Study on the Proportion Regulation of Cell Types in Dictyostelium(粘菌における細胞種の比例制御に関する研究)

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号	181
発行年	2000
URL	http://hdl.handle.net/10097/12871

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学位の種類	博士(情報科学)
学位記番号	情 博 第 181 号
学位授与年月日	平成13年3月26日
学位授与の要件	学位規則第4条第1項該当
研究科、専攻	東北大学大学院情報科学研究科(博士過程) システム情報科学専攻
学位論文題目	Study on the proportion regulation of cell types in Dictyostelium discoideum
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論 文 内 容 要 旨

1 Introduction

Tissues of multicellular organisms are in general formed by cell types whose relative populations are actively regulated. The maintenance of these tissues, for instance in a regeneration process, is achieved by cell type transdifferentiation, dedifferentiation and/or differentiation of pluripotent cells (the so-called stem cells) into finally differentiated cell types. Examples of capital biomedical relevance include the blood cell system and the epidermis. While the molecular details of the cell-cell signalling involved in these processes are being unveiled, the global mechanims that regulate the populations remain obscure. Using as a model system the social amoebae *Dictyostelium discoideum*, I have inquired into the principles of cell-type regulation at the tissue (collective) level both from an experimental and a theoretical perspective.

In *Dictyostelium* a multicellular aggregate, the mound, is formed by the cAMP-mediated aggregation of $\sim 10^2 - 10^5$ cells. This mound elongates into a migrating cylindrical finger, the slug, which under appropriate conditions becomes a mushroom-like structure, the fruiting body, which has dead vacuolated cells in the stalk and spore cells on top of it. Cell type pattern in the slug is highly organized along an anterior-posterior axis (see Fig. 1). The $\sim$ 20% anterior is mainly composed of prestalk (pst) cells (which finally differentiate into stalk cells). The remaining $\sim$ 80% posterior region is mainly made of prespore (psp) cells (which become spores) (see review [1]).

Slugs display the same qualitative cell type patterning for sizes varying more than 3 orders of magnitude in volume [2]. On the other hand, they can regenerate a normal cell-type pattern after removal of one of the cell types [3]. These observations led Bonner to propose that the proportion of cell types is regulated to have a constant value [2]. Several studies of Dictyostelium fruiting bodies, however, have suggested that proportioning in Dictyostelium differs systematically from true constancy [4].

2 Experimental results

2.1 Lack of regulation of cell type proportion within a 2.5 fold tolerance range

We have confirmed the dependence of proportion on size in the slug stage using a short-lived β-galactosidase as a reporter of the prestalk specific ecmA gene expression: the prestalk proportion decreases from $24 \pm 5\%$ in slugs of 10^3 cells to $10 \pm 3\%$ when 10^5 cells are present (see Fig. 1,2). Regeneration experiments suggest that this difference is not due to a modulation of the proportioning set-point by size, as one might have expected; instead there appears to be a regulatory "tolerance zone" at all sizes. After amputation of the whole posterior region, transdifferentiation stops after the fraction of prestalk has been reduced from 100% to $28 \pm 2\%$, well above the initial value of $10 \pm 3\%$, while after prestalk cells removal the transdifferentiation endpoint is about 10% (see Fig. 3). Most strikingly, we find no regulation at all after partial amputations of the prespore region. It seems that any prestalk proportion is stable between a $\sim$ 10% lower threshold and a $\sim$ 30% upper threshold.

2.2 The shape of the prestalk region is regulated

We now turn our attention to the issue of slug shape. Fig. 1, in which slugs of various sizes have been magnified to the same apparent size, suggests that large slugs are proportionately thinner than small ones. Quantitatively, this can be characterised by the aspect ratio (Length/Width). Fig. 4 shows that slug aspect ratio indeed increases with size, but that the aspect ratio of the prestalk region is size-independent. In Fig.5, which shows gal stained slugs at different times after posterior amputation, it can be seen that slugs progressively elongate as transdifferentiation proceeds, but that the aspect ratio of the prestalk region is always constant.

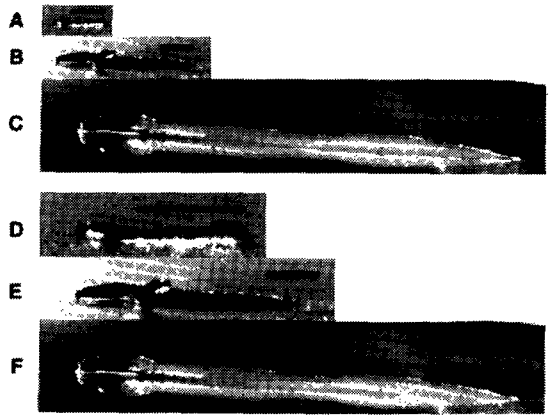

Figure 1: NC4-*ecmAO*-iα-gal transformant slugs of different sizes. The same slugs are shown at the same magnification in **A**, **B** and **C**, and after appropriate scaling in **D**, **E** and **F**, respectively. All scale bars represent 200 μm.

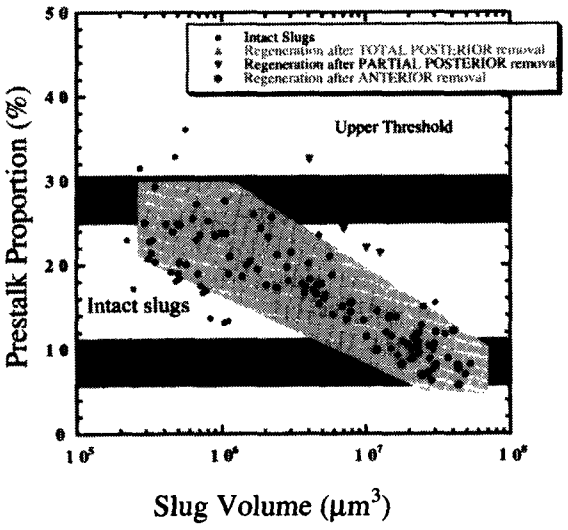

Figure 2: Prestalk proportion vs. slug volume. For intact slugs, proportion decreases with size. Slugs regenerated after removal of prestalk cells have similar proportions to intact slugs. Slugs regenerated after partial and total amputation of prespore cells display a prestalk proportion at the upper threshold of proportions observed in intact slugs. These results suggest that there is a tolerance region (10-30 %), within which there is no regulation.

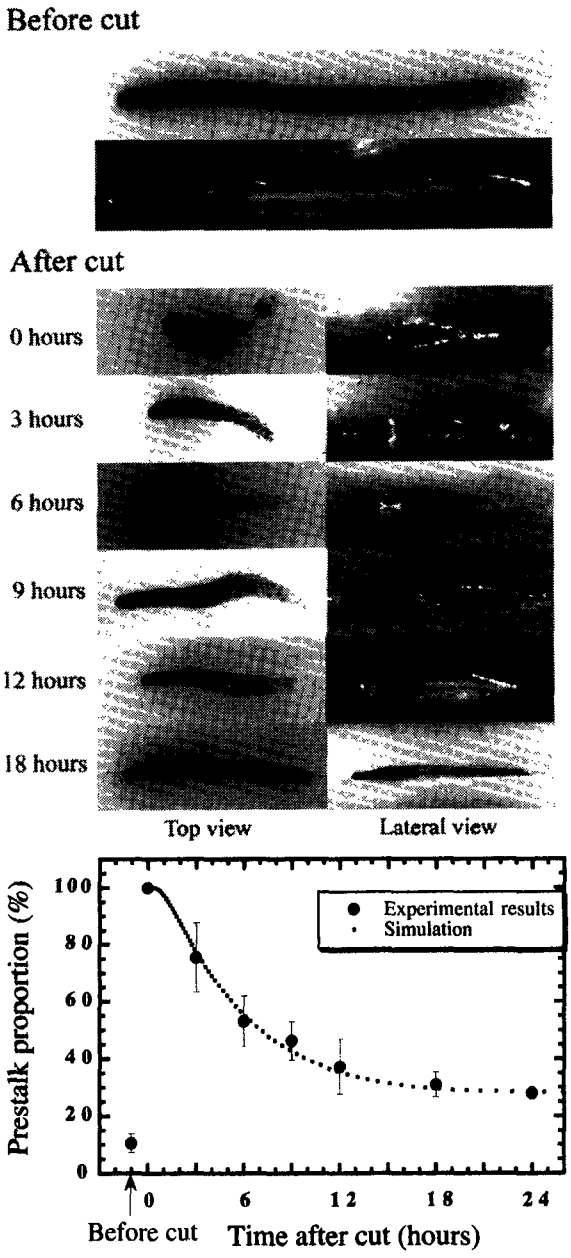

Figure 3: **Top.** Slugs of *Dictyostelium discoideum* before and during regeneration after total removal of the prespore cells. **Bottom.** Prestalk proportion before and during regeneration. Small dots show the results of simulation. Proportion is partially but not completely recovered.

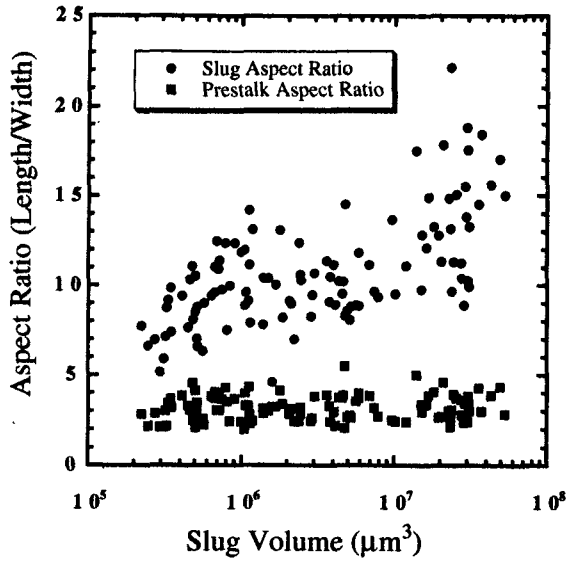

Figure 4: Aspect ratio of whole slug and anterior region vs. slug volume for intact slugs. A correlation is found between slug aspect ratio and slug volume: big slugs tend to be relatively slimmer than small slugs. However, the aspect ratio of the prestalk region remains constant.

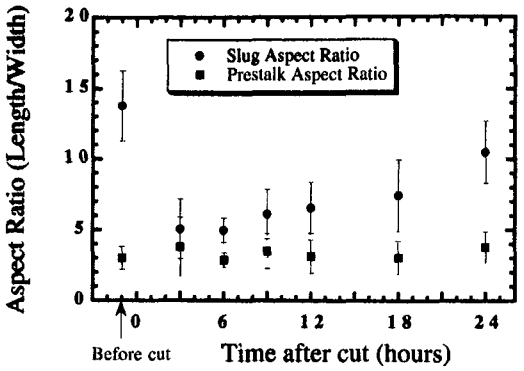

Figure 5: Aspect ratio of slug increases as regeneration proceeds while aspect ratio of prestalk region remains roughly constant at a value slightly above that of intact slugs.

At present we cannot explain this constancy in the aspect ratio of the prestalk region. In principle one expects the slug shape to be determined by the cell motion, which depends on differential chemotaxis (or motive force) between cell types and differential cell adhesion.

3 Modelling

Several studies have presented evidence that prestalk cells require a chemical secreted by prespore cells to remain in their prestalk differentiated state [5]. Based upon these facts, it has been proposed that a small diffusible molecule acting as prespore inhibitor/prestalk inducer might be regulating the proportion of cell types [6].

3.1 Evidences against a regulation based on positional information

In spite of the diffusive nature of this molecule and the self-organized spatial structure, the cell patterning in the slug does not appear to be regulated by a positional information scheme: (i) slugs don't display any size-independent characteristic length; (ii) cell sorting occurs faster than cell transdifferentiation; (iii) proportion regulation without spatial pattern has been observed [5]. Therefore it appears that the regulation is global and the concentration of the prespore inhibitor can be assumed to be rather homogeneous along the slug. The spatial segregration of cell types may be simply the result of cell sorting by means of differential cell adhesion and/or differential chemotaxis to cAMP signalling [7].

3.2 Global negative feedbacl plus cell-autonomous positive feedback model

To explain the finding of a tolerance region for proportion regulation, we present a model based on: (i) A global negative feedback for the regulation of proportion mediated by a diffusible molecule. (ii) A cell-autonomous positive feedback represented by a hysteresis-like behaviour (i.e. bistability) in the induction of differentiation.

The negative feedback is mediated by a diffusible prespore inhibitor u which is produced by prespore cells and degraded by prestalk cells. The prestalk proportion is represented by η. Cell type transdifferentiation is represented by the decreasing function $f(u)$ for $pst \Rightarrow psp$ conversion and the increasing function $g(u)$ for $psp \Rightarrow pst$. After appropriate transformation, the model reduces to the following equations.

$$\frac{du}{dt} = (1-\eta) - u\eta \qquad (1)$$

$$\tau\frac{d\eta}{dt} = g(u)(1-\eta) - f(u)\eta \qquad (2)$$

The main novelty of the model is given by hysteresis-like behaviour produced by the assumptions on g and f. First we assume that there is a cell-autonomous positive feedback in the differentiation process. This means that differentiation is bistable, i.e. $psp \Rightarrow pst$ transdifferentiation begins only at $u > u_2$, but the reverse conversion requires to decrease u below a much

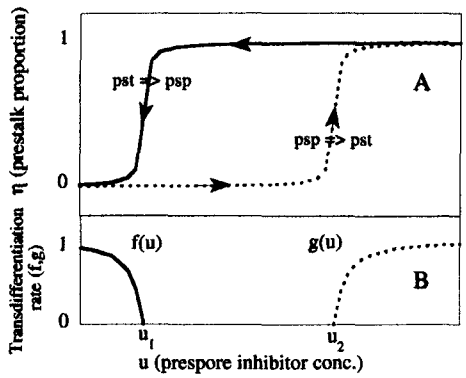

Figure 6: (A) Prestalk proportion dependence on the prespore inhibitor/prestalk inducer. The hysteresis-like behaviour can be seen as the fingerprint of bistability in cell type differentiation (B) Cell type transdifferentiation rates $f(u)$ and $g(u)$. To ensure stability it is postulated that they only take a positive value below (above) some threshold u_1 (u_2).

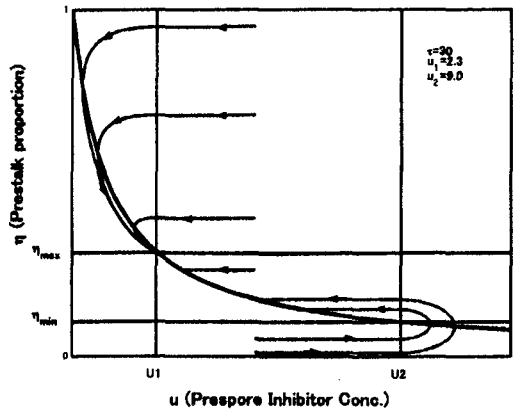

Figure 7: Trajectories obtained by simulations starting at different initial conditions. The nullcline $\frac{du}{dt} = 0$ is shown as a solid line. Starting with proportions above $\eta > \eta_{max}$ or below η_{min} , the proportion is regulated. Within the tolerance range, however, there is no regulation.

lower threshold u_1. Second, as illustrated by Fig. 6B, we assume that this behaviour applies not only for the single cell, but for the whole population (this can be shown to be the case as far as the distribution of thresholds u_1 and u_2 don't overlap). Under this assumption, if u is taken as an external parameter, it folllows from the equation 2 that the proportion will display a hysteresis-like behaviour such as shown in Fig. 6A.

Fig. 7 shows the results of simulations. A whole segment of fixed points is found for $u_1 < u < u_2$ and $\eta = \frac{1}{1+u}$. In consequence the range of stable proportions extends from $\eta_{min} = \frac{1}{1+u_2}$ to $\eta_{max} = \frac{1}{1+u_1}$.

4 Discussion anc conclusion

A general lesson may be drawn from this study. In tissue maintenance, it is both important to control the cell type proportions and to keep each of these cell types in a well differentiated state. However, there seems to a conflict of between these two requirements. On the one hand proportion regulation is better served by signalling that involves fast global negative feedback. On the other hand, a robust cell differentiation requires a strong cell-autonomous positive feedback to operate the "switch" between cell types. Without positive feedback cell differentiation would result in continuous spectrum of cell phenotypes. As a result of the positive feedback, cell differentiation will always display some hysteresis in respect to the control exerted by the global regulative mechanism. And this hysteresis poses a limit to the precision of the proportion regulation. In other words, it looks as though the more robust is the cell differentiation the less precise is the proportion regulation and vise versa.

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論文審査の結果の要旨

多細胞生物の組織は一般に異種の細胞から成っていてその比率は制御されている。この制御過程における細胞-細胞間のシグナル伝達の分子機構については解明されつつあるが、その比率を制御するグローバルなメカニズムの研究は進んでいなかった。著者は細胞性粘菌（Dictyostelium discoideum）をモデルシステムとして用い、細胞種の制御と組織レベルでのパターニング機構の研究を行ってこのメカニズムの解明に貢献した。本論文はこの成果をまとめたもので全編7章からなる。

第1章は序論であり、研究の動機とその意味付けについて述べている。

第2章では、研究の背景について述べている。10^2～10^5の範囲において粘菌移動体中の2種類の細胞の比率が制御されているというBonnerの提案とその問題点について述べている。

第3章では、本研究に用いた実験手法について述べている。特に半減期の短いβ-galactosidaseを予定柄細胞特定遺伝子ecmAのリアルタイムレポーターとして用いる新しい手法について述べている。

第4章では、細胞種の比率と空間パターンの定量的研究の結果について述べている。その結果、細胞種の比率は移動体の大きさに依存すること、及び予定胞子細胞を切除すると切除前の状態に復帰しないことを述べ、これらの結果から細胞種の比率の制御は上限と下限があること、その制御は本質的に厳密ではないことを結論している。これは重要な知見であり高く評価できる。

第5章では、移動体の形の定量的解析について述べている。インタクト及び再生中の移動体の両方において、その形態が摂動後すばやく制御され、予定柄部分の長さと幅の比率が一定になることを記している。この制御の速さは、二つの細胞種の易動度の違いが移一定になることを記している。この制御の速さは、二つの細胞種の易動度の違いが移動体の形態を決定している可能性を示唆している。このことは新しい知見で評価できる。

第6章では、本研究で明らかにした本質的に厳密でない制御の機構を説明するため、シグナル分子に基づいたモデルを構築したことについて述べている。シグナル分子による細胞種の比率変化に対する抑制と細胞種の相互転換に対する二つのしきい値の存在を基本としたこのモデルは、実験の結果よく説明しており評価できる。

第7章は結論である。

以上要するに本論文は、多細胞生物の細胞種の比率に関する制御機構の研究をモデル生物である細胞性粘菌を用いて初めて定量的に行い、その理解を進めたもので、生物物理学およびシステム情報科学の発展に寄与することが少なくない。

よって、本論文は博士（情報科学）の学位論文として合格と認める。