Morphogenesis in the cellular slime mould *Dictyostelium discoideum*; the formation and regulation of aggregate tips and the specification of developmental axes

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SUMMARY

A general discussion of ‘organizing regions’ and the specification of biological patterns is followed by introducing the idea that the tip of the slime mould cell mass is such an ‘organizer’. This view is supported by a discussion of the developmental ubiquity of the tip and its effects.

A staging system is described which assigns numbers to sequential morphological changes during development.

A set of experiments investigating the role of the tip are described, using techniques of cell labelling, grafting and bisection of cell masses with barriers, and the manufacture and use of cylindrical barriers of permeable cellulose.

The results of such experiments show:

1. That the tip of the cell mass is made of the same group of cells from stage 10 (late aggregate) to stage 20+ (culmination).
2. That a stage 10 aggregate will regenerate a new tip in an average time of 32 min.
3. That if a stage 10 aggregate is bisected by an impermeable barrier two tips, indicating two new developmental axes, develop in an average time of 34 min.
4. That if a stage 10 aggregate is bisected for 40 min, the barrier removed and one of the tips removed, the remaining tip inhibits the re-formation of the second tip, and the polarity of the aggregate is again reorganized with respect to the remaining tip.
5. That if experiments (3), (4) and (5) are repeated with a stage 9 aggregate, which is an hour younger, all the regulation times are increased by about 60 min. Similarly a stage 8 aggregate takes over 120 min longer to show the effect.
6. That if part or all of a cell mass from any stage is placed inside a cellulose tube, all the enclosed cells differentiate into stalk cells.

These results are then discussed in relation to pattern formation and the role of the tip in polarization and the specification of new developmental axes in cell masses.

A model for culmination in the slime mould is proposed which takes account of the above results. The essence of this model is that at no time are stalk and spore cells ‘determined’ in the classical sense, and that, by a non-signalling positional information system, the size invariance of the ratio of stalk to spore cells seen in the fruiting body is a result of the mechanical process of culmination.

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INTRODUCTION

Much theoretical consideration is now being given to possible mechanisms for the ordering of cells and their differentiation into the complex patterns of biological structures (Symposium of the Society for Experimental Biology, 1971) and the problem has been formally stated by Wolpert, using the concept of positional information (Wolpert, 1969, 1971). For some models it would appear necessary for a graded parameter to arise or be generated within a developmental field, from which cells gain information about their position or their neighbours, the interpretation of this information being reflected in the pattern of differentiation seen in the cell mass as a whole. Many models can be formulated to explain such processes, but one striking feature is the need for some special organizing or boundary regions within the ‘homogeneous cell mass’. For example, one type, using a gradient of a substance set up by diffusion requires a ‘source’ — a special region to produce the substance. The problem in all such models is the origin of special regions from which signalling occurs, or more generally, the origin of the region which acts as a reference for spatial or temporal information. In many regulating systems one finds a dominant, organizing region, the assembled list of which is very impressive (Wolpert, 1971), and there is a large body of evidence to support the addition of the apical tip of the developing sorocarp of the cellular slime mould Dictyostelium discoideum to this list. Several authors have described the role of the tip in particular parts of the life-cycle, and a summary of the evidence for its apparent fundamental significance throughout the whole morphogenetic period in controlling, or being necessary for the control of, all spatial organization is presented below. Throughout normal morphogenesis, from early aggregation onwards, a topologically distinct region, defined solely by its sharp change in radius of curvature, is obvious, as shown in Fig. 1. The ubiquitous effect of the tip can be quite impressively demonstrated by considering each stage of the life-cycle and its dependence upon a tip: during early aggregation a small tip develops at the aggregation centre, and if this is removed the aggregate disperses and is reorganized around new centres (Bonner, 1950). If the tip is removed from a migrating slug, locomotory and morphogenetic movements cease until a new tip develops (Raper, 1940). During the rearrangement of the slug into a post-migration cell mass the only gross morphological feature common to the two stages is the tip. If this tip is removed, or even disturbed, morphogenesis and differentiation are arrested whilst a tip re-forms. The same is true during fruiting body construction; the differentiating spore mass will stop rising and stalk formation ceases until a new tip develops (personal observation).

It has also been reported that the attitude of a fruiting body to the substrate is effected by a gaseous hormonal system which orientates the fruiting body during culmination so that the distance between the spore head and all non-adsorbant surfaces is maximum on all sides (Bonner & Dodd, 1962); the first
Fig. 1. The stage numbering system used throughout the text. Each scale line equals 1 mm. The stage numbers correspond approximately to the number of hours after deposition of washed interphase cells on to non-nutrient agar that each distinct form appears.
step to be seen during experimental reorientation of a cell mass which is about
to culminate is the movement of the tip over the surface, so that the resultant
frUITING body will be correctly orientated when formed. Mechanically agglo-
merated amoebae will undergo normal morphogenesis if they are put on to a
moist surface and allowed to develop a tip (Gerisch, 1968), but if they are
physically or chemically prevented from doing so, they differentiate to give an
abnormal pattern of cells (personal observation). Aggregated amoebae will
form fruiting bodies without prior migration if they are, for instance, placed
into drops or films of water. The fruiting bodies so formed are always out into
the air, but here again a tip-like process must appear before normal development
ensues. It can thus be seen that the tip is essential for normal development at all
stages.

An even more direct demonstration of the organizing ability of the tip has
been made by Robertson (1972), who has shown that, if the tip from any
developmental stage is transplanted into a field of amoebae which are competent
to receive and relay periodic signals, the amoebae form streams towards the
tip following the same defined sequence of responses irrespective of the stage
from which the tip was removed. Robertson et al. (1972) have also demonstrated
the effects of transplanting tips between developmental stages and have shown
that, in all the 25 combinations they tried, a tip transplanted from a specified
stage of development organized the development of at least a portion of the
field into which it was transplanted.

The development of the tip and its effects on the pattern of cells seen after
differentiation have been investigated. Experiments have been performed to
provide information about the permanence of the tip and its constituent cells
throughout the life-cycle. The nature of the possible differentiation of the cells
in the tip to provide a ‘special region’ and their control of morphogenesis has
been investigated by observing the effects of the addition or removal of these
tips from cell masses. Division of the aggregate into two fields with the sub-
sequent regeneration of tips has proven useful in providing an estimation of the
time for the specification of polarity and the determination of developmental
axes in the cell mass of slime-mould cells.

MATERIALS AND METHODS

Cells of Dictyostelium discoideum NC-4 were grown on Sussman’s SM agar
in association with Escherichia coli B/r. Cells were harvested after 40 h incuba-
tion at 22 °C and washed three times by centrifugation in distilled water at 5 °C.
They were then deposited on 2 % agar in distilled water in Petri dishes, at
0·5 ml of 5 × 10⁷ cells/ml per plate, or on to Millipore filters as described by
Sussman (1966), and reincubated at 22 °C. All manipulations of cell masses were
performed in a Perspex cabinet at 18–22 °C at a relative humidity over 75 %.
The development was observed with a binocular microscope (× 15) and described
by the system of stage numbers shown on Fig. 1. These numbers correspond approximately to the time in hours after deposition of the cells at which each morphologically distinct stage appears, in lightly buffered conditions on Millipore filters as described by Newell, Telser & Sussman (1969). The cells were labelled by suspending them for 15 min in a suspension of Whatman CM 32 resin particles to which acridine orange had been adsorbed, as previously described (Farnsworth & Wolpert, 1970), before being deposited on to filters.

Impermeable barriers were cut from polyester film 19 µm thick (Melanex, S, gauge 75, ICI Ltd.) and inserted into cell masses which had been individually transferred to non-nutrient agar plates.

Cellulose tubes were prepared by the evaporation of cellulose nitrate in ether ('Necolodine' solution (stanvis) B.D.H.) on to nickel wires 0.5 mm diameter, at room temperature and humidity (Grabar & De Loureiro, 1936).

RESULTS

(i) Permanence of the tip

Initial experiments have been performed to determine whether the tips seen at the various stages in the developmental sequence are composed of the same cells. Tips were removed from cell masses, the cells of which had been labelled with a fluorescent marker as described above, and then grafted intact in place of the tips on unlabelled cell masses. By this method it has been shown that the tip is composed of almost the same population of cells throughout normal morphogenesis following aggregation. It has been observed that at least 70% of the cells in the tip of a stage-20 post-migration cell mass are the same cells which formed the tip on the stage-10 pre-migration one. However, if the tip-like structure which forms the centre of the streams of aggregating amoebae during stages 3–8 of aggregation is replaced by a labelled one, the labelled cells are randomly distributed in the early aggregate from stage 10 onwards.

(ii) The effect of grafting more than one tip on to a cell mass

An attempt was made to graft the tips from labelled cell masses on to others which had not had their tips removed in order to investigate the possibility of differentiation in the tip. This was found to be difficult to accomplish because of the sheath of slime which encases the cell mass, as indicated by Robertson et al. 1972, and apparently holds it in its hemispherical form. If the slime is not broken before the new tip is grafted on, the additional cells are not in close enough contact to be integrated and they slide down the side of the cell mass, carried by the descending flow of slime, as are inanimate markers when they are applied to the surface (Shaffer, 1965). If, on the other hand, the host cell mass is grossly disturbed, its organization is lost and the cell mass collapses into a loose pile of cells which then re-aggregate, or in the special case of a stage 14 or 15 cell mass (the migrating slug), where additional tips could be easily grafted on, the new tip takes control of a portion of the grex and the original aggregate
splits into separate entities, each with a tip, as reported by Raper (1940). This result was of little importance in these investigations as it failed to show the pattern of differentiation of one cell mass with two tips; it does, however, demonstrate a difference in mechanical stability of the hemispherical aggregated forms (stages 10–13 and 16–18) and the slug form (stages 14 and 15). The mechanical disturbance required to integrate new tissue into the cell mass is tolerated by the cylindrical forms but nearly always causes loss of integrity in the hemispherical forms. It was found possible in 13 cases, however, to add an intact tip to an intact stage-10 aggregate and in every case the labelled cells from the new tip were integrated into the cell mass of the host and eventually, because of their position, gave rise to spores (cf. Farnsworth & Wolpert, 1970).

(iii) Aggregate fusion

Since the previous experiment failed to demonstrate the effect of having two functional tips on one cell mass, an experiment was designed in which two of them, complete with tips, were fused side by side. Again mechanical difficulties prevailed; if the cell masses were disturbed sufficiently they fused but collapsed, to reorganize themselves over 3 h into one or more (usually two) separate
Morphogenesis in Dictyostelium

(iv) The effect of impermeable barriers

The regulative powers of pieces cut from aggregated cell masses of Dictyostelium has long been known and it is easy to show that, when a cell mass is split into two, each half develops a new tip (Raper, 1940). Experiments were designed to make use of this property to form cell masses with two tips. Small barriers of impermeable plastic 2 mm × 0.5 mm × 18 μm were inserted, so as to effect a vertical bisection into stage 9–11 and 16–18 cell masses which had had their tips removed (see Fig. 2). As expected, the two halves each developed a tip and produced two separate fruiting bodies. However, it was found that the position of the barrier was a critical factor in the determination of the pattern of the resultant differentiation. If the barrier was only partially inserted into the mass, less than a third of the way down the vertical axis, the barrier was avoided by being pushed aside or completely ejected by the subsequent morphogenetic movement of the cell mass, so that only one tip and one fruiting body formed. If the barrier was inserted more than two-thirds of the way into the cell mass the two halves took on individual identity and each developed a tip, which led to the formation of two fruiting bodies. This procedure thus gave a system for the formation of cell masses with two functional tips. The physical presence of the barrier between the two newly formed cell masses prevented slime from forming or accumulating between them, so that when the barrier was removed intimate contact between the cells of the two masses was possible, leading to successful fusion.

(v) The time for the specification of polarity

If the tip is removed from a cell mass between stages 10 and 12, it rounds up and becomes very smooth. After an average period of 32 min, with a range between 25 and 40 min (see Fig. 4A), a small blob appears on the surface which becomes, within 10 min, a complete new tip. The further development of the cell mass is polarized with respect to this new tip; that is, this region becomes the front of the grex and the new tip from stage 16 onwards (see section (i) above) and its final position governs the attitude of the fruiting body by directing the site of stalk formation. The new tip can thus be said to indicate, if not instigate, the polarity for the subsequent development of the aggregate. Therefore, this first appearance of a small distension, in early tip formation, was used to indicate when the polarity of a cell mass had been specified. The time after the removal of a tip at which a new tip began to reappear was thus scored as the time for the re-specification of polarity.

If a barrier was inserted all the way into a stage 10 (late aggregate) which had had its tip removed, each of the two new cell masses had developed a tip on
Fig. 3. Effects of barrier insertion and removal on stage-10 late aggregates.

Fig. 4. Effects of experimental procedures.

- Percentage of a population of stage 10 aggregates showing reappearance of a new tip at times after tip removal
- Percentage of a population of bisected stage 10 aggregates showing reappearance of a tip at times after bisection and tip removal
- Percentage of a population of bisected stage 10 aggregates showing the appearance of two developmental axes with varying times of mechanical bisection by a barrier
- As for C
  - Stage 9 aggregates
  - Stage 8 aggregates
average after 34 min (see Fig. 4B for the time distribution of this event), this time corresponding to the time of determination of the new developmental axis, as outlined above, and being only 2 min longer than the time taken for an unbisected aggregate to re-specify its polarity (Fig. 4A), a difference which cannot be considered significant.

If the barrier was removed less than 30 min after its insertion, the aggregates fused again to give one cell mass, which developed one tip, and gave rise to one fruiting body. If the barrier was removed after a 30 min period had elapsed, two tips were just beginning to develop, and the two halves of the aggregate later took on separate identities, rounding up into two distinct aggregates, exactly as it would have been had the barrier remained. (See Fig. 4C for the time distribution of this event.)

If, however, the barrier is removed after 40 min, at which time all the aggregates have specified two new axes (Fig. 4C) and one of the tips cut off, the two halves re-fuse, the remaining tip taking control and a single fruiting body is constructed along an axis aligned with respect to it (see Fig. 3). This was seen to happen in 25 out of 28 cases, the only condition being that the barrier was removed from the cell mass within about 3 h of its being inserted. If it was left after this time, the two halves had rounded up and moved so far apart that they were no longer in close enough contact to re-fuse when the barrier was removed.

(vi) The effect of age on the time for the specification of polarity

The time for the specification of new axes, as determined by the above method, is about 37 min for a stage 10 (late aggregate). If a stage 9 aggregate is used, which will not form a tip for another hour, the specification time is lengthened to about 100 min (60 + 40); similarly a stage 8 aggregate will not show new axes until the residence time approaches 160 min (60 + 60 + 40) (see Fig. 4D for this data).

(vii) The effect of permeable barriers

As Raper & Fennel described in 1952, during culmination a cellulose tube is formed down the centre of the culminating cell mass, and as this can reasonably be supposed to have some connexion with the normal pattern of differentiation, tubes of cellulose were constructed as described and inserted into cell masses. The pattern of differentiation was altered, with all stages and all pore sizes of the cellulose giving the same result; if the tip was undisturbed, one or more normal fruiting bodies were formed, either outside or occasionally along the lumen of the tube, and if the tip was removed or destroyed all the cells included within the tube had, within 18 h, all become stalk cells. That this result is not due to direct induction by the cellulose is shown by the fact that the insertion of rods or sheets of the cellulose, or of stalks from already formed fruiting bodies, failed to affect the pattern of differentiation. Similarly, other tubes of similar diameters like the spines from the rear legs of cockroaches or the hairs from nettle leaves failed to induce stalk cells, as did the introduction of glass tubes of
similar diameter, even if they were coated with cellulose. This phenomenon is
under further investigation and will be reported in more detail elsewhere.

DISCUSSION

The reported experiments with impermeable barriers demonstrate both tempo-
ral and spatial aspects of regulation in cell masses. Removal of a stage-10
tip leads to the formation of a new tip with a T 50 of 32 min; bisection of a stage
10 late aggregate will give two new tips with a T 50 of 34 min. Once a barrier
has been in an aggregate, 10 h old, for 40 min, the same pattern of differen-
tiation was seen whether the barrier was removed or not, this demonstrates that
this time for tip development is also the time for the re-specification of the two
new developmental axes. It appears therefore that the process which determines
the axes for subsequent development and the site of the initiation of tip formation
are the same, or at the least occur simultaneously, on a similar time scale. In
aggregates that are less than 10 h old, this determination time is increased by a
period which corresponds approximately to the time it takes for the aggregate
to become 10 h old, showing that this specification of pattern depends upon a
cellular competence which arises with age.

These bisection experiments also present good evidence that the mechanism
by which the tip dominates the cell mass is one involving the suppression of
further tip formation. A cell mass having been bisected for 40 min and allowed
to develop its two new axes for up to 180 min will, if the barrier and one of
the tips are removed, regulate to come under the control of the remaining tip
(Fig. 3). This tip will suppress not only the re-formation of the second tip but
also halt and redirect development from the second axis.

The organizing properties of the tip may not be those of the individual cells
of which the tip is composed but are characteristic of the group of cells. Although
the tip of the slime mould is composed of the same cells throughout the life-
cycle, the cells' past history and individual properties may be irrelevant; a tip
cell can be replaced by any other cell and can itself be integrated anywhere. The
integration of additional tips into established cell masses similarly shows that
the properties of the tip are those of an organized group of cells rather than a
collection of differentiated cells, since tip cells are integrated and behave
developmentally as all other amoebae when grafted on to intact cell masses. This
observation also suggests an inhibitory system by which an established tip
prevents others from forming. The fusion of whole cell masses and their sub-
sequent resumption of individual integreties demonstrates clearly that there is a
mechanism by which aggregate size is sensed and controlled. It may be that
this is due to the failure of the tip inhibition system over a certain distance
(systems involving diffusion and threshold levels immediately spring to mind);
this would allow more than one tip to develop on a large cell mass, each tip
taking control of a region giving it polarity and individual identity. Certainly in
Morphogenesis in Dictyostelium

Fig. 5. Sequential stages in the model for culmination. (A) Stage 17 aggregate. (B) Cellulose tube forming. (C) Stalk cells forming at base. (D) Stalk forming in tube, amoebae entering top of tube and spores beginning to differentiate at periphery. (E–G) Process continues, spore formation spreading nearer centre of rising cell mass, region of undifferentiated amoebae being depleted by both the encroachment of the developing spores and by loss of the cells entering the tube to become stalk. (H) Process has limited itself; all the amoebae except those in the tip have either started to become spores or have entered the tube. Closed symbols = fully differentiated cells; open symbols = cells in process of differentiation.

normal cultures a small proportion of the aggregates spontaneously form more than one tip, the aggregate subsequently splitting into several smaller cell masses.

The causality of the relationship between the site of a tip and the resultant pattern of differentiation is equivocal but there are many observations which suggest a direct control. It is easily shown that though differentiation, per se, is independent of morphogenesis, the establishment of the normal pattern of differentiation requires normal morphogenesis. It is noticeable that stalk cells will only form when a tip is present during differentiation (except following treatment with $10^{-3}$ M cAMP or when the cells are enclosed in a cellulose tube (Bonner, 1970)). Even when morphogenesis is abnormal, as in cell masses hanging in drops of 0-1 M urethane, the stalk cells formed are in discrete regions often in long lines resembling stalks, although they are surrounded by spores or dead
cells (Gerisch, 1968). If such aquatic cell masses do not develop a tip, or tip-like process, no stalk cells are formed, the cells either dying or forming spores (personal observation).

From these observations together with those in the literature, one may tentatively propose a mechanism for the control of differentiation by the tip. The aggregate develops a tip which dominates its surrounding area, such area becomes a field organized by the tip as demonstrated by Raper (1940) and Robertson et al. (1972). The tip is formed at the top of the aggregate in response to some external stimulus. A cellulose tube which encloses a central core of cells (as in Raper & Fennel, 1952) is formed beneath the tip. This sheath acts as a selective filter restricting the flow of a certain molecular species, the resultant chemical information about position being interpreted by the cells and used to determine whether they become stalk or spore. Cells enter the forming stalk, at the top, near the tip, in a reverse fountain movement, and are added to the top of the stalk as they differentiate as described by Raper & Fennel (1952). The cell mass which is not included by the lengthening tube progressively differentiates into spores, beginning from the outside. The differentiating spore mass is carried away from the substrate by the extending stalk, this process continuing until all the amoebae in the centre have either been added to the stalk or turned into spores (see Fig. 5). It can be seen that this type of mechanism will give an approximately constant ratio of the two cell types. The bigger the cell mass, the longer it takes for all the cells to differentiate, hence the stalk is longer, but as a consequence of the increased time the number of spores that are formed increase (see Appendix for more formal description). The process is in fact self-limiting, and is attractive because it is a dynamic non-signalling positional informational system, a very simple method of imparting information to cells which fits nicely with the apparent position of the cellular slime mould in the early evolution of truly multicellular organisms. Such a model is consistent with all the observations of this paper. The coincidence of the times for the initiation of tip formation and the determination of developmental axes, and the induction of stalk cells by artificial cellulose tubes, are all predictable from the model.

The regulatory processes leading to the size invariance of the ratio of stalk to spore cells are, I believe, inherent in the physical processes of morphogenesis, there being no need for a signalling mechanism providing positional information for each cell and determining its developmental fate at an earlier stage.

Such a hypothesis indicates a direct experimental approach for the investigation of the specification of pattern. It explains the extreme developmental lability seen in the morphogenesis and it directs attention to the culminatory stages. Certain very definite predictions can be made from this class of model and these are now being tested and will be reported elsewhere.

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REFERENCES


APPENDIX

Considering three basic assumptions for the model:

(1) The radius of the cellulose tube at the site of formation in the tip is proportional to the size of the cell mass at any given time.

(2) The rate of spore deposition is proportional to the surface area of the spherical cell mass as it rises up the stalk.

(3) The rate of stalk formation is proportional to the diameter of the cellulose tube at the tip where the cells enter it.

If \( R_1 = \) radius cellulose tube, \( V = \) volume of the undifferentiated ‘sphere’ of cells, \( V_0 = \) volume of the stalk, \( V_1 = \) volume of the spore, \( J = \) rate of flow of cells into the stalk at the tip, thus:

(1) \( R_1(t) = \alpha V(t)^{\frac{1}{3}}, \)

(2) \( (dV_1/dt) t = \beta V(t)^{\frac{4}{3}}, \)


\( J2\pi R_1(t) = dV_0/dt(t) = - (dv/dt)(t). \)

Eliminating \( R_1 \) from (3):

\[ -V(t)/V(t)^{3/2} = 2\pi \alpha J. \]

\[ \therefore \quad V(t)^{3/2} = (-4\pi/3) J_\alpha t + V^* \tag{4} \]

Substituting into (2):

\[ (dV_1/dt)(t) = (-4\pi/3) J_\alpha \beta t + BV^*, \]

\[ \therefore \quad V_1(t) = (-2\pi/3) J_\alpha \beta t^3 + BV^* t. \tag{5} \]

The culminating process stops when volume of the sphere raised by the developing stalk consists only of spores, i.e.

\[ V_1(t) = V(t). \]

Let this equal \( \bar{V} \), then

\[ (4\pi/3) J_\alpha t = V^* - \bar{V}^*. \]

\[ \therefore \quad \bar{V} = (-2\pi/3) J_\alpha \beta \frac{V^* - \bar{V}^*}{(4\pi/3) J_\alpha} + \beta V^* \left( \frac{V^* - \bar{V}^*}{(4\pi/3) J_\alpha} \right). \]

\[ \therefore \quad \frac{\bar{V}}{V} V^{3/2} = \frac{-3 \beta}{8\pi J_\alpha} \left[ 1 - \left( \frac{\bar{V}}{V} \right)^{3/2} \right]^{3/2} + \frac{3 \beta}{8\pi J_\alpha} \left[ 1 - \left( \frac{\bar{V}}{V} \right)^{3/2} \right], \]

\[ \frac{\bar{V} - \bar{V}^*}{V} \left( \frac{\bar{V}}{V} \right)^{3/2} = \frac{3 \beta}{8\pi J_\alpha} \left[ 1 - \left( \frac{\bar{V}}{V} \right)^{3/2} \right]. \]

Let

\[ \frac{3 \beta}{8\pi J_\alpha} = \gamma, \]

\[ \left( \frac{\bar{V}}{V} \right)^{3/2} = \frac{\gamma}{\bar{V}^{-1/2} + \gamma} = \frac{\gamma \bar{V}^{-1/2}}{1 + \gamma \bar{V}^{1/2}}, \]

\[ \frac{\bar{V}}{V} \left( \frac{\gamma \bar{V}^{-1/2}}{1 + \gamma \bar{V}^{1/2}} \right)^2. \]

Thus for ‘small’ \( \bar{V} \),

\[ \bar{V}/V \approx \gamma^2 \bar{V}^{1/2} \quad \text{or} \quad V \approx (\bar{V}/\gamma)^{3/2} \quad \bar{V} \approx \gamma \bar{V}^*_3. \]

It can thus be seen that for this particular set of initial assumptions the ratio of stalk to spore should vary only by one order of magnitude in four, this being well within the observed limits of the experimental determinations of the size invariance of this ratio (see Bonner, 1967, for a summary of the available figures). Specific predictions about the time course of stalk formation can also be made from this model and these are now being tested.

It is also interesting that a model using much less specific initial assumptions, i.e. assuming only that the rate of spore formation and the size of the tip and hence the rate of stalk formation are related in some fashion to an unspecified spatial dimension of the cell mass, one can prove that the ratio of stalk to spore will again be size invariant.

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