Differentiation and replication of spots in a reaction-diffusion system with many chemicals

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Abstract. – The replication and differentiation of spots in reaction-diffusion equations are studied by extending the Gray-Scott model with self-replicating spots to include many degrees of freedom needed to model systems with many chemicals. By examining many possible reaction networks, the behavior of this model is categorized into three types: replication of homogeneous fixed spots, replication of oscillatory spots, and differentiation from "multipotent spots". These multipotent spots either replicate or differentiate into other types of spots with different fixed-point dynamics, and, as a result, an inhomogeneous pattern of spots is formed. This differentiation process of spots is analyzed in terms of the loss of chemical diversity and decrease of the local Kolmogorov-Sinai entropy. The relevance of the results to developmental cell biology and stem cells is also discussed.

A simple chemical reaction-diffusion system, the Gray-Scott (GS) model [1], has recently been studied extensively. This model exhibits self-replicating spots within some range of parameter values [2]. Similar replicating spots have been found in experiment [3].

In each model system studied to this time, only a single type of spots appears, and there is no differentiation to create different types of spots. To search for possibility in complex pattern formation as in biological systems, it is interesting to study a reaction-diffusion system displaying not only the division but also differentiation of such cell-like spots. It is more necessary to consider chemical reaction dynamics within each spot which are more complex than those adopted in the GS model. Also, in order for spots to continue replication and differentiation, their division process and their internal complex chemical dynamics must somehow organize a proper relationship. We consider a model with such sufficiently complex dynamics and study how this relationship is organized. In so doing, we find the connection between self-replicating spots and cell differentiation in dynamical systems models assuming the cell itself [4–6]. We do this by adopting a many-degree-of-freedom version of the GS model, with the goal of realizing spot differentiation [7].

As a variant of the GS model we study the following model, possessing the many degrees of freedom needed to model a complex catalytic reaction network:

$$\frac{\partial u_i(x,t)}{\partial t} = D_u \nabla^2 u_i(x,t) + A(1 - u_i(x,t)) - u_i(x,t) \sum_{j=1}^{Q} \sum_{k=1}^{Q} W_i^{(j,k)} v_j(x,t) v_k(x,t),$$

$$\frac{\partial v_i(x,t)}{\partial t} = D_v \nabla^2 v_i(x,t) - B v_i(x,t) + v_i(x,t) \sum_{j=1}^{P} \sum_{k=1}^{Q} W_j^{(i,k)} u_j(x,t) v_k(x,t).$$

Here $u_i(x,t)$ denotes the concentration of the i-th inhibitor and $v_i(x,t)$ that of the activator chemical. Each inhibitor is produced at a constant rate, A, and both the activators and inhibitors decay at rates proportional to their concentrations (with different coefficients). The diffusion constants of inhibitors and activators are denoted by D_u and D_v, respectively. We assume that these constants are equal for all chemical species for simplicity. This model is a variant of that proposed by Cronhjort and Blomberg [8], and is reduced to the GS model by setting $P = 1$, $Q = 1$, $W_1^{(1,1)} = 1$. Here we fix $P = 3$ and $Q = 20$, and choose the reaction matrix W randomly, under the constraint that the reaction for each activator's replication is catalized by k ($0 \leq k \leq K$) randomly chosen activators. We have explored the cases $K = 4, 5, 6$. Within this choice of K values, there is no qualitative difference in our results depending on K. Hence we show mainly the results of $K = 4$. The specific numbers of chemicals P and Q are not important, but we note that it is very difficult to realize the differentiation we study, when these numbers are small. While here we present results for the case of one spatial dimension, numerical results for the two-dimensional case indicate similar behavior.

The behavior of our model depends on the choice of the reaction network W (which may be regarded to represent a prototype of a complex intracellular autocatalytic reaction network). We carried out numerical computations using a variety of randomly chosen reaction networks W, and with these we classified the possible types of dynamics of our system. Although we have studied a large number of networks, the observed behaviors belong to each of the four classes, as will be shown.

For each set of simulations, this matrix was fixed, while the parameter values were set throughout as $D_u = 1.0$, $D_v = 0.010$, $A = 0.020$, and $B = 0.060$ or 0.070. (Below we discuss bifurcation with the change of the parameters A and B.) These parameters were chosen so that the spot structure displayed by the GS equation is displayed by the presently considered model also. We used $\Delta t = 0.010$ and $\Delta x = 1.0$ for the numerical integration, while we have confirmed that the numerical results are unchanged by using smaller values for Δt.

In general we begin from some initial conditions from which two simple spots are formed. These spots then produce additional spots, and the process continues until eventually spots are distributed throughout the entire system. When such a state is realized, spot division ceases. Here, we first classify the (transient) dynamics of our model starting from such initial conditions. These results were obtained by considering several thousand randomly chosen reaction matrices.

To characterize the spot dynamics quantitatively, we have introduced two quantities measuring the diversity of these dynamics. One is the chemical diversity $S_i(nT)$, which is defined by

$$S_i(nT) = -\sum_{j=1}^{Q} P_i^j(nT) \log(P_i^j(nT)),$$

with $P_i^j(nT) = \overline{v_i^j}(nT) / \sum_{j=1}^{Q} \overline{v_i^j}(nT)$, and $\overline{v_i^j}(nT) = (1/T) \sum_{t=(n-1)T}^{nT} v_i^j(t)$, ($n = 1, 2, ...$),
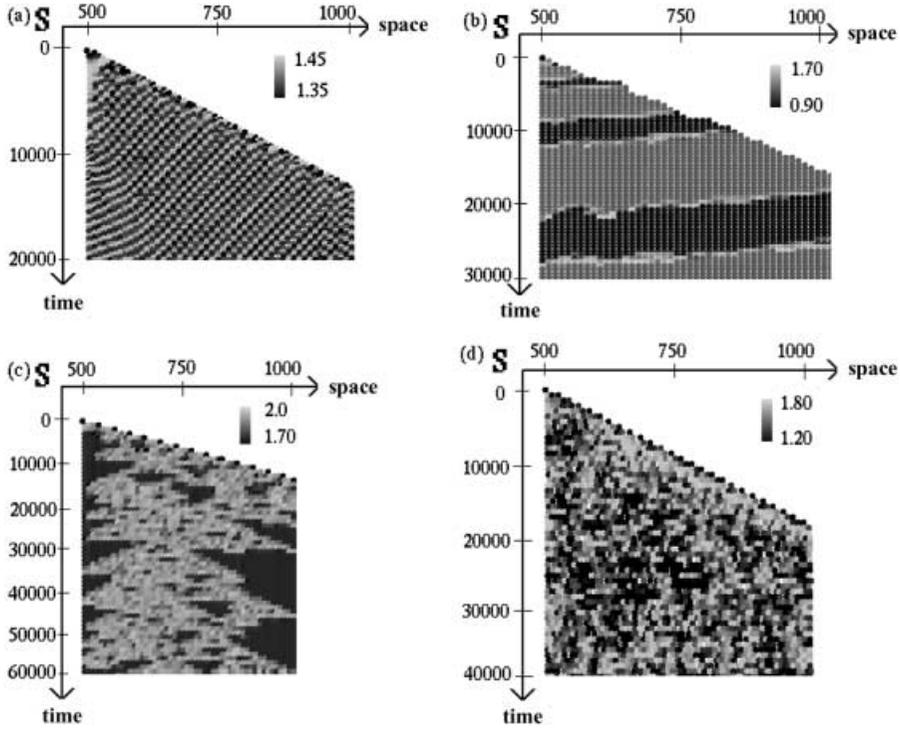
Fig. 1 – Chemical diversity $S_i(nT)$ for some patterns, that are made by different reaction networks ($K = 4$ is adopted in all the examples). The diversity $S_i(nT)$ of the half-space (from the center to the right edge) pattern is plotted using a gray scale. All of them adopt the same initial conditions, *i.e.*, at $t = 0$ only a single spot with $u_i = 0.50$ and $v_j = 0.250$ for all i and j exists. (a), (b) The chemical dynamics of spots are non-chaotic; (c) spatiotemporal intermittency is observed for chemical dynamics in spots; (d) fully chaotic dynamics for spots. For all these examples, spot structures are preserved.

where v_i^j is the j-th chemical concentration at the center of the i-th spot. The interval T used for the average is chosen to be on the order of a time scale for spot division [9].

The second quantity is the "local KS-entropy" h_i of the i-th spot. The local KS entropy here is defined as the sum of positive local Lyapunov exponents [10], obtained by using the tangent vectors corresponding to the chemical concentrations of the spot in question [11].

The spot dynamics are classified into the following four types [12]:

i) *Fixed-point case.*
In this case, the set of concentrations within each spot converges either to the same fixed point or to a couple of fixed points. In the former case, each spot has identical, fixed chemical concentrations, and they are separated by equal distance. In the latter case, different spots are formed, which use different sets of v for a given resource u_i ($i = 0$–P), and the interaction between the different spots through chemicals are negligible. Here, again, spots are separated by equal distance.

ii) *Oscillatory case.*
This case is further divided into non-chaotic, intermittent, and highly chaotic cases by the choice of reaction networks. In the non-chaotic oscillation case, the spatial pattern of spots
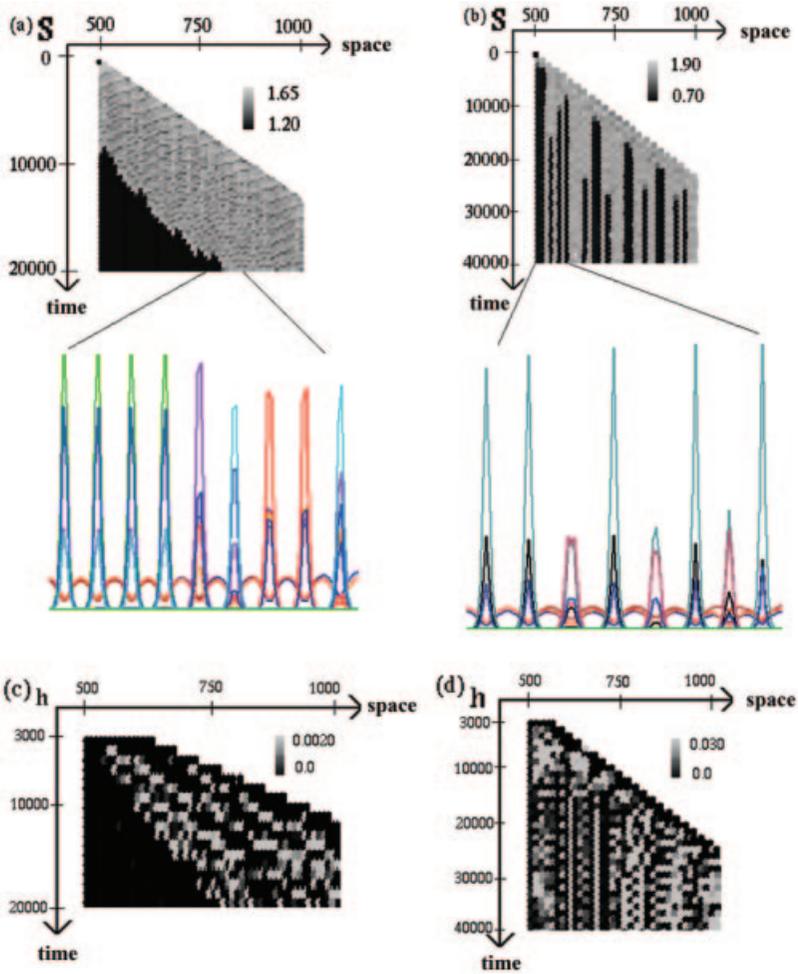
Fig. 2 – Chemical diversity $S_i(nT)$ and local KS entropy h_i for differentiation cases (since the average of local KS entropy requires some time steps, h is plotted only for time > 3000). Panels (a) and (c) are examples of case-I differentiation, while (b) and (d) are examples of case-II differentiation. A part of snapshot patterns of $u_i(x,t)$ and $v_j(x,t)$ is also plotted for each case of panels (a) and (b). Here we use a different color for each chemical species. Only half of the pattern from the center to the right edge is also plotted as in fig. 1. For the specific form of each reaction network W, see `http://chaos.c.u-tokyo.ac.jp/~takagi/GS.html`, although each of two cases appear for a wide variety of networks. Here, (a) is an example of $K = 6$ case, while (b) uses $K = 4$.

is fixed in time. In some reaction networks, there exist propagating waves (see fig. 1a, b). In spatio-temporal intermittency, the spatial pattern of spots is clearly separated into laminar and burst regions to form some characteristic patterns (see fig. 1c). Those patterns are expected to be in the same class that had been studied extensively in CML [13]. Contrastingly, in the chaotic case, the concentrations of each spot change chaotically in time, around the heteroclinic orbit (see fig. 1d). At any given time, the chemical concentrations vary from cell to cell, but their averages over time are almost identical for each cell. In this case, spots not only replicate but also sometimes annihilate.

iii) *Case-I differentiation.*
In this case, with time, spots differentiate into different types, namely, inner and outer types (see fig. 2a). For the inner type, the set of chemical concentrations converge to a fixed point, with less resource chemicals. Outer-type spots exhibit either fixed-point, periodic, or chaotic oscillations, depending on the reaction matrix and the parameter values. Here, the diversity is larger for the outer type. The inner type has smaller chemical diversity and null local KS entropy, as the dynamics fall onto fixed points. For the parameter values we used, the local KS entropy of the outer part can be either positive or zero, depending on the network (see fig. 2a, c). (The sign also changes depending on the parameter values.)

iv) *Case-II differentiation.*
Here, spots differentiate into two types, as in case I. Spots of the initial type exhibit chaotic oscillations. The division of these spots produces either the same type of spots with chaotic oscillations or different types with fixed-point dynamics. Here, differentiation is not governed by the inner/outer distinction, but rather spots with chaotic oscillations appear periodically, with some interval (see fig. 2b). Thus in this case there is pattern formation on two distinct spatial scales, that of the spot size and that of the average distance between two chaotic spots. In this case, the chaotic spots have a large chemical diversity, while other types have much smaller diversities. Complex dynamics are stable within spots of the first type, with positive local KS entropy, while for the other types, the local KS entropy is zero (see fig. 2b, d).

Let us study the differentiation process in more details. In both cases, spot types with smaller diversities and fixed-point dynamics are differentiated from the initial type, with high diversity. In fact, there exist initial conditions for which there results no differentiation of spots. Depending on the initial conditions and their chemical diversity, the first spot will either be a chaotic type or a fixed-point type. In the former case there will exist differentiation, but in the latter case there will be none, as such a spot can only replicate. Thus, the differentiation from the first type is irreversible. For initial conditions with sufficiently large chemical diversity, a chaotic spot will appear, while for smaller initial chemical diversities the basin structure is so complex that it is not feasible to predict which type of spot will appear initially. Statistically, however, the ratio in which differentiation occurs is in proportion to the chemical diversity of initial conditions. If a chaotic spot does appear, the differentiation process we have been discussing will take place.

The difference between the two cases I and II regards the ability for spontaneous differentiation. In case I, spots located in the outer region are maintained only through the flow of chemicals from the outmost part. The differentiation from the outer region into the inner region continues if the dynamics in the outer region are chaotic. However, if the dynamics of the outer spots converge to a fixed point, the differentiation is terminated, and, as a result, the growth of the inner part ceases.

On the other hand, in case II the number ratio of the two types of spots remains nearly fixed (with small fluctuations). In this case, differentiation from chaotic spots continues with some rate, and the spot is distributed in space at some rate. The pattern formed by the different types of spots is independent of the boundary conditions.

The differentiation here is reminiscent of that exhibited by stem cells in biological systems. In a biological stem cell system, initial cell types can either replicate or differentiate into different types. These differentiated cells can themselves differentiate, or they can replicate. Upon such repeated differentiation, multipotency comes to be lost, and eventually cell types that can only replicate are produced. In ref. [6], such stem cell is found to be a natural
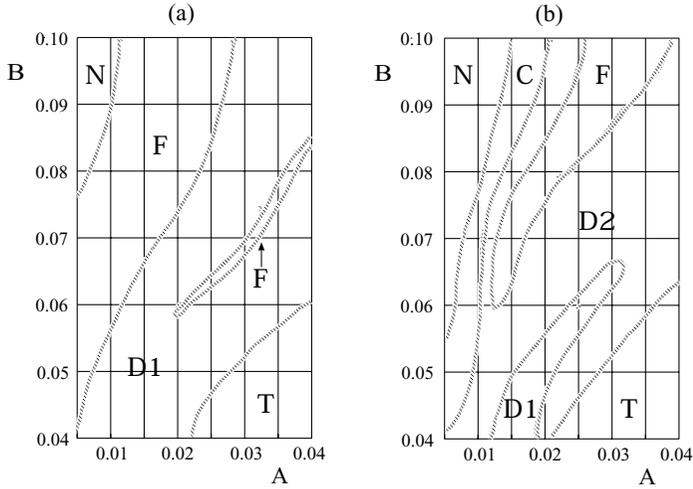
Fig. 3 – Rough phase diagrams for the network adopted in fig. 2(a) (case I, (a)), and fig. 2(b) (case II, (b)). The phases are denoted as follows: N corresponds to the spatially uniform state with $u_i = 1$ and $v_j = 0$ and no spots. F corresponds to the fixed-point case. C corresponds to the chaotic case. $D1$ corresponds to a case-I differentiation pattern. $D2$ corresponds to a case-II differentiation pattern. T corresponds to turbulent chemical dynamics, with no clear spots. (Initially, a single localized spot, with $u_i = 0.50$ and $v_j = 0.250$ for all i and j, was prepared.)

characteristic of dynamical systems in which there exists cellular structures and cell division. Here it is shown that such stem cell systems naturally appear in a reaction-diffusion system, without assuming the cellular structure in advance.

In case I of our model, these "stem-type" spots are located only in the outer region, while in case II, such "stem-type" spots exist within the inside of the pattern. In both cases in which differentiation shows up in our model, the diversity and local KS entropy decrease as the differentiation progress [6]. Differentiation into a spot with fixed-point–type dynamics is irreversible, as mentioned above. This is consistent with the loss of multipotency observed in real cellular systems.

Finally, we examine the parameter dependence of each type of dynamics. Here we consider altering the parameters A and B, while keeping the other parameter values fixed. In the homogeneous case, the bifurcation that results is qualitatively similar to that for the original GS equation. Typical phase diagrams for differentiation in cases I and II are shown in fig. 3. As we see, as the decay rate for v_i is decreased, there are bifurcations from a uniform system to a system with a single type of spots to a system with turbulent spatial structure and no clearly defined spots. This set of bifurcations exists quite generally, for a variety of reaction networks W. Of course, local structures of these phase diagrams depend on the choice of reaction network. However, the differentiation event is commonly observed in a wide range of parameters.

There has been a great deal of effort dedicated to relating reaction-diffusion systems to morphogenesis in biological systems, since the pioneering study of Turing [14]. However, these models have not yet been able to describe the complexity observed in biological pattern formation, which includes cell differentiation from stem cells, determination of fixed types, irreversibility and robustness in development. In this work, we have found that the behavior which can be interpreted as representing the irreversible differentiation from stem cells to

fixed cell types is displayed quite generally for a particular set of reaction-diffusion equations. With regard to the broader context of the general study of reaction-diffusion equations, it is interesting to note that a pattern with two distinct spatial scales is organized in case II. Also, it is interesting to note that the behaviors from a variety of reaction networks are classified just into the four cases. It will be important to classify possible types of spatiotemporal patterns that appear when we increase the complexity of the internal reaction dynamics.

Since our model includes only reaction and diffusion, without any other type of mechanism, the present study is also relevant to the study of prebiotic evolution leading to proliferation and diversification of cells. Indeed, the autocatalytic nature of the reaction networks studied here is similar to that of the hypercycle studied by Eigen and Shuster [15], while the relevance of the spatial structure in resisting parasites [16] and short cuts of network structure [17] has been discussed [8,18–20] . In contrast to previous studies, the present study explains not only the robust replication process but also the diversification to different cell types.

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