Wilhelm Roux's Archives (1981) 190:361–364

Roux's Archives of Developmental Biology

Rapid Communication

The Scale-Invariance of Spatial Patterning in a Developing System

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Summary. Regulating systems, that is, those which exhibit scale-invariant patterns in the adult, are supposed to do so on account of interactions between cells during development. The nature of these interactions has to be such that the system of positional information ("map") in the embryo also regulates. To our knowledge, this supposition regarding a regulating map has not been subjected to a direct test in any embryonic system. Here we do so by means of a simple and novel criterion and use it to examine tip regeneration in the mulicellular stage (slug) of *Dictyostelium discoideum*. When anterior, tip-containing fragments of slugs are amputated, a new tip spontaneously regenerates at the cut surface of the (remaining) posterior fragment. The time needed for regeneration to occur depends on the relative size of the amputated fragment but is independent of the total size of the slug. We conclude from this finding that there is at least one system underlying positional information in the slug which regulates.

Key words: Regulation – Positional Information – Regeneration – *Dictyostelium*

Introduction

Developmental systems are traditionally classified as *regulative* or *mosaic* (Graham and Wareing 1976). For the purposes of this article, a regulative system is understood to be one in which the relative proportions of component parts in the adult are independent of embryonic size. In contrast, in mosaic systems, removal of a small region of the embryo shows up as a missing part in the adult. Experiments have provided evidence for both regulation and mosaicism, at times in the same organism at different stages (Arnold 1968; Rose 1970). The occurrence of regulation implies that because of reasons having to do with cell-cell interactions, a cell differentiates according to its relative position in the embryo, while mosaicism is probably due to other overriding influences – for instance, those originating from cell lineage or from the existence of cytoplasmic determinants in the egg. The nature of the intercellular communication system involved in regulation is still an open question, though models have been proposed, focusing on *positional information* (Wolpert 1969; Robertson and Cohen 1972).

The system of positional information – the nature of the map – can be described formally by using the concept of a state function (Cohen 1971). In a developing system, corresponding to every cell in position x and at time t, there is a variable S which depends on x and t in some fashion; $S=f(x, t)$, say. S is called the *state-function*; its value depends on interactions amongst cells as well as those between the cells and the environment, and a knowledge of it at any time permits one to predict the future fate of the cell: one might say that the state-function of a cell *is* its fate. The point of interest to us is that the state-function depends on position; and for a regulative system, this dependence refers to relative – and not absolute – position. These ideas are not new, and were first clearly stated by Driesch (discussed fully in Wilson 1904) when he hypothesised that the prospective fate of a cell was a function of its relative position. Thus, in an embryo which is approximately linear and of overall size L, the function $S=f(x, t)$ must depend on position x only through the ratio x/L whenever x is measured from one of the boundaries of the system. Though this is an inference of fundamental interest, it does not appear to have been tested; vast as the literature on regulation is, as far as we are aware it only pertains to the scale-invariance of adult patterns. Part of the reason for this omission surely lies in the difficulty of identifying an appropriate state-function for an embryo, not to speak of making quantitative measurements on such a function. The purpose of this article is to point out that in the case of the multicellular slug of *Dictyostelium discoideum*, we are able to identify a suitable statefunction and to verify that it is scale-invariant. Verification depends on the requirement that on loci of constant S, t depends on x only via the ratio x/L.

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Materials and Methods

The Experimental System

The phenomenon studied was tip regeneration in the embryonic stage (slug) of the cellular slime mold *Dictyostelium discoideum*. The slug is a long and thin cylindrical multicellular structure formed when starved amoebae are allowed to aggregate on a substratum. It is characterised by a smooth, tapered protrusion at its anterior end, called the *tip*; this tip is similar to a classical amphibian organiser (Raper 1940; Rubin and Robertson 1976). After variable periods of migration the slug erects itself into a fruiting body, the equivalent of an adult stage. The cells comprising the slug differentiate into one of three types in a position-dependent manner, with cells in the anterior forming the future stalk, those further posterior giving rise to spores, and the posteriormost ones forming a basal disc supporting the entire fruiting body. This pattern regulates; the relative sizes of stalk and spore tissue vary only slightly from one fruiting body to another. Direct counting has shown that the ratio of the number of stalk cells

0340-0794/81/0190/0361/$01.00

to spores is not constant, but varies within certain limits (Stenhouse and Williams 1977). However what is striking is that when a slug is fragmented by transverse cuts, each of the small slugs so formed differentiates into a miniature fruiting body complete with spores, stalk and basal disc (Raper 1940). All this is preceeded by a re-specification of the pattern in the fragments, the anterior part of each giving rise to stalk and the posterior to spore cells (Bonner et al. 1955; Sakai 1973). None of these processes is accompanied by any discernable change in overall size or cell number. It must be mentioned that a predisposition for future positions in the slug – and therefore for different fates – exists even before the onset of aggregation (Takeuchi 1969), that is, before the presumed beginning of cellular interactions. What is of interest to us here is that the normal outcome of such tendencies can still be overridden by position-dependent effects, which have to necessarily be a consequence of cell–cell interactions. Here we shall use the *Dictyostelium* slug as the prototype of a regulating embryo, with regulation occurring due to intercellular communication.

Our study concerns one easily recognisable feature of pattern re-specification in fragmented slugs, and that is the appearance of a new tip at the anterior margin of tipless fragments (Raper 1940). In particular, we focus on that state of cells as a consequence of which they form a tip; thus the value of the state-function of interest to us is whatever it is that corresponds to cells being in the "tip state". We try to answer the following two questions: (a) If a slug is cut transversely, how long does it take for the posterior fragment to regenerate a new tip? (b) How does the time needed for regeneration depend on the level of the cut and the overall length of the slug? The hypothesis being tested is that the time for tip regeneration depends only on the *relative* level at which the slug is cut. If verified, this will imply that the map – or system of positional information – underlying this aspect of pattern in the slug is indeed regulative.

Methods

In order to induce aggregation and slug formation, exponentially growing cells of *Dictyostelium discoideum* NC-4(H) were washed free of bacteria and spread evenly on 10 cm petri dishes containing 2% agar made up in distilled water (Bonner 1967). The initial density of spreading was $7 \pm 1 \times 10^5$ cells/cm^2. Plates were incubated at 21° C–22° C in the dark for varying periods; the slugs used by us ranged in age from 4 h to 60 h and in size from 0.63 mm to 3.3 mm. Migrating slugs were examined under a microscope with a graduated scale in the eyepiece, and an anterior fragment of the desired length was cut off cleanly with a microscalpel and removed. The lids of the petri dishes were thereafter closed and the slugs continually observed for signs of regeneration. Since the morphology of a tip is unambiguous, the time of regeneration can be scored fairly accurately; a further help is that immediately after a new tip forms, the fragmented slug either resumes migration or proceeds to erect itself into a fruiting body; at any rate, movement, which is absent in the interim, starts again (Raper 1940). An additional objective estimate of regeneration times was provided in other experiments which showed that tipless posterior fragments would accept donor tips as grafts right upto the time that they regenerated tips of their own (Lokeshwar and Nanjundiah, manuscript submitted for publication).

Results

A new tip invariably regenerates from the cut surface of a posterior fragment, and the smaller the size of the fragment, the longer it takes to do so. Figure 1 displays the dependence of tip regeneration times (t^*) on the absolute lengths of the anterior fragment cut (x^*) from slugs of various overall length (L). The results have been grouped so as to allow a test of the hypothesis that t^* depends on x^* only through the ratio x^*/L. For each class of relative lengths x^*/L, Table 1 indicates the distribution of regeneration times about the mean. Taken together, these results suggest that the time needed for tip regeneration does scale with the relative length of the cut fragment.

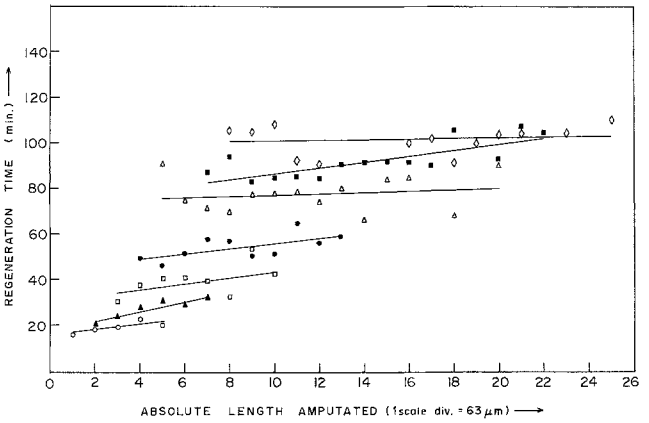

Fig. 1. Variation of tip regeneration times with the absolute length cut, holding the relative length fixed. Abscissa, position of the cut x^*, measured from the anterior margin of the slug, in units of microscope eyepiece scale divisions (1 division = 63 μm); ordinate, mean regeneration time t^* in minutes. The symbols stand for the percentage of total length cut off, that is, to $(x^*/L) \times 100$ where L is the overall slug length. Symbols ○ (5–10), ▲ (11–20), □ (21–30), ● (31–40), △ (41–50), ■ (51–60), ◇ (61–70). The straight lines are based on a linear regression of t^* with respect to x^* (correlation coefficient >0.93 in all cases). Error bars have not been shown for the sake of clarity. Each point in the figure represents an average of about 12 experiments, and the standard error of the mean regeneration time for a fixed x^*/L and x^* is about 7% of the mean. There is a separate validation of our measurement of tip regeneration times. In the course of independent experiments conducted in parallel with these, we have amputated anterior fragments of slugs at different levels and studied how well a foreign tip, transplanted at various times following amputation, gets accepted and thereby inhibits autonomous tip regeneration (Lokeshwar and Nanjundiah, submitted for publication). Rejection of a graft is on account of autonomous tip regeneration in the host. For a set of experiments corresponding to the same relative level of cut x^*/L, we have shown that the time course of tip regeneration in a population is the same as the time-course of graft rejection. This correlation indicates to us the reliability of the morphological criteria based on which we have recorded tip regeneration

Consider for simplicity an embryo of developmental age t and made up of a line of cells of total length L, the position of the cells in it being defined by the variable x. Suppose that the developmental map in this embryo is expressed by the functional relationship $S = f(x, t)$. Each pair of values (x, t) corresponds to a certain state of the cell. If one focusses attention on a particular cell state S^*, the set of variables (x^*, t^*), for which $S = S^*$, will themselves be linked through some functional relationship. Now if the state S depends on x only through the ratio x/L, it follows that a similar dependence holds for the relationship between x^* and t^* referred to above; that is, t^* varies only with x^*/L and not with x^* by itself. Therefore in order to test whether the map given by $S = f(x, t)$ is regulative or not it suffices to verify whether the prediction $t^* \propto x^*/L$ holds or not. (Clearly, the result of such a test is applicable only to that aspect of pattern relevant to the cell state S^*, and the

Table 1. This gives the distribution of the regeneration times shown in Fig. 1. Inspite of a slight overlap between results for successive ranges, it is clear that the mean regeneration time t^* steadily increases with the relative length cut x^*/L. The fourth column gives confidence limits for the slopes of the regression lines drawn in Fig. 1. A slope of exactly zero would imply that for a given x^*/L, t^* is independent of x^*. Within 95% confidence limits, such an implication is valid for the fifth (41%–50%) and seventh (61%–70%) classes. However, even for the remaining classes, the coefficient of variation of t^* practically independent of x^*. Direct measurements confirm that the slugs are all more or less of the same breadth (by choice). The number of cells in a slug is then directly proportional to length, for instance, slugs 0.75 mm, or nearly 12 scale divisions long, had 3.0×10^4 cells (mean of three independent measurements)

Class	Percentage cut $(x^*/L) \times 100$ (mean $\pm$ s.d.; no. of cases)	Regeneration time t^* in min, averaged over all cases (mean $\pm$ s.d.)	Length of anterior fragment amputated (units: micrometer scale div.; 1 div. = 63 μm)	95% confidence limits for slope $\partial t^*/\partial x^*$ (min/scale div.)	Coefficient of variation of regeneration times (s.e.m./mean) $\times 100$
1	5–10 (9.0 ± 1.2; 62)	18.5 ± 5.8	1–5	1.20 ± 0.87	5.99%
2	11–20 (15.9 ± 2.7; 148)	25.7 ± 9.7	2–7	2.35 ± 0.70	7.37%
3	21–30 (26.0 ± 2.8; 108)	38.5 ± 12.0	3–12	1.33 ± 0.56	5.64%
4	31–40 (36.1 ± 3.2; 117)	54.3 ± 13.8	4–13	1.20 ± 0.40	3.28%
5	41–50 (45.6 ± 3.3; 99)	76.2 ± 17.1	5–20	0.26 ± 0.29	2.74%
6	51–60 (55.7 ± 2.7; 84)	90.8 ± 14.4	7–22	1.27 ± 0.20	2.39%
7	61–70 (65.8 ± 3.0; 59)	103.1 ± 15.3	8–25	0.11 ± 0.25	1.78%

test will in theory have to be repeated for every possible cell state before it can be stated that the entire map is regulated.) In our case, the state S^* refers to a group of cells forming a tip. Therefore whenever a new tip regenerates, we assume that the cells comprising the tip have state function S^*. t^* is the time taken for a new tip to form when a slug of length L is cut transversely at a distance x^* from the anterior margin. Our results demonstrate that t^* does indeed scale with the ratio x^*/L.

Discussion

Since Raper's pioneering experiments in 1940, work on regulation in *Dictyostelium* has primarily been concentrated on the pattern of proportions within the fruiting body, a subject that Bonner (1967) has termed "the supreme problem in the differentiation of the cellular slime molds". When studying pattern, it is not immediately obvious what the relevant physical measures of size ought to be – linear dimension, volume, or cell number. All three have been examined in various slime mold species. Recently, Spiegel and Cox (1980) have made a persuasive case for geometrical length rather than cell number as the important variable underlying the spacing pattern in the fruiting body of another cellular slime mold, *Polysphondylium pallidum*. Whatever the case, it is undeniable that there is a fair amount of adjustment in the relative sizes of spore mass, stalk, and possibly basal disc, though careful counts show that the relative proportions of *numbers* of cells vary (Stenhouse and Williams 1977). Thus the fruiting body does exhibit a rough regulation.

Our results show that even before this, there is a regulative map in the slug: for a given relative length of cut x^*/L, the time t^* for tip regeneration is more-or-less the same even when the absolute length cut x^*, and therefore the length of the slug L, varies by a factor of over three to five (Fig. 1, Table 1). Typically, for a fivefold variation in size, the coefficient of variation in regeneration times is about 6% (averaged).

At this stage we will not speculate on the basis of the system of signalling between cells which leads to a regulative map. Beginning with the pioneering work of Turing (1952), a number of theories, postulating a controlling role for diffusible morphogens, have been proposed. Gierer and Meinhardt (1972) have been able to account for quite a few features of patterning – including regulation – in *Hydra* on the basis of a model similar to Turing's but involving non-uniformly distributed sources of morphogen. A qualitatively different theory, requiring the existence of intracellular oscillations and wave propagation, was put forward by Goodwin and Cohen (1969). None of these theories has gone through detailed experimental testing, meaning that at the present time it is impossible to clearly identify the variables in any model with known substances or phenomena. The same comment applies to models constructed with special emphasis on the *Dictyostelium* slug (Loomis 1972; McMahon 1973; Lacalli and Harrison 1978; Sussman and Schindler 1980; Othmer and Pate 1980). In any case, in the absence of external influences, the spontaneous generation of pattern within a homogenous system gives rise to basic theoretical difficulties if it is required that the pattern is regulated (Wolpert 1971; Robertson and Cohen 1972). The case of the slug is important because it brings out the essential features of regulation in a particular fashion: within a contiguous group of genetically identical cells raised in a common uniform environment, cells follow different fates according to their relative positions.

The present study has demonstrated that in addition to this, the kinetics of tip-regeneration in the slug shows scale-invariance. The underlying causes are unknown. If regeneration is indeed a consequence of mutual signalling amongst the cells of the slug's posterior fragment, it follows on purely dimensional grounds that the speed of this signal must increase with overall slug size: curious in itself, and apparently a requirement of catastrophe-theory models of morphogenesis (Cooke 1975). Alternatively, the signal could move at a constant speed but the cell's response time could be graded with total size, though this seems somewhat implausible. On the other hand, if the time needed for regeneration is a reflection of an earlier gradation of potentialities, our findings indicate that such a pre-pattern of cell fates also exhibits regulation. In either case, we have evidence for regulation in an embryonic system of positional information.

Acknowledgements. We thank Th. M. Konijn for supplying the *Dictyostelium* stock. This work was supported by grants to V.N. from the Department of Science and Technology and the Indian National Science Academy.

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Received September 3, 1981
Accepted October 10, 1981