost the same as Simfly. Production rate is fixed to 1 unit for all the proteins. Diffusion rate is fixed for each hierarchy: maternal-effect, gap, and pair-rule. For efficiency, new version has decaying rate (no “lifetime” as in Simfly); in each time step for all the proteins, 20% of the total amount is deleted. This shortcut does not change the maximum amount of protein in Simfly. We confirmed, by checking each pattern produced in Simfly2, that this shortcut made little change in the simulated pattern.

4.1 Threshold Model

Each protein is activated by, repressed by, or not related with other proteins. These relations are represented with two inequalities as in described in Introduction. Two threshold values range integers from 0 to 5. An example for gap proteins are shown below.

\[
\text{Rule 1}
\]

\[
\begin{array}{cccccc}
\text{bcd} & \text{caudal} & \text{hb} & \text{Kr} & \text{kni} & \text{giant} \\
\text{[hb 25 4 } & \text{[((A 2) (N .) (N .) (R 3) (O .) (O .)]]} \\
\text{[Kr 25 4 } & \text{[((O .) (R 3) (N .) (N .) (R 4) (R 2)]]} \\
\text{[kni 25 4 } & \text{[((N .) (S 4 1) (R 3) (R 4) (N .) (O .)]]} \\
\text{[gt 25 4 } & \text{[((N .) (N .) (N .) (R 4) (R 2) (N .)]]} \\
\end{array}
\]

Two integers right after protein names specify diffusion constants. These values do not change in the course of simulation. According to threshold values, there are four types of relations.

S: Squelching. Next two integers are the threshold of both the upper and the lower limit.

A: Activation. The threshold of the upper limit is always 5 (max-threshold).

R: Repression. The threshold of the lower limit is always 0 (min-threshold).
N: No relation. Proteins do not interact. If the lower threshold is larger than the upper in S relation, corresponding two proteins do not interact, either.

A relation \((S 4 1)\) in \(kni\) rule of caudal column is interpreted as

\[
\text{if } 4 > \text{amount(caudal)} > 2 \text{ then create(kni)}.
\]

From Rule 1, you can tell that \(hb\) emerges only when \(bed\) is more than 2 and \(Kr\) is less than 3. 

Pair-rule proteins may have multiple regulatory loci. Each relation in a rule is interpreted as conjunct, and each juxtaposed rule as disjunct.

\[
\begin{align*}
\text{name } &\left[ ((S1) (S2) (S3)) \right. \\
\text{name } &\left[ ((S4) (S5) (S6)) \right. \\
\rightarrow & (S1 \land S2 \land S3) \lor (S4 \land S5 \land S6)
\end{align*}
\]

For example, assume protein \(hairy\) and \(runt\) have 3 regulatory loci each. Following is an example for these pair-rule proteins.

\[
\text{Rule 2}
\]

\[
\text{hairy 10 1} [ \\
[(N .) (N .) (N .) (N .) (S 5 4) (N .) (N .) (N .)] \\
[(N .) (N .) (N .) (N .) (N .) (S 3 1) (N .) (N .)] \\
[(N .) (N .) (N .) (S 4 2) (N .) (N .) (N .) (N .)]] \\
\text{bed caudal hb Kr kni giant hairy runt} \\
\text{runt 10 1} [ \\
[(N .) (N .) (N .) (N .) (S 4 2) (N .) (N .) (N .)] \\
[(N .) (N .) (N .) (N .) (N .) (S 4 3) (N .) (N .)] \\
[(N .) (N .) (S 4 2) (N .) (N .) (N .) (N .) (N .)]]
\]

The expression pattern with this rule is shown in Display 1. Note that there is no threshold of 0. No threshold of 0 as the lower limit means every protein must have at least one activator; no relation can be written only with repression.

4.2 Trimming Branches

\[
\text{Rule 3}
\]

\[
\text{ID EVAL} [ \text{h}b 10 1 [[[A] (R) (N) (R) O O ]]] \\
\text{Kr 10 1} [[O O O (N) (R) (R) ]]] \\
\text{kni 10 1} [[[O O (R) (R) O) (R)]]] \\
\text{gt 10 1} [[[O O O (R) (R) O) ]]]]
\]
Figure 2: Guessing Relations

From observations in genetics, we can logically guess the possible relation among proteins. From expression patterns reported in literature, many relations are determined to be never repressed, never promoted, or not related.

These restrictions can be used as in Rule 3. Blank braces are regarded as $S$ relation, in which two thresholds are required, while restricted braces requires only one or no threshold.

4.3 Genetic Algorithm

SIMFLY2 repeats the following cycle.

1. Create initial population.
2. Evaluate each individual and sort the population.
3. Leave better individuals and kill others.
4. Duplicate individuals, then crossover and mutate them. (Figure 3)
5. Goto step (2)

GA can search for pseudo-optimal values for a given problem, though its mechanism of performance has not yet fully analyzed. GA is a variant of hill-climbing method, and if the climbing terrain contains many sheer cliffs, it does not well work. The terrain of the problem depends on evaluation function, so what is important is definition of evaluation function. Evaluation function in SIMFLY2 is the same as that in SIMFLY; the number of matching interval relation of proteins. If the matching number of two rules are the same, the rule with less valid relations has a better score. (Remember that simpler rules are better.) With this evaluation function, the terrain still have cliffs; a single change in a rule may reduce the number of matching score by 3 or 4. Therefore, GA often fails to find the best answer. But note that GA is not an optimizing algorithm. It suffices if GA finds a new interaction among proteins in a several times of runs.
4.4 Result

First we tested the rule of previous Simply, and confirmed that GA did find the optimal relations we had found using exhaustive search in the last version.

![Optimal Pattern Diagram]

Figure 4: Optimal Pattern

**Rule 4**

\[
\begin{array}{cccccc}
\text{bcd} & \text{caudal} & \text{hb} & \text{Kr} & \text{kni} & \text{gt} \\
[\text{hb 10 1 [[(A 13)(R 1235)(N .)(R 3)(N .)(N .)]]}]
[\text{Kr 10 1 [[(N .)(R 3)(N .)(N .)(R 45)(R 13)]]}
[\text{kni 10 1 [[(N .)(S 4 1)(R 34)(R 4)(N .)(R 145)]]}
[\text{gt 10 1 [[(N .)(N .)(N .)(R 4)(R 13)(N .)]]}]
\end{array}
\]

Started with the total population 300, mutation rate 5%, crossover rate 80%, and survivors’ population 40 for each generation, GA converges within 20 generations to Rule 4. For R and A, only one threshold is shown. (The other is fixed.) Juxtaposed numbers mean that the threshold can take any of these numbers. Note that GA does not always find this result.
From this result, we can tell that repression of kni by gt and that of hb by nos are redundant. See the threshold takes from 1 to 5. We can draw Rule 1 from this result. For each run of GA, we can find a new regulatory patterns in this way. Some other patterns are shown in Appendix.

Next, we tested how pair-rule stripes can emerge from the optimal gap protein pattern. With relations for gap proteins fixed, 3 rules for hairy protein and 3 for runt were searched. If we do not allow 0 to be a threshold, Rule 2 are produced. If we allow, Rule 5 is produced. The expression pattern with this rule is shown in Display 2.

![Display 2](image)

### Rule 5

```
[haired 10 1 [ [N .] (N .) (N .) (R 4) (R 2) (R 3) (N .) (N .)]]
```

Note that both proteins contain many N rules. Simpler rules seem to emerge easier than complex ones. In Rule 5, runt does not regulate hairy. More closer look reveals that as much as 5 hairy stripes emerge through only repression by gap proteins (see the first rule for hairy). With repression only, many stripes can hierarchically emerge in this way, but it is unlikely that a protein has no activator. With activation, however, neither hierarchical regulation nor mutual interaction between pair-rule proteins is observed as in Rule 2.

## 5 Discussion and Future Work

### 5.1 GA Parameters

In GA, most important is the parameters set for each problem: crossover rate, mutation rate, and initial population. In fact, without knowledge of terrain of a given problem, we cannot properly determine these values. Currently the population is set to 300 ~ 400. Larger population is better, but we need to make the program more efficient to simulate with more population. Implementation on a more efficient machine is our future work.

### 5.2 Pair-rule Proteins

Primary pair-rule proteins, (eve, hairy, runt), interact with one another. We have simulated only hairy and runt protein, but other proteins need to be simulated to find the real interaction.
With 2 proteins only, however, the number of threshold values exceeds 40. To cope with more proteins, the simulator needs to be much more efficient.

6 Conclusion

We propose threshold model for qualitative simulation of proteins. After this model, Simfly2 is implemented for the simulation of DNA-binding proteins in Drosophila embryogenesis. Using GA, Simfly2 found the relation among gap proteins, interaction which Simfly found in our last work. Simfly2 also suggests following relation among gap and pair-rule proteins.

- Pair-rule proteins are regulated by a small number of proteins.
- Pair-rule 7 stripes emerge in hierarchical manner, if a protein may not have its own activator. If the protein must have its activator, stripe region is simultaneously specified through subtle changes in threshold values.

Acknowledgment

We thank Prof. Iba for his helpful comment and his suggestion about GA. We also thank Prof. Takagi at Human Genome Center (HGC) for letting us use the facilities in HGC. This work was supported by a Grand-in-Aid for Scientific Research on Priority Areas, ‘Genome Informatics’, from the Ministry of Education, Science, Sports and Culture of Japan.

References


Appendix

Another rule for gap proteins

Protein hb is known to be repressed by nos, but in our simulation, activation by bcd is sufficient for its localized expression.

\[
\begin{align*}
\text{hb} & \quad 10 \quad 1 \\
\text{Kr} & \quad 10 \quad 1 \\
\text{En} & \quad 10 \quad 1 \\
\text{gt} & \quad 10 \quad 1
\end{align*}
\]

Another rule for pair-rule proteins

(Activation always required. Display 2)

Hierarchical regulation is clear in this rule. First hairy emerges in and from between gap proteins. Next, runt comes out where there is no hairy.

\[
\begin{align*}
\text{hairy} & \quad 10 \quad 1 \\
\text{runt} & \quad 10 \quad 1
\end{align*}
\]

Another rule for pair-rule proteins

(Activation always required.)

This produces similar result as that of Rule 2. There is no hierarchical interaction between pair-rules.