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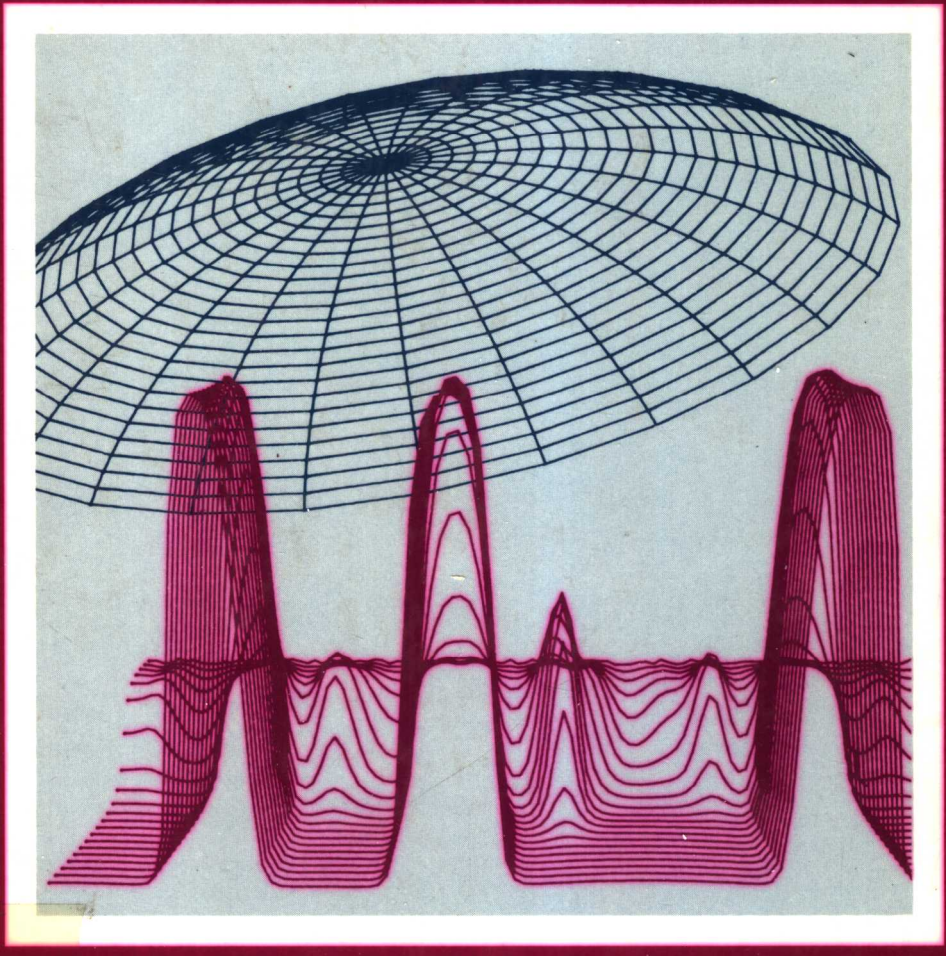
VOLUME 23

SPATIAL ORGANIZATION IN EUKARYOTIC MICROBES



EDITED BY R K POOLE AND A P J TRINCI

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Cover illustration. The cover design is based on Figures 4 and 6 of Chapter 1 and shows a simulation cytoskeleton at the apex of a regenerating *Acetabularia* plant and a periodic calcium pattern.

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This book is based on a symposium of the Cell Biology Group of the SGM held in December 1986.

Abbreviations

cdc	cell division cycle
DAPI	4′,6-diamidino-2-phenylindole
DIF	differentiation inducing factor
cpDNA	chloroplast DNA
mtDNA	mitochondrial DNA
HMM	heavy meromyosin
MF	microfilaments
NETO	new end take off
RIM	reflexion interference microscopy
TLCCD	thin-layer countercurrent distribution
ts	temperature sensitive

Preface

During the last two decades a great deal has been learnt about the genetics, biochemistry and molecular biology of the regulation of temporal events in microbial cells and in particular about the regulation of the cell cycles of prokaryotic and eukaryotic microorganisms. By contrast, comparatively little is known about the mechanisms involved in regulating the spatial organization of cells. The Cell Biology Group of the Society of General Microbiology therefore decided to hold a meeting in Swansea on 'Spatial Organization in Eukaryotic Microbes', both to summarize our present knowledge of this subject and to stimulate further research into this important aspect of cell biology. The present volume is based upon the proceedings of this meeting.

The importance of cytoskeletal elements is evident in a number of chapters. In the first, Brian Goodwin discusses how the spatial organization of cells may be regulated and assesses the role of Ca^{2+} in modulating the mechanical state of the cytoskeleton of *Acetabularia* by influencing the polymerization of microtubules and actin. Jeremy Hyams describes changes in the location of microtubules and actin during the cell cycle of *Schizosaccharomyces pombe*, and draws our attention to the correlations between actin location and septation, and between actin location and cell elongation. William Birky details the importance of the cytoskeleton in partitioning organelles between daughter cells during cell division and describes the role of microtubules in organelle migration.

Conrad King reports the role of cytoskeletal elements in the crawling polarized movement of amoebae, whereby the cytoskeleton is locked onto the substrate to provide the necessary grip. Paul Markham examines the evidence that Woronin bodies may be involved in the spatial organization of fungal hyphae, but finds little to support the hypothesis that these structures are involved in regulating hyphal polarity. Their ability to move into and block septal pores is well-established but whether a contractile system or some other mechanism operates to effect such movement remains an open question.

The use of vibrating probes to detect electrical current around the tips of fungal hyphae is described by Franklin Harold who shows that the appearance of new zones of inwardly directed current precede and predict the appearance of cell polarity and branching in *Achyla*. Polarity in this system can be explained in terms of the non-uniform distribution along hyphae of proton-translocating ATPases and amino acid uptake systems. Neil Gow supports the general hypothesis by demonstrating that the imposition of an electrical field around fungal hyphae influences their direction of growth, and he suggests that this may result from the relocation of charged proteins in the membranes. Cell polarity is readdressed by Donald Watts, who emphasises the role of morphogens in providing undifferentiated cells with positional information.

The Group and the Editors take this opportunity to thank all who spoke and attended, and the meetings staff of the Society for their excellent support.

We hope that this selection of topics illustrates the thrust of research in this challenging field and that the well-referenced chapters will provide a starting point for those who wish to read more widely.

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CHAPTER 1

Mechanisms underlying the formation of spatial structure in cells

B.C.GOODWIN, C.BRIERE[1] and P.S.O'SHEA

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Introduction

Growth and morphogenesis in higher plants result from a complex interplay of processes, chief among which are rate and orientation of cell divisions and specific, controlled changes of shape of individual cells. These require spatial control over intra- and inter-cellular events affecting the mechanical state of the cytoskeleton and the cell wall. We have some understanding of how simple changes in cell shape may be regulated by, for example, hormone-induced changes in microfibril orientation which alter the ratio of lateral to longitudinal cell expansion (Ridge, 1973, 1975); but the origin of more complex cell shapes as in root hair cells or stellate cells is poorly understood. Localized weakening of cell walls must presumably arise and localized expansion follow; for root hair cells this is a process of tip growth. Investigating these processes in multicellular systems is, however, extremely difficult.

We have therefore used the unicellular marine alga, *Acetabularia mediterranea,* to study the control mechanisms governing the detailed and regular changes of shape which this cell undergoes during the regeneration of apical structures, mediated via the cytoskeleton and the plasmalemma. What particularly recommends this species for morphogenetic studies is its structural simplicity, the spatial symmetry of the generated forms, and the ease with which it lends itself to physiological and morphological investigation. There is also now a very extensive literature on many aspects of the growth and regeneration of this species (Bonnotto *et al.*, 1979; Woodcock, 1977). Furthermore, present evidence (Green *et al.*, 1971; Métraux and Taiz, 1977) suggests that the processes controlling growth in algal cells do not differ fundamentally from those in higher plant cells.

Research on *Acetabularia* and selected systems indicates that four important variables are immediately implicated in localized tip growth, namely electrical currents, mechanical strain activation of plasmalemma ion pumps, proton or enzyme secretion and calcium localization. This evidence comes from studies of different plant species,

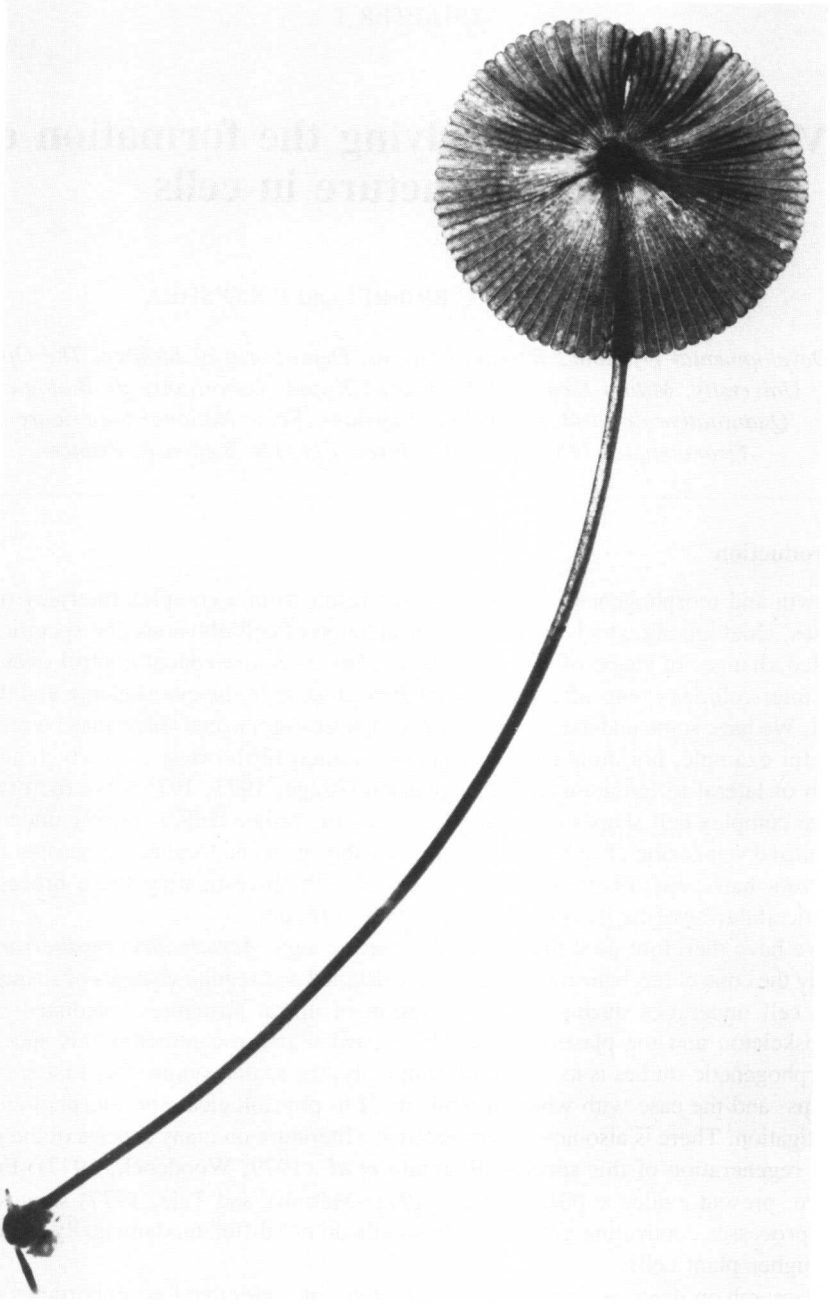

Figure 1. The mature form of *Acetabularia mediterranea.*

but makes a coherent picture. The assocation of electrical currents with tip growth comes primarily from the work of Jaffe (1981) and his co-workers who, by use of the vibrating probe (Jaffe and Nuccitelli, 1974), have shown in the germinating lily pollen grain and

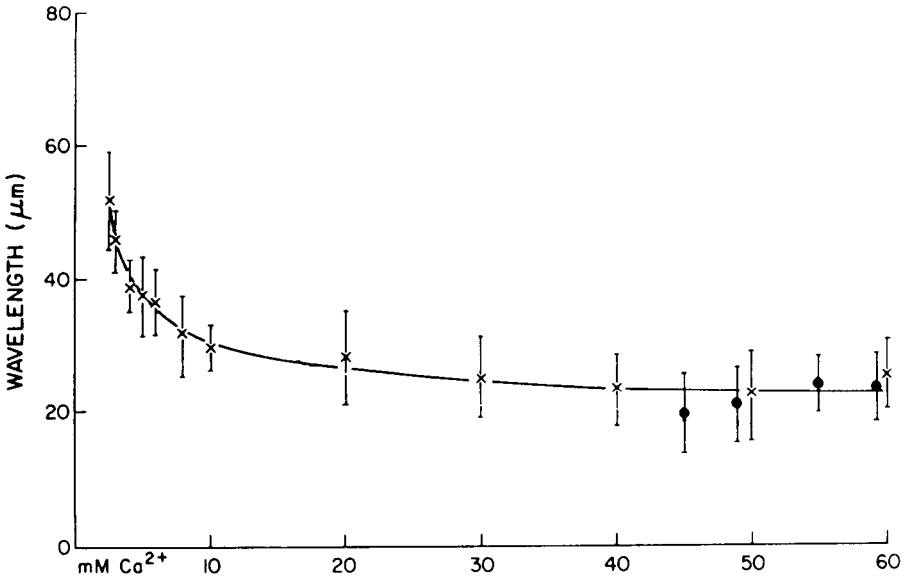

Figure 2. Changes in the wavelength of the whorl pattern as a function of external calcium in regenerating *Acetabularia* plants (×). The mean wavelengths between hairs in whorls with gaps are also shown (•).

in *Fucus* and *Pelvetia* eggs that the growth point is predicted by the point of greatest density of a steady inward-directed current that starts well before any visible growth. These and other studies (Weisenseel and Kicherer, 1981) have indicated that one of the ions carrying the current is calcium, and that there may be differential localization of calcium ion channels and pumps within the plasmalemma, creating the sources and sinks for an intracellular electric current. The electric circuit is completed by extracellular current flow, which can be measured by the vibrating probe.

Identification of the mechanical component of the growth process comes from the recognition that turgor pressure is a prerequisite for cell expansion (Green *et al.*, 1971; Ray *et al.*, 1972). There is evidence that the maintenance of turgor pressure is dependent upon pressure-sensitive ion pumps in the plasmalemma by a mechanism such as that proposed by Zimmerman and Steudle (1978). A relationship between acid-secretion, increase in wall extensibility, and cell expansion has also been suggested (Tepfer and Cleland, 1979), while there is evidence of a direct correlation between external acidification and localized cell expansion in *Chara* and *Nitella* (Lucas and Smith, 1973; Taiz *et al.*, 1981). These observations suggest, but do not yet clearly establish, a causal relationship between local mechanical strain and proton pump activation. It remains a possibility that other mechanisms of wall loosening may be involved in the process, such as hydrolase secretion. These are questions we are currently investigating in *Acetabularia* (*Figure 1*).

The occurrence of increased levels of calcium at the tip of the growing lily pollen tube has been demonstrated by Reiss and Herth (1979) using chlorotetracycline. This correlates well with the electrical current observations of Jaffe (1981) and the evidence

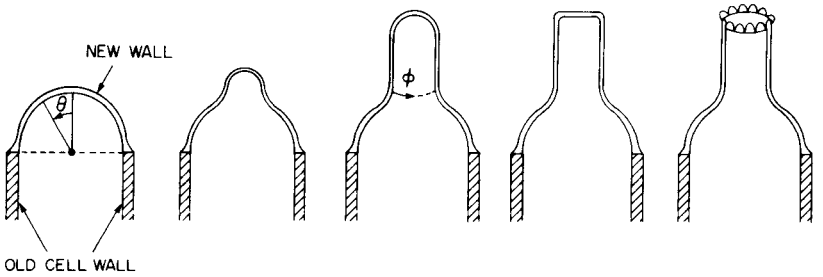

Figure 3. Sequence of morphogenetic shape change at the regenerating tip of *Acetabularia*, from initial repair after cap removal to the beginning of whorl formation.

that calcium is the major ion involved. Furthermore, Goodwin *et al.* (1983) have presented evidence that transitions between the different phases of regeneration in *Acetabularia* can be controlled by extracellular calcium concentration localized in the tip region. Experimental evidence implicating bound calcium as a 'morphogen' in whorl formation has also been presented (Harrison and Hillier, 1985).

The results of a similar study of variation in the wavelength of the whorl pattern as a function of extracellular calcium are shown in *Figure 2*. The wavelength is measured as the mean distance between hair primordia soon after they become visible in whorl formation (see *Figure 3e*). This is determined by measuring the diameter of the whorl, counting the number of hairs and calculating the mean radial distance between hairs. The wavelength decreases in a systematic manner as the external calcium concentration varies over the range in which whorl formation is possible ($2-60$ mM Ca^{2+}; see Goodwin *et al.*, 1983). Interesting evidence that the whorl pattern arises as a global periodicity in some variable(s) comes from the observation of incomplete whorls that form occasionally towards the upper end of the permissive calcium range. Despite the presence of extensive gaps in the pattern where hairs failed to form, the regions of continuous hair primordia had the same wavelength as that of complete whorls. This insensitivity of wavelength to deletions of pattern suggests that whorls are initiated by complete rings of periodicity in variables which could include cytosolic free calcium as a primary morphogenetic agent. The gaps may then be due to regions of the pattern where the amplitude of the periodicity falls below a threshold value for hair formation, as might be expected near a threshold of whorl formation.

These observations all make a *prima facie* case for a connection between electrical, mechanical and ionic variables, primarily calcium and protons, in the control of tip growth and morphogenesis. The experimental system which we have been studying is the sequence of regenerative events following cap removal in *A. mediterranea*. After the wound reaction and recovery, the apical cytoplasm and plasmalemma assume a hemispherical form, with a thin cell wall forming outside the plasmalemma. The first phase of regeneration is the formation of a conical tip which elongates. The geometry of these shape changes are shown in *Figure 3*, where only the cell wall is drawn. After a period of elongation, the tip flattens and a ring of hair primordia forms, as shown in the last two drawings of *Figure 3*. The hairs of the whorl, as the circular structure is called, grow and branch, producing a delicate structure which later dies, the hairs falling off the stalk and leaving a scar. From the centre of a whorl, a new tip arises

and axial growth resumes. Depending upon growth conditions (ion concentrations, nutrients, light, etc) this process may be repeated a number of times, giving a sequence of whorls. The final stage of regeneration occurs when the tip flattens and a cap primordium is formed, a structure which is considerably more complex than a whorl of hairs but has the same radial symmetry. This grows radially to produce the adult cap, an intricately-sculpted form which reaches a characteristic size. Upon cessation of cap growth, the plant reaches a state of morphogenetic equilibrium (for a comprehensive description of these processes, see Puiseux-Dao, 1970).

The morphogenetic field

The question we address in this chapter is the extent to which these morphogenetic changes may be described by a field theory based upon the spatio-temporal properties of the cytoskeleton in the cortical cytoplasm of *Acetabularia*. Field equations describing the state of this structure and the kinetics of cytosolic free calcium, with which it interacts, were derived in a paper by Goodwin and Trainor (1985). These equations are basically the same as those first used by Odell *et al.* (1981) to describe certain morphogenetic processes in animal embryos, with modifications arising from our interpretation of cytoskeletal and calcium dynamics. The equations are applicable to any type of cell, and so in principle could describe the basis of morphogenesis in any organism, with secondary modifications to take account of features characteristic of different types, such as plants or animals, unicellular or multicellular. Applications of this analysis to a variety of different morphogenetic processes have been described by Oster *et al.* (1983) and Murray and Oster (1984). The proposition, then, is that mechanochemical fields based upon cytoskeletal-ionic interactions may be the universal basis of morphogenesis.

An implication of this proposition is that fields based upon the kinetics of diffusion-reaction may not play the primary role in morphogenesis proposed first by Turing (1952), so that there may be no 'morphogens' in this sense. This would account for the remarkable difficulty that has been encountered in identifying such substances in developing organisms.

Starting with the equation derived by Goodwin and Trainor (1985), we carried out a study of their behaviour using a modified finite-element method to describe the inter-

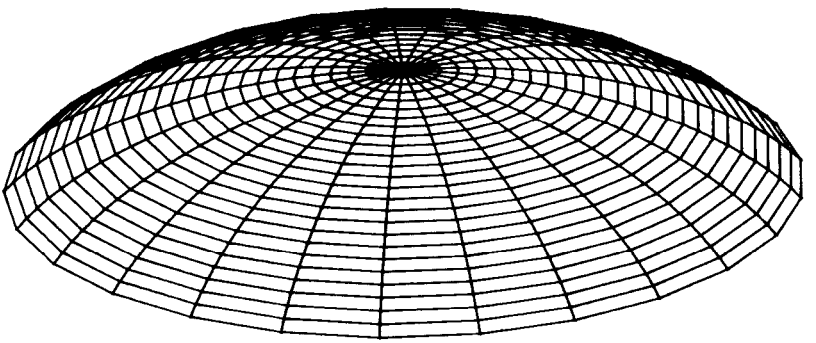

Figure 4. Mesh of viscoelastic filaments simulating the cytoskeleton in the cortical cytoplasm at the apex of a regenerating *Acetabularia* plant.

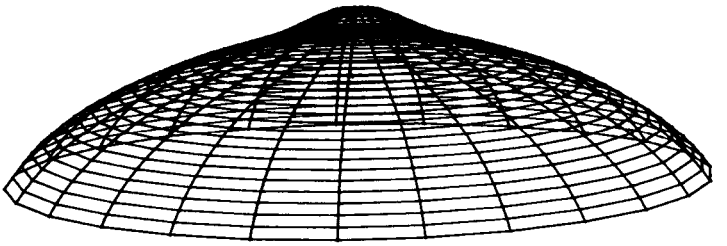

Figure 5. Initiation of tip formation by a bifurcation in the cytoskeleton-calcium system, with a decrease in the elastic modulus of the wall at the apex and buckling out due to turgor pressure.

active dynamics of the cytoskeleton and calcium in the cortical cytoplasm (the thin gel-like, non-streaming region of cytoplasm lying immediately beneath the plasma membrane, which is pressed against the cell wall by turgor pressure from the central vacuole). We began with an analysis of the behaviour of a two-dimensional shell, simulating the regenerating apical tip of an *Acetabularia* plant. This shell is described by a network of finite elements as shown in *Figure 4*, each of which is assumed to obey the visco-elastic and calcium equations of Goodwin and Trainor (1985). For purposes of computation, these take the form described elsewhere (Goodwin and Brière, 1987). The cell wall, external to this network, was described simply by a substance with a variable elastic modulus whose value was defined by a function of the mechanical strain in the underlying cortical cytoplasm. The reasoning behind this coupling is explained in Goodwin and Trainor (1985): where the cortical cytoplasm is strained, so will be the plasmalemma, which could then either activate proton pumps, resulting in local wall acidification and softening; or facilitate hydrolase secretion. The result of the simulation is shown in *Figure 5*. The geometry together with the boundary conditions have the consequence that maximum strain occurs at the tip, the cell wall elasticity decreases here and bulges out under the action of the outward-acting turgor pressure.

However, we now face the question of how the mechanochemical model produces the whorl pattern. We have shown in a simulation of a one-dimensional version of the non-linear equations that growth of a periodic mode does indeed occur for parameter values satisfying the bifurcation condition, as shown in *Figure 6*. However, we have not yet demonstrated this in two dimensions, although we expect to obtain this in the full non-linear simulation. Whether or not the geometry of the growing tip dictates the whorl pattern as the most stable bifurcating solution remains to be seen.

Extracellular ion currents

A recent study we have carried out using a vibrating probe to measure extracellular electrical currents has extended the analysis above. Both mature and regenerating plants in the light show large (up to 380 $\mu A.cm^{-2}$) currents directed out of the plant (coventional current polarity) everywhere except the rhizoid, where the currents are directed inwards. Ion substitution studies have revealed that most of this current actually consists of an outward flux of chloride at the rhizoid, inwards elsewhere. This current is greatly reduced in the dark, to between 0 and 4 $\mu A.cm^{-2}$. Previous experimental studies (Goodwin *et al.* 1983) had shown that regeneration can be selectively inhibited

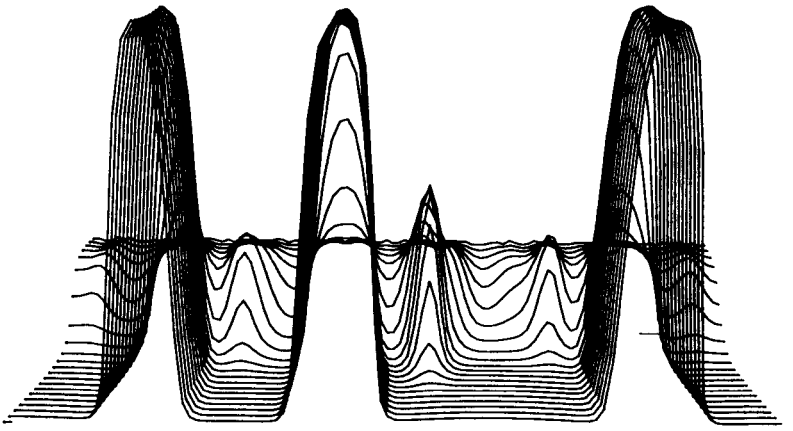

Figure 6. Development of a periodic pattern in calcium in a one-dimensional simulation of the cytogel with parameter values in the bifuraction range.

at different stages (tip, whorl, cap formation) by either reduction in external calcium to specific levels in the external medium, or addition of ions such as cobalt or lanthanum which block calcium entry into the cell. It was therefore of interest to see if the addition of cobalt to the medium in which the plants were regenerating had an effect on the current.

Plants regenerate in the dark as rapidly as in the light, providing they are on a light–dark regime. Since the dark current is much smaller than the light, the effect of added cobalt (0.5 μM $CoCl_2$) was measured and found to reduce the current density by 5–15%, amounting to less than 0.4 $\mu A.cm^{-2}$, which is nevertheless a substantial calcium flux in absolute terms. A similar current reduction in response to added cobalt was observed in the light, but it was more difficult to measure accurately because of the large current levels under these conditions. A calcium outflow from the stalk and into the rhizoid thus appears to be a component of the current flux in *Acetabularia*.

A further observation on regenerating plants was very interesting. It was found that the extreme apex of the regenerating tip is electrically silent, with no net current entering or leaving the plant in this region. This is where most of the growth occurs, new membrane and cell wall being actively produced as the cell elongates. It thus seems most likely that electrical neutrality is maintained by a balanced rate of ion movement across this growth domain, with a net calcium influx here balanced by other cation efflux. However, a resolution of these details requires further study. This work will be reported *in extenso* in a forthcoming paper by O'Shea *et al.* (1987).

Thus, our combined experimental and theoretical study of a rather basic example of tip growth and morphogenesis in the unicellular giant marine alga, *Acetabularia*, provides a model system for the study of more complex forms of apical growth. There are still many questions that require resolution in the unicellular case, foremost among which are the conditions for sustained growth after tip formation in the model (*Figure 4*) and the realization of the whorl pattern, a three-dimensional version of the bifurcation in *Figure 6*. On the experimental side, major questions concern the exact nature