**REVIEW
ARTICLE****From morphogenes to morphogenesis**

Franklin M. Harold

Tel: +1 970 491 0433. Fax: +1 970 491 0494.

Department of Biochemistry and Molecular Biology, Colorado State University, Fort Collins, CO 80523, USA

Keywords: morphogenesis, growth, polarity, cytoskeleton, cellular continuity

If the concept of organization is of such importance as it appears to be, it is something of a scandal that biologists have not yet begun to take it seriously but should have to confess that we have no adequate conception of it. The first duty of the biologist would seem to be to try and make clear this important concept.

J. H. Woodger, *Biological Principles – A Critical Study* (1929)**The Genetic Program**

There are many generalizations in biology but precious few universal laws; and of these, the least controversial may well be that like begets like. Offspring resemble their parents in form as well as function: roses and rabbits, yeast and *Escherichia coli* display the same forms, generation after generation, within a narrow range of variations. How does that come about? The object of this essay is to consider how far we have come in the search for answers; and to persuade you that the answer is not known, not even in principle, for the quest reaches deep into the abiding mysteries of organized complexity.

Biology has few laws but innumerable instances; which forms should we think about? Microbiologists learned from Francis Bacon that the nature of things is oft better perceived in small than in great; and will therefore be receptive to the proposition that, as in the case of metabolism, energetics and heredity, the essential principles of morphogenesis are most plainly displayed by the simplest creatures. So we shall speak here, not of frogs and flies but of bacteria and fungi, and seek a fresh perspective on that ancient conundrum.

Some may suspect that I am setting up a straw man: surely, we know how like begets like! Form, like other facets of structure and function, must be encoded in the genes. Indeed, if you delve into the literature you will soon encounter the idea of a 'genetic program': the proposition that the genome encodes, not form *per se*, but instructions for generating particular forms. No one has given a more explicit statement of this premise than François Jacob (1973): 'The whole plan of growth, the whole series of operations to be carried out, the order and the site of synthesis and their co-ordination are all written down in the nucleic acid message'. It is a sweeping claim, and we shall later consider how to interpret it. For the present, let us note that the genetic program is a metaphor.

To give the proposition meaning, the word must be made flesh: one must describe concrete and explicit mechanisms by which linear, one-dimensional nucleotide sequences determine living forms in three dimensions or in four.

Historically, biologists have taken two distinct paths toward this goal. One approach asks how genetic changes affect form, by isolating mutants whose form is aberrant and then working out the nature of the defect. This approach, a productive and fashionable one, directs attention to individual genes and gene products that play a role at particular stages of morphogenesis. The alternative procedure is to ask how organisms produce successive shapes as they traverse their life cycle. This query focuses attention on structures, forces and flows that modulate form, rather than on molecules and genes. The chief message of the present argument is that these two modes of inquiry are complementary, not successive. It is emphatically not true that research at the 'molecular' level supersedes the physiological studies of earlier times because it affords deeper insight into the nature of things. On the contrary, if we are ever to understand how one cell of *E. coli* gives rise to two daughter cells shaped just like their parent, we must marry the bottom-up view of the molecular biologist with the top-down view of the physiologist. Even the simplest cells are systems of mind-boggling complexity; we will not understand how forms are inherited until we understand how they are produced.

What follows is an essay, the presentation of a point of view, rather than a literature survey. I have cited chiefly recent papers and review articles; references to earlier work will be found in these, and also in a previous effort of my own (Harold, 1990). But I must not fail to acknowledge the inspiration of colleagues, whose writings lent clarity to ideas that I present here as though they were entirely my own, notably John Tyler Bonner, Joseph Frankel, Brian Goodwin, Lionel Jaffe, Paul Green and Gunther Stent.

The tactics of morphogenes

The uses of morphological mutants

The term 'morphogene' was coined, as far as I know, by William Donachie to designate genes involved in the production of organismal form; expression of the mutant phenotype results in abnormal shape. Many such mutants have been isolated from bacteria, fungi and other micro-organisms, but in most instances the biochemical nature of the deficiency is not known. Only in the case of the budding yeast *Saccharomyces cerevisiae* do we possess a substantial collection of genes (most of them temperature-sensitive) whose mutant phenotype is grossly mis-shapen, and for which the biochemical activity of the gene product is known in some detail. Many of these were isolated as *cdc* mutants, which arrest at particular stages of the cell cycle with reproducibly abnormal morphology (Pringle & Hartwell, 1981); others were selected or constructed in recent years, with various objectives in mind. Table 1 presents a sample of these genes, intended to illustrate both the power and the limitations of the genetic approach to morphogenesis.

Inspection of the set shows that the mutants fall into three classes. The first consists of genes that specify the structure or production of primary cellular building blocks such as actin, tubulin or chitin. The second contains genes that encode familiar housekeeping enzymes, such as pyruvate kinase or phosphoglucose isomerase; why a defect in one of these enzymes should result in morphological abnormalities is quite unknown. The third, and potentially most informative class, consists of genes that specify elements of the cell's regulatory circuitry: protein kinases, guanosine triphosphatase, calcium-binding proteins. Every one of these assignments represents a major research accomplishment, yet from the viewpoint of morphogenesis the outcome is disappointing. A glance at Table 1 suffices to show that identification of a genetic lesion with a particular biochemical deficiency in no way explains, or accounts for, either the normal form or the aberrant one resulting from the mutation.

The failure of these molecular discoveries to connect with cellular forms is, in my judgement, not some temporary shortfall but an unconformity that demands explicit recognition. It calls into question the presumption, taken

Table 1. A gallery of genes and shapes in *S. cerevisiae*

Gene/genotype	Phenotype	Morphology	Reference
Wild-type	Normal budding		Gimeno <i>et al.</i> (1992); Blacketer <i>et al.</i> (1994) Chenevert <i>et al.</i> (1992)
Wild-type	Forms shmoos in response to mating factor		
Wild-type	Diploid cells subjected to nitrogen starvation elongate and grow as pseudohyphae		
<i>BEM1</i>	Defective response to mating factor, fail to make shmoos		
<i>CDC3, 10, 11, 12</i>	Grow as multinucleate cells with multiple abnormal buds. Defective in making chitin ring and neck filaments.		Adams & Pringle (1984); Kim <i>et al.</i> (1991); Flescher <i>et al.</i> (1993) Sloat <i>et al.</i> (1981)
<i>CDC24</i>	Fail to polarize or bud, grow into huge multinucleate cells. Probably defective in a calcium-binding protein.		
<i>CDC30</i>	Arrest as mononucleate, single-budded cells. Deficient in phosphoglucose isomerase.		
<i>CDC42</i>	Fail to bud but continue to grow, arresting as large unbudded cells. GTP-binding protein, may be involved in actin assembly.		Dickinson & Williams (1987)
<i>CAP2</i>	Similar to actin-deficient mutants: grow slowly, size and shape abnormal. Deficient in capping protein.		
<i>PFY2</i>	Similar to <i>CAP2</i> mutants, deficient in profilin		Adams <i>et al.</i> (1990); Li <i>et al.</i> (1995)
<i>PKC1</i>	Arrest in division with small buds. Deficient in a homologue of protein kinase-C.		
<i>MYO2</i>	Grow as large, unbudded cells with abnormal microfilaments. May be defective in a motor protein.		Amatruda <i>et al.</i> (1990)
<i>TPM1</i>	Similar to <i>MYO2</i> , also fail to make shmoos. Deficient in tropomyosin.		
			Haarer <i>et al.</i> (1990)
			Levin & Bartlett-Heubusch (1992)
			Johnston <i>et al.</i> (1991)
			Liu & Bretscher (1992)

by many scientists as an article of faith, that when enough is known of the molecular parts their organization into a whole will take care of itself. Morphological mutants obviously supply powerful tools for the dissection of the molecular mechanisms that underlie morphogenesis, and we can confidently expect ever more detailed accounts of these processes. But the integration of the constituent molecules into the higher-order function of growing a bud does not leap to the eye. For the most part, morphogenes encode quite mundane proteins, elements of the catalytic or structural ensemble. There is no obvious 'genetic program', no clear distinction between one subset of genes whose products execute morphogenesis and another subset that directs the operation. One would expect such supervisory genes to be transcribed at particular stages of morphogenesis, and examples of stage-specific genes have been reported (Andrews & Herskowitz, 1990; Johnston, 1992). But these turn out to be concerned with DNA synthesis and the cell cycle, not morphogenesis *per se*. As a general rule, morphogenes (like other genes) appear to be transcribed continuously, often in advance of need; and in many instances morphogenesis proceeds in the absence of new protein synthesis altogether (Kropf *et al.*, 1989; Soll & Sonneborn, 1971). But if one cannot distinguish genes whose products govern the work from those that execute it, what meaning can one assign to the glib assertion that this or that gene 'controls' some particular process? Nor does it do much good to mumble that, once all the genes and their products have been characterized, the problem will vanish; for the limitations of the results at hand are too glaring to wither away of their own accord.

My own reading of the outcome is that there is a wide gap between the genes, which specify the structure and synthesis of molecular components, and the spatial articulation of these components. Cellular forms emerge as a collective expression of the activities of many gene products, in a manner that has yet to be spelled out. The study of morphogenes is useful, indeed necessary, but not sufficient; our goal must be to unravel the network of epigenetic processes that generate actual forms, but also to visualize it as an integrated whole. Only when this has been accomplished can we understand how forms are transmitted from one generation to the next; and a narrow focus on individual genes distracts from this task.

In search of a better metaphor

The belief that cell shape is somehow prescribed by the genome has taken a grip on the scientific imagination, no doubt because this metaphor does illuminate the relationship between nucleic acids and proteins (Stent, 1981, 1985; Goodwin, 1986). The sequence of nucleotides specifies the sequence of amino acids, and the chain folds spontaneously into the correct three-dimensional form. It is quite appropriate, then, to envisage the shape and function of an enzyme as being directly prescribed by the 'program' of bases in DNA. Indeed, that program's writ often runs much further. Many large and intricate biological structures arise by self-assembly of pre-formed protein and nucleic acid subunits, with little or no input of

Table 2. Some self-assembling structures

Viruses, including bacteriophages
Ribosomes
Bacterial flagella and basal bodies
Lipid bilayer membranes
Some cell walls (S-layers, glycoprotein walls)
Microtubules (tubulin)
Microfilaments (actin)
Thick filaments (myosin)
Nucleosomes
Nuclear-membrane pores
Nucleus as a whole, including lamina and membrane

additional energy or information (Table 2). One can thus think of ribosomes or bacterial flagella as being fairly directly specified by the genes that encode their components – though in reality, this generalization skates briskly over some very thin patches (see Kellenberger, 1990, for the case of viruses). If the form of organelles can be said to be instructed by genes, why should we not extrapolate this insight to whole cells and organisms?

The reason is that the processes of cellular construction are quite different from self-assembly, and in quite fundamental respects. Cells never arise by aggregation, but by the growth of pre-existing cells; they model themselves upon themselves. Their form is not related in a simple fashion to those of their molecular constituents, which are smaller by three to six orders of magnitude, but is the product of generative processes organized in space. These generative processes are typically energy-consuming, directional and dependent upon pre-existent structures (localized deposition of cell wall as a consequence of targeted secretion is a case in point). To be sure, molecules make up the structures and mediate all the processes; but their articulation in cellular space is a function, not of intrinsic molecular shapes but of localized signals and markers. We can see that, when applied to the organismic level of organization, self-assembly becomes another metaphor – and an inadequate one at that.

In fact, a more illuminating metaphor has been at hand for nearly 40 years, with the special merit that it can be represented pictorially. Conrad Waddington, in his prescient work entitled *The Strategy of the Genes* (1957), spoke of the 'epigenetic landscape' illustrated in Fig. 1(top). A developmental process, such as the unfolding of a yeast cell's bud, is represented in an abstract manner by the path taken by the ball as it rolls downhill towards the front of the scene. Its course is guided by the chief valley, which steers the ball down the normal route. But a small change in the configuration of the landscape, or a judicious nudge, could deflect the ball into an adjacent valley, and thus elicit the production of an aberrant form.

If morphogenesis is channelled by hills and glens, where do the genes come in? Look at the landscape again, this time from beneath (Fig. 1, bottom). It is an undulating surface, shaped by struts and stays like a tent at the fair;

Fig. 1. The epigenetic landscape. Top, the course of morphogenesis is represented by the path of the ball as it rolls downhill towards the observer. It will normally keep to the valley on the left, but may be diverted into an alternative one by genetic or environmental changes. Bottom, the landscape as a surface, seen from beneath. The pegs in the ground represent genes, and the strings that connect to the surface correspond to gene products and their activities. Reprinted from Waddington (1957) with permission from George, Allen & Unwin, an imprint of HarperCollins.

and the genes are represented by the pegs that hold down the guy ropes. If one of these were pulled out by mutation, there may or may not be consequences for the shape of the surface above, and hence for the path taken by the ball; but these consequences are governed, not only by the peg that was pulled but also by many others that remain in place. Even so, morphogenesis results from the interplay of hundreds of gene products deployed in a spatially structured manner. To understand the phenotype of a morphological mutant, its particular lesion must be examined as one strand of a web. Morphogenes have much to teach us about the tactics of morphogenesis; for the strategy we must turn elsewhere.

Digression on the uses of metaphor

Hard-headed readers are apt to wonder what to make of the repeated reference to metaphors. To put it charitably, is not a metaphor just a temporary expedient, to be

discarded as soon as definite and certain information has come to hand? My own experience of scientific procedure suggests otherwise. The only questions we can ask of Nature that do yield definite and certain information are quite narrowly phrased. Which protein does gene X encode? What second protein does the first bind to? What structural features define the substrates of a particular enzyme? Such information, though often useful, gains biological meaning only when it has been woven with many other strands into a broad, causal, dynamic and comprehensible account that connects with living organisms. Information comes in bits; understanding requires us to integrate the bits, and that remains a task for the imagination. In science, as elsewhere, imagination is impotent without metaphor. Metaphors allow us to rephrase the new and puzzling in terms of the homely and familiar, and so provide a staircase for the mind to climb. If you doubt this, look at the technical terms that make up scientific discourse and consider how many of them began their career as metaphors.

Rambling the epigenetic landscape

The merits of the epigenetic landscape as a guide to reflection become apparent when one delves beneath the surface to ask which aspects of morphogenesis are represented by the peaks and valleys of Fig. 1(top)? On the premise that morphogenesis is not primarily about molecular building blocks but about their assembly into complex structures on a much larger scale, I take it that the landscape's topographic features correspond to the processes that generate form on the cellular scale, micrometres to millimetres. These are necessarily epigenetic, for they require the coherent workings of large numbers of molecules, producing effects that are directional and localized both in space and in time. In short, morphogenesis lies in the domain of the physiologist, the student of complex systems.

In order to keep this discussion coherent and directional let me centre it on a particular organism, the budding yeast *S. cerevisiae*. How far have we come towards understanding how these cells grow buds, constant in shape and placement? A meaningful answer must reach beyond the perpetuation of individual genes and gene products, to the processes that generate the bud and to the persistence of their timing and localization in space.

Life-history of a bud

Fig. 2 plots the initiation of a bud, its enlargement and eventual separation from the mother cell. It is useful to envisage this as a developmental itinerary, consisting of concurrent but intrinsically independent processes that shape the bud, duplicate the genome and separate cellular individuals. These processes are coordinated with the aid of 'checkpoints', to ensure that (for example) closure of the septum does not proceed until mitosis has been completed (Hartwell & Weinert, 1989; Lew & Reid, 1995).

The genesis of a bud begins with the selection of a locus

Fig. 2. Landmarks of the cell cycle of *S. cerevisiae*. Note that deposition of new cell wall is restricted to the bud.

on the mother cell's surface where outgrowth will later take place. Placement of the bud is governed by rules, and can be predicted from the genotype: haploid α and a cells bud axially, as do homozygous diploids, but heterozygous diploids (αa) bud in a bipolar manner. Genes required to execute these rules have been identified and shown to encode proteins of a GTPase cycle (Chant & Stowers, 1995). Just what transpires during selection of the bud site remains uncertain, but the propensity of haploid cells to place the new bud adjacent to a previous division site strongly suggests that a spatial marker of some kind persists from one cell cycle to the next. A different marker is thought to identify the poles of diploid cells (Chant & Pringle, 1995). When this marker is 'activated', it guides the localized assembly of an annular structure that marks the new bud-initial; this includes a ring of chitin, another of 10 nm neck filaments and several additional proteins (for recent reviews see Drubin, 1991; Madden *et al.*, 1992; Nelson, 1992). It appears that the new bud normally emerges within this ring, although no one of its constituents is thought to be essential.

A new-born yeast cell grows and deposits wall polysaccharides uniformly all over its surface until it attains some critical size and initiates a bud; thereafter, deposition of new wall material is confined to the growing bud. Expansion of the wall is patterned in space and in time: to begin with, growth takes place chiefly at the tip of the forming bud, then it becomes uniformly distributed, and eventually expansion halts. The mature bud is distinctly smaller than its mother. It must be remembered that it is the wall that determines a yeast cell's shape; the manner of wall growth is therefore the crucial ingredient in morphogenesis.

Expansion of the wall is a secretory process, most of whose secrets remain to be revealed. Membrane-bound

Fig. 3. Physiology of bud growth. The diagram identifies processes known or surmised to contribute to the enlargement of the bud's surface.

secretory vesicles are prominent in every electron micrograph (Fig. 4). The general consensus is that these carry precursors and enzymes for wall biosynthesis: mannosylated proteins, chitin synthase and β -glucan synthase. When the vesicles reach the plasma membrane they fuse and undergo exocytosis (Figs 3 and 4). But the contents of these vesicles have not been established; recent findings suggest that wall precursor vesicles are distinct from those that transport secretory enzymes (Govindvan *et al.*, 1995). Precursor vesicles are likely to comprise a diverse family, of which only a single member has been characterized: chitosomes, whose cargo is a precursor form of chitin synthase (Bartnicki-Garcia *et al.*, 1984; Gozalbo *et al.*, 1987). Once inserted into the plasma membrane, chitin synthase is believed to catalyse a vectorial reaction, such that chitin precursors come from the cytoplasm while the polysaccharide elongates at the external surface.

Precursor vesicles mature in Golgi equivalents, travel to the cell surface and undergo exocytosis. At the present time, neither the pathway of vesicle transport through the cytoplasm nor their targeting to the site of fusion are clearly understood, but both subjects are under intensive investigation (Novick & Botstein, 1985; Baba *et al.*, 1989; Adams *et al.*, 1990; Liu & Bretscher, 1992; Gabriel & Kopecká, 1995). Cramming into a thimble the findings of several laboratories (Fig. 3), it appears likely that the prominent cables of actin microfilaments that course through the cytoplasm and reach into the bud serve as tracks upon which wall precursor vesicles move towards the site of exocytosis. A particular form of myosin, encoded by the *MYO2* gene, may serve as the motor for this transport (Johnston *et al.*, 1991; Govindvan *et al.*, 1995). The cables are thought to target the vesicles to specific fusion sites, which can perhaps be identified with prominent plaques upon the plasma membrane that contain actin and actin-binding proteins. The distribution of these plaques is generally correlated with sites of wall

synthesis. Recent spectacular electron micrographs (Mulholland *et al.*, 1994) display local invaginations of the plasma membrane, bounded with actin, that may possibly represent the loci of exocytosis and of new wall deposition. Confirming the existence of these invaginations by means of freeze-fracture electron microscopy, Kopecká & Gabriel (1995) propose that they represent the sites of β -glucan incorporation.

While all the foregoing has been happening at the cell surface, the nucleus has not been idle. Just before the bud primordium forms, the spindle-pole body (a centriole-equivalent situated upon the nuclear membrane) undergoes duplication. The products separate and migrate, followed by rotation of the nucleus, bringing the spindle-pole bodies into line with the axis of the emerging bud. The two spindle poles then nucleate development of the mitotic spindle, which guides separation of the duplicated chromosomes. Another set of microtubules sprouts from one spindle pole to reach deep into the bud. Somewhat surprisingly, microtubules (unlike microfilaments) are not required for the localization or emergence of the bud (Huffaker *et al.*, 1988; Jacobs *et al.*, 1988), but they are needed to guide migration of the nucleus into the neck of the developing bud, and presumably serve to move other cytoplasmic organelles into the bud as well.

Upon completion of mitosis, the maturing bud separates from its mother by ingrowth of the septum. Unlike the bulk of the cell wall, which is made of β -glucans and mannoproteins, the septum is composed of chitin. Its formation is a lengthy process that requires two distinct chitin synthases (Shaw *et al.*, 1991). Septation begins prior to the emergence of a visible bud (Kim *et al.*, 1991), and culminates with cytokinesis and splitting of the septum to liberate the newly formed daughter cell. The site of septum formation is marked by a permanent bud scar upon the mother-cell's surface.

Force and compliance

The identification of molecular elements of the physiological machinery that effects the development of a bud represents genuine progress, yet it leaves the problem of morphogenesis *sensu stricto* very nearly where it stood two decades ago. We can describe the succession of forms displayed by a developing bud, but lack explanatory principles to account for that succession. One reason for this mismatch lies close to hand. Form is an expression of the spatial and temporal organization of physiological activities, and these higher levels of biological order are the first to be disrupted when cells are homogenized in preparation for molecular analysis. To understand how forms are generated and change with time, one must step back from the accumulation of molecular detail and consider the physical properties of biological materials, the forces that confer shape upon them, and the means employed to localize the action of these forces. Events at this level of organization are not readily reducible to molecular structures and couplings, but they bridge the gap between the nanometre scale of molecules and the micrometre scale of cells.

Every object, living or inanimate, owes its form to physical forces of one kind or another. D'Arcy Thompson earned enduring fame by his elegant exposition of this theme (Thompson, 1961). In the case of the bud, expansion must at minimum overcome the forces of molecular cohesion. In other words, the wall that exists at any moment must be subjected to forces tending to stretch it (stress); and it must yield to these forces (strain), usually by the insertion of new wall elements, while maintaining the cell's osmotic integrity. Stress and strain will both be non-uniformly distributed, for reasons both geometrical and biological, and the changing form of the growing bud probably owes much to the pattern of stress and strain. Unfortunately, the structure of yeast cell wall is only partially understood (Klis, 1994); as to its physical properties and manner of expansion, both are areas of near-total darkness. Reflecting on this matter early in the development of the surface-stress theory, Koch *et al.* (1982) and Koch (1983) proposed that a bud is initiated by local softening of the cell wall; it expands uniformly by the insertion of new wall elements, in response to tension exerted by turgor pressure as the volume of cytoplasm increases. The general principle is the marriage of global force and local compliance (Harold, 1990).

The idea, though plainly simplistic, can serve as a starting point for more sophisticated inquiry. Yeast cells do maintain turgor pressure, and some insight into its regulation has recently been attained (Maeda *et al.*, 1994), but no attempt seems to have been made to relate hydrostatic pressure to wall expansion. One would want to know how wall expansion is effected, what regulates its plasticity, and particularly how wall expansion is localized. These are questions with which students of hyphal fungi have had considerable experience. There is a broad consensus that turgor pressure supplies the driving force for apical extension and shapes the growing tip (Saunders & Trinci, 1979; Koch, 1994). Nascent wall, produced at the apex by freshly exocytosed synthases, is plastic and stretches under the force of hydrostatic pressure; but it soon hardens, becoming rigid and capable of supporting the cylindrical trunk (for a lucid and penetrating discussion of this conception see Wessels, 1986, 1993). But the relationship between pressure, wall plasticity and morphogenesis is not straightforward. The oomycetes *Achlya* and *Saprolegnia*, which are particularly well suited to research in this area because they do not regulate turgor, can extend and even generate normal hyphae in the absence of measurable turgor by depositing a more plastic apical wall (Money & Harold, 1992, 1993; R. L. Harold, N. P. Money & F. M. Harold, unpublished observations). The simplicity of nature, like so much else, seems to be in the eye of the beholder!

We are just beginning to explore the interplay of wall plasticity and hydrostatic pressure in shaping tip or bud. The point to be emphasized is that no student of morphogenesis can ignore this level of inquiry, whose concepts and techniques are far-removed from the molecular. I am keenly aware that physiology (in fact, all of non-molecular cell biology) is out of favour and allegedly unfundable. One must hope that this is not altogether

true, lest scientific fashion join with political myopia to foreclose the subject of morphogenesis to experimental scrutiny.

The challenge of localization

Cell biology differs from biochemistry in that it revolves around events that happen in one place but not in another. To illustrate the issues that must be tackled, consider dimorphic growth of the pathogenic fungus *Candida albicans*. On certain growth media the population consists entirely of single yeast cells that reproduce by budding, much as described above. Relatively small changes in the temperature or pH of the growth medium trigger an abrupt transition to the hyphal form: the cells cease to separate but continue to elongate, producing cylindrical tubes whose extension is restricted to the apex (Gow, 1994). How does this come about? One can seek insight into the matter at more levels than one, by asking about the nature of the environmental signals that trigger the transition (Odds, 1985), or by identifying genes that are differentially required for one phase or the other (Elorza *et al.*, 1994; Liu *et al.*, 1994). But if our goal is to understand the transition from the yeast shape to that of a hypha, the most pertinent approach is that pioneered by Staebell & Soll (1985) and Soll *et al.* (1985), and recently extended by Merson-Davies & Odds (1992). The former authors examined surface expansion directly, by monitoring the successive positions of latex particles bound to the cell surface. In budding yeasts, about 70% of the expansion took place in a tiny apical region; when the bud had attained about two-thirds of its final length the apical site shut down, leaving isotropic expansion to complete the bud. By contrast, in the hyphal phase the apical site remained active indefinitely and delocalized expansion made but a minor contribution.

How these two spatial patterns arise must be the crux of the matter, the key to dimorphic growth. The question is one facet of the more general one, how is wall expansion localized? The simplest hypothesis proposes that the wall expands at the locus where precursor vesicles undergo exocytosis (this, in turn, might be the site to which they are delivered by tracks, or else a fusion site identified by a local signal or marker). Yeast and hyphae would differ in that exocytosis is diffuse in the former, tightly focused in the latter. The hypothesis is consistent with the distribution of peripheral actin plaques, which tend to be the most abundant in regions of active wall synthesis (Adams & Pringle, 1984; Anderson & Soll, 1986; Gabriel & Kopecká, 1995; Yokoyama *et al.*, 1994). Mulholland *et al.* (1994) propose that the plaques, which they believe to represent actin-bounded invaginations of the plasma membrane, may be loci of wall synthesis that are shielded from the stress of hydrostatic pressure. It is not a long leap to suggest that in due course the shelter is dismantled; at this instant the particle of nascent wall becomes part of the stress-bearing fabric, allowing the latter to expand. Differential rates of wall hardening may play a secondary role in shaping the cells: the slower the rate at which nascent wall sets, the more rounded the final shape is likely to be.

Localized wall expansion and re-modelling appear in many guises. When a yeast cell selects a bud-site, exocytosis is one of the functions that will be called for. Haploid cells placed in a gradient of mating pheromone change shape, developing into 'shmoo' (Fig. 4) whose pointed end faces the high side of the gradient (Segall, 1993). And diploids of the proper genotype, when subjected to nitrogen starvation, give rise to elongated cylindrical cells (Table 1) that bud from the distal end, generating pseudohyphae (Gimeno *et al.*, 1992; Blacketer *et al.*, 1994). Both these morphological variants presumably represent alterations in the spatial and temporal pattern of wall expansion. Whether they also reflect changes in the localization of secretion remains to be seen, and the underlying molecular mechanisms are largely unknown.

So far, so good. Many unanswered questions remain concerning the assembly of wall precursors, the expansion of wall and how these processes are localized to particular sites. But the terms of the discussion are coming together. This is a story about vesicle transport, localized exocytosis, actin assembly and transmembrane signals; and it is a story that, at least in outline, may be common to many walled eukaryotic microbes. This makes it imperative to state that progress on the mechanistic plane cannot, by itself, lead to an understanding of morphogenesis, which requires global organization. Suppose, for example, that the actin plaques of *C. albicans* turn out to represent sites of local cell wall synthesis and expansion. What, then, makes these plaques cluster together under conditions that induce hyphal growth, and keeps them clustered, while conditions that favour the yeast phase allow the plaques to disperse? A local signal, you say, and that may be so – but what activates the signal just at the tip? There is no doubt that we are gradually learning how the walls of unicellular organisms are produced. Now we must ask how cells orchestrate these processes globally; and that takes us further along the hierarchy of complexity, where the light grows murky and there are few firm stepping stones.

Polarity and self-organization

At the end of the day, the study of morphogenesis is an exercise, not of scientific analysis but of synthesis. Morphogenesis defies 'reduction', for the very coherence of many molecules and their integration into a large-scale purposeful pattern are its essence. If genes specify building blocks and physiologists examine construction, pattern formation is akin to architecture.

How do organisms impose order on the confusion of the construction site? Here is the crux of the matter, the point where we transcend chemical means for biological ends. Most microbiologists, it appears, cling to Jacob's (1973) dictum, at least in spirit: they look to the genome to play the architect's role. From where we now stand, this seems to me a rejection of reason. All that we have learned points to the conclusion that several epigenetic layers intervene between genes and form. Morphogenesis cannot be orchestrated by the genome, but makes manifest a

Fig. 4. A polarized cell. Three-dimensional reconstructed images of a single *S. cerevisiae* 'shmoo', treated with α mating factor for 80 min. The rough endoplasmic reticulum that lies beneath the plasma membrane has been omitted. (a) Top view; (b) side view. f, filasome; G, Golgi body; l, lipid granule; m, mitochondrion; n, nucleus; nu, nucleolus; rer, rough endoplasmic reticulum; v, vacuole. Note the accumulation of precursor vesicles at the apex. Reprinted from Baba *et al.* (1989) with permission from the Company of Biologists.

higher level of order, corresponding to the cellular scale of size and organization.

If a single phrase can stand for the whole riddle, it may be 'cell polarity'. The term refers to the visible directionality, not only of discrete physiological processes such as vesicle transport, but of cellular operations as a whole. Polarity is particularly conspicuous in tip-growing organisms: fungal hyphae, pollen tubes, also yeast and germinating algal zygotes, orient their activities towards a unique locus, the apex (Fig. 4). Products of biosynthesis, often translocated from afar, are assembled there into new plasma membrane and cell wall. Cellular organelles become oriented towards the apex and migrate into it, apparently along tracks supplied by the cytoskeleton. The accumulation of secretory vesicles is conspicuous in Fig. 4, as it is in other tip-growing cells. Metabolic activities, biophysical properties and organelle distribution vary in a graded fashion along the axis of polarity (recent reviews on apical growth include Wessels, 1986, 1993; Harold, 1990, 1994; Heath, 1990, 1994; Kropf 1992, 1994). The

form taken by tip-growing organisms is surely not a free-standing character, but emerges as another expression of the polarized organization of the entire economy. And when we understand how this large-scale global order is generated, maintained and modulated, the riddle of morphogenesis will be seen in an altogether friendlier light.

Embryologists have grappled for decades with large-scale patterning, which led them to formulate the concept of a 'field'. For present purposes, a field may be sufficiently defined as 'a territory within which developmental decisions are subject to a common set of co-ordinating influences' (Frankel, 1989, 1992). Developmental fields in this very general sense exist also in single cells, ciliates in particular. The evidence, marshalled by Frankel (1989, 1991, 1992), identifies two quite distinct levels of spatial order. Some features of the cortex of ciliates are specified by local interactions at the molecular level: ciliary units, for example, whose duplication may represent some manner of self-assembly. But other features are patterned

in relation to the cell as a whole, such as the placement of oral apparatus and contractile vacuole pores, or the cell's overall handedness. These appear to be established by reference to a global cellular grid that specifies the placement of organelles independent of their molecular architecture. The physical nature of this field remains quite unclear, but its existence is not in doubt.

Whether a global field of spatial organization also plays a role in the outgrowth of a yeast-cell's bud, or in the propagation of a hyphal tip, is simply not known. When applied to organisms in general, the concept of a field loses the precision that ciliatologists can demand. Nevertheless, like other biological metaphors, this one has the virtue of concentrating the mind on what stands in need of explication, which is the coordination of molecular processes on the cellular scale.

Embryonic fields leapt into prominence with the discovery of their underlying physical basis, in gradients of informational molecules (signalling proteins or transcription factors) that instruct cells regarding their position in the embryo and specify their developmental destiny (St Johnston & Nüsslein-Volhardt, 1992). In the same vein, it is tempting to imagine the propagation of a hypha, or the outgrowth of a bud, as being directed by a map or pre-pattern, represented by the distribution of one or more 'morphogens'. The general idea has been quite extensively discussed, with particular reference to transcellular electric fields, electric currents, protons and calcium ions. This line of enquiry was inspired by Lionel Jaffe nearly 30 years ago (Jaffe, 1968, 1981; Jaffe *et al.*, 1974), and continues to generate experiments and debate (Harold & Caldwell, 1990; Herth *et al.*, 1990; Kropf, 1992, 1994; Harrison, 1993; Jackson & Heath, 1993; Harold, 1994).

Technology in this field is advancing rapidly, making it increasingly feasible to measure extracellular electric currents and intracellular differences of potential, also to visualize the spatial distribution of protons and Ca^{2+} by means of fluorescent dyes. Data from pollen tubes, algal filaments and zygotes, and also from fungal hyphae, are converging on the conclusion that free cytosolic Ca^{2+} is commonly (always?) distributed in a graded fashion, highest at the extreme tip because influx is maximal there (Rathore *et al.*, 1991; Miller *et al.*, 1992; Kühtreiber & Jaffe, 1990; Berger & Brownlee, 1993; Garrill *et al.*, 1993). The findings do not yet add up to a coherent thesis; they certainly do not support a simple pattern of morphogen gradients, like those that block out the developing fly. But they strongly suggest that a growing tip is the site of localized calcium influx, and that this flux performs an important function in determining the locus of growth. To put it another way, an influx or perhaps a gradient of calcium ions may serve as a primary signal for the establishment of spatial global order. Whether calcium ions are uniquely identified with this role, or represent but one of several parallel signals, is one of the many uncertainties that must be resolved.

It's a long road from a localized flux, or even an ion gradient, to the kind of global vectorial organization of the cytoplasm that Fig. 4 displays, and there is not much

in the way of facts to guide (or restrain) speculation. The general presumption is that information carried by the ion flux gives direction to the extension of the cytoskeleton, which in turn positions enzymes and transport systems in the plasma membrane. The pivotal event seems to be the localized assembly of actin into meshwork or filaments at a locus from which outgrowth takes place. This formulation is grounded in the extensive evidence that links actin-rich structures to outgrowth in a variety of tip-growing organism: *Pelvetia* embryos and oomycete hyphae generate striking apical actin caps, and the association between actin-rich plaques and wall growth in yeast was mentioned above. That cytochalasins characteristically block apical growth and disrupt cell polarity is another straw in the wind. It bears repeating here that localized growth of walled cells reflects localized wall expansion; we do not know whether this is accomplished by targeting secretory vesicles, or by localizing fusion, or by some other mechanism. All we can really say is that actin-based structures play a critical role in whatever happens.

With solid information scarce, speculation has sought to bridge the gap. In an important early attempt, based on researches with *Pelvetia* embryos, Brawley & Robinson (1985) proposed that secretory vesicles carry Ca^{2+} channels. Upon fusion with the plasma membrane these admit Ca^{2+} , stimulating further exocytosis and recruiting actin monomers into local filaments that guide additional vesicles. A more elaborate model, devised by Goodwin and his colleagues in the context of *Acetabularia* (Brière & Goodwin, 1988; Goodwin & Brière, 1992), is rooted in the mechanochemical properties of the cell cortex. These depend in a complex manner on the calcium concentration and on the degree of crosslinking among actin filaments; loci of maximal cortical strain are assumed to activate proton pumps that induce wall expansion. It is noteworthy that the model can be simulated on a computer, and spontaneously generates forms that resemble those of regenerating stalks.

Such models do not pretend to be more than first attempts to carry a coordinating field from ion currents to cellular organization, and they will surely prove to be overly simple and abstract. But they illustrate principles that are bound to feature in the evolution of all such models: a cytoskeleton whose members are interconnected into a mechanical continuum (Ingber, 1993); initiation of outgrowth at the cell cortex; feedback loops, such that the initiating signal is simultaneously a cause of localized secretion and its consequence; a matrix of multiple parallel gradients, no single one of which is necessary and sufficient; and the hierarchical elaboration of order upon order. It is customary to envisage cellular order as fragile, dependent upon exquisite regulation at every point, but that is certainly a misapprehension. On the contrary, biological forms are robust (Goodwin *et al.*, 1993) in the sense that much redundancy is built into their making; the loss of one molecular component (one of several chitin synthases, for instance, or one of multiple actin-binding proteins) can often be tolerated with minimal disruption of the final product. Forms, it seem, arise naturally out of

the dynamic properties at work in the developing organism.

The intellectual ferment in this field owes little to research with yeast, whose practitioners have been more inclined to praise the genes. But yeast belongs to the same universe of discourse. A primary signal (carried by the mating pheromone, or by a marker for the bud-site) initiates the establishment of a polarized cellular organization; its ultimate purpose is localized wall expansion, apparently based on targeted vesicle transport or fusion (Lew & Reed, 1995). Not only do actin plaques cluster at sites of wall expansion, so does calmodulin (Brockhoff & Davis, 1992; Sun *et al.*, 1992). That does not necessarily imply a local calcium flux, for the function of calmodulin in this instance does not depend on its capacity to bind Ca^{2+} ! A normal system of microfilaments is required for the regeneration of protoplasts (Gabriel & Kopecká, 1995), and for the dimorphic transition in *Candida* (Akashi *et al.*, 1994; Yokoyama *et al.*, 1994). The perturbation of budding by cytochalasins is undoubtedly due to the aberrant deposition of new cell wall (Gabriel & Kopecká, 1995). And recent research with permeabilized cells documents directly that localized actin assembly is an early step in the initiation of a bud (Li *et al.*, 1995). The pressing task now is to clarify just how microfilaments are arranged, and what the functions of such patterns may be. Progress along this line should also shed light on the vexing question of how *Candida* switches between two modes of wall expansion, and two morphologies.

Readers who have not yet thrown up their hands in despair will doubtless have noticed that, even as we advance toward higher levels of complexity, hard data are increasingly left behind. Not without reason do experimentalists like to begin by grinding spatial heterogeneity into a pulp! It is not unreasonable, then, to ask how models about morphogenesis can possibly be verified, let alone falsified. I have been impressed by the power of computer simulation, especially when it is grounded in realistic physiological parameters. To be sure, the fact that a program generates a biological shape is no proof that it represents the way organisms grow (remember fractal trees?). But it may well incorporate critical postulates that can be verified experimentally. For example, the remarkable computer simulation of hyphal growth put forward by Bartnicki-Garcia (1990) rests upon the postulate of a vesicle-supply centre capable of autonomous mobility; the model will stand or fall, not on its mathematical merits but on the existence of such an organelle.

Cellular continuity

A ramble is the better for a destination, even a nominal one. We began this one from the universal law that the form of organisms is transmitted from one generation to the next, not precisely but within a narrow range of variation, with the object of learning how that comes about. Now, it is an article of the popular scientific credo that heritable information is encoded in the genome. Distressingly, what has been learned of morphogenesis

from both the genetic and physiological approaches challenges that simple faith. Organismic form is plainly not the direct product of distal genetic instructions but of proximal physiological processes. How, then, is one to account for the reproducibility of these forms? If not the genome, what orchestrates the hubbub of enzymes, filaments, membranes and polymers into a recurring pattern? We really do not know, and this is just what lends the study of microbial morphogenesis its fascination and significance. Unlike most of the projects that engage the attention of researchers, it presents not a puzzle but a mystery.

Let us be very clear about what genes do and do not contribute to the perpetuation of form. Genes encode the primary structure (and at some remove, the activity) of cellular building blocks, including the enzymes that put the blocks in place. Genes also play a large role in the regulation of biosynthesis; that is pertinent, for the overproduction of some cell constituents can be as disruptive of normal form as a shortfall. What the genes encode must also set the bounds of morphological plasticity: evidently, the yeast genome does not hold out the options of flagella or pseudopods. What never ceases to amaze me is how many scientists believe, or profess to believe, that genetic information is not only necessary but also *sufficient* to determine cellular morphology. The unstated premise is that a cell is at bottom a self-assembling structure, and I hope to have persuaded the reader that this notion carries reductionism to the point of absurdity. Genes are linked to living shapes by an intervening hierarchy of epigenetic procedures that execute the instructions and integrate them into a functional unity. Molecular variation supplies the raw material of evolution, but natural selection operates upon cells rather than on molecules. Consequently, when it comes to the propagation of form, the issues of conceptual interest centre on the workings and perpetuation of the higher levels of biological order.

We make contact here with another of biology's few universal laws, formulated in the mid-19th century in the context of the emerging cell theory. Cells, Rudolf Virchow proclaimed, never arise spontaneously but invariably descend from a pre-existing cell. The meaning of this fundamental restriction on what is possible in biology goes far beyond the requirement that genetic information be replicated, transcribed and translated. It asserts a requirement for the continuity of the structural organization displayed by every cell. Only a cell has the capacity to construct another cell. This, incidentally, is why the origin of life stands as the most profound mystery in biology; and we note in passing that, despite all the achievements of investigators in pre-biotic biochemistry, it remains utterly beyond our comprehension.

Just what does a cell pass on to its daughter, over and above the information contained in its genome? Years ago, Gunther Stent (1978) pointed out that every communication carries two levels of meaning: one explicit, embodied in the symbols that make up the message, and another implicit, which depends upon the context. Cell heredity is about the implicit meaning of the genetic

record. It does not take the form of a linear string of symbols, and may be impossible to quantify; its essence appears to be the spatial setting within which genetic information is expressed and deployed. For a simple example, consider biological membranes. The lipid bilayer is a self-assembling structure, and even the insertion of integral membrane proteins is very nearly a spontaneous process. But the function, the biological meaning of the plasma membrane, demands that proteins span the membrane in a uniform direction ('right-side out'). This polarity derives from the cell as a whole, which cunningly supplies precursors to the cytoplasmic surface only.

The principle is a general one, but takes a variety of particular forms. As a cell grows and makes itself, the macromolecules specified by the genes are released into a context that is already spatially structured. The functions of new gene products reflect both their intrinsic molecular properties and the places into which they come; chitin synthase can make cell wall only if it is inserted, with the proper orientation, into an active patch of plasma membrane. Natural selection, acting at the organismic level, will preserve only those molecular variants that enhance, or at least do not disrupt, global order. Sometimes, structural guidance is plainly visible in the form of spatial markers: persistent markers play a role in the selection of the budding site by yeast (see above), and in many other well-studied organisms (Frankel, 1989; Harold, 1990). But the structural context of the whole cell is, I maintain, required even when no landmarks carry over from one generation to the next. In the extreme case, the fertilized *Pelvetia* egg has no pre-formed axis. But its cytoplasm, compartmentalized and structured in the standard eukaryotic manner, is so laid out that it can perceive directional cues from the environment and thus establish an axis of polarity. Once development is underway, linkages between cytoskeleton and cell wall serve as fixed points to guide the later stages of morphogenesis (Kropf, 1992, 1994).

If the continuity of spatial organization looms so large in the propagation of form, one would expect to encounter instances in which a structural modification passes down the generations in the absence of genetic alteration. In fact, structural inheritance has been familiar to ciliatologists for more than 30 years. The subject was pioneered by the late Tracy Sonneborn, who discovered that experimental inversions of the ciliary units were transmitted for hundreds of generations in strains isogenic with their normal fellows. Indeed, doublet cells (created by back-to-back fusion of the products of abortive cell division) propagate indefinitely as doublets, even though no gene has mutated. A more recent case-in-point is the propagation of left-handed and right-handed organization (for a description of these and many other astonishing examples see Frankel, 1989, 1991). Whatever the underlying mechanisms, known only in part, these discoveries document that the transmission of structure and form is partly independent of the transmission of genetic information. These are separate channels, different in kind. It must be added that the evidence for non-genic structural inheritance comes entirely from ciliates, probably because

their elaborate architecture favours detection of the phenomenon. Demonstration that extra-genic structural inheritance is indeed universal would add immeasurably to our comprehension of morphogenesis.

So powerful has been the impress of the central dogma on the scientific imagination that biologists are disposed to see the hand of a genetic program throughout the discipline. Yet the natural world shows numerous phenomena that are reproducible without being programmatic: the ecological succession following a forest fire, for example, or the daily ebb and flow of city traffic. Stent (1981) designates these as *historical* in nature. Each stage of the pageant is at once the effect of earlier events and the cause of subsequent ones; and the sequence recurs time after time because like causes elicit like effects. The historical character of morphogenesis is plainly displayed in Waddington's landscape (Fig. 1): the rolling ball's course is not prescribed by a program, but responds to the local situation from moment to moment.

The proposition, that the continuous propagation of cellular organization represents a mode of heredity distinct from (and ancillary to) that encoded in the genes, will strike some readers as an outrageous assault on established truth, and tinged with vitalism to boot. To others it may seem no more than a trivial and self-evident gloss upon current orthodoxy. Is structural order anything more than an intermediate stage in the expression of information carried in the genes? Common sense, I believe, lies somewhere between these two extremes. To highlight cellular continuity implies no conflict with that bedrock of biological understanding, the central dogma. It is simply an acknowledgement of practical necessity. In the four decades elapsed since the nature and functions of the genetic material were established, our knowledge of the architecture and dynamics of cells has also increased astronomically. Does anyone really believe that the replication, transcription and translation of DNA supply an adequate capsule summary of the intricacies of cell reproduction? Nor is the idea particularly novel. 'Cytoplasmic heredity' has a lengthy and somewhat unhappy history (Sapp, 1987); and some of the ways in which biological order perpetuates itself have been considered by others, notably Riedl (1978), Webster & Goodwin (1981) and Kauffman (1993). But the fact remains that cellular continuity is not part of most scientists' mental universe, and the more's the pity. For we urgently need a conception of the organism to complement our preoccupation with genes, and the persistence of structural order must take its place in any such philosophy.

It would be misleading to imply that organizational continuity has been forgotten altogether. Textbooks of cell biology do, as a rule, mention Sonneborn's classic experiments on the inheritance of acquired characteristics in ciliates; the perpetuation of centrosomes and centrioles; and the common experience that biological membranes seldom (if ever) arise *de novo*, but grow by the extension of existing membranes. But the fragmented treatment of such matters leaves the impression that they are peripheral rather than central, and here is where I beg to differ. The simple fact that we cannot presently specify how bio-

logical order (including form) is propagated implies, at the very least, a great lacuna in our comprehension of biology. The question, whether organizational continuity is fundamentally different from genetic continuity or is but one facet of the latter, may be of greater interest to philosophers than to scientists. But as Woodger saw in 1929, organization is central to the very nature of living things. It has many levels, of which the order of the nucleotides is but one. And unless we explore them all, we shall fail in our duty to make clear what biological organization means.

Acknowledgements

This essay is based on a series of public lectures, delivered at the University of Aberdeen as part of the celebration of its quincentenary. Let me then extend my best wishes to that admirable institution, and fond thanks to the members of the Department of Molecular and Cell Biology for making both Harolds welcome in the granite city on this and many previous occasions. In the words of the toast of Bon Accord: happy to meet, sorry to part, happy to meet again. Preparation of this paper has been supported in part by grant MCB-9017130, from the National Science Foundation.

References

- Adams, A. M. & Pringle, J. R. (1984). Relationship of actin and tubulin distribution to bud growth in wild-type and morphogenetic-mutant *Saccharomyces cerevisiae*. *J Cell Biol* **98**, 934–945.
- Adams, A. E. M., Johnson, D. I., Longnecker, R. M., Sloat, B. F. & Pringle, J. R. (1990). CDC42 and CDC43, two additional genes involved in budding and the establishment of cell polarity in the yeast *Saccharomyces cerevisiae*. *J Cell Biol* **111**, 131–142.
- Akashi, T., Kanbe, T. & Tanaka, K. (1994). The role of the cytoskeleton in the polarized growth of the germ tube in *Candida albicans*. *Microbiology* **140**, 271–280.
- Amatruda, J. F., Vannon, J. F., Tachell, K., Hug, C. H. & Cooper, J. A. (1990). Disruption of the actin cytoskeleton in yeast capping protein mutants. *Nature* **344**, 352–354.
- Anderson, J. M. & Soll, D. R. (1986). Differences in actin localization during bud and hypha formation in the yeast *Candida albicans*. *J Gen Microbiol* **132**, 2035–2047.
- Andrews, B. J. & Herskowitz, I. (1990). Regulation of cell-cycle dependent gene expression in yeast. *J Biol Chem* **265**, 14057–14060.
- Baba, M., Baba, N., Ohsumi, Y., Kanaya, K. & Osumi, M. (1989). Three-dimensional analysis of morphogenesis induced by mating pheromone α -factor in *Saccharomyces cerevisiae*. *J Cell Sci* **94**, 207–216.
- Bartnicki-Garcia, S. (1990). Role of vesicles in apical growth and a new mathematical model of hyphal morphogenesis. In *Tip Growth in Plant and Fungal Cells*, pp. 211–232. Edited by I. B. Heath. San Diego, CA: Academic Press.
- Bartnicki-Garcia, S., Bracker, C. E. & Ruiz-Herrera, J. (1984). Chitosomes from the wall-less 'slime' mutant of *Neurospora crassa*. *Arch Microbiol* **139**, 105–112.
- Berger, F. & Brownlee, C. (1993). Ratio confocal imaging of free cytoplasmic calcium gradients in polarising and polarised *Fucus* zygotes. *Zygote* **1**, 9–15.
- Blacketer, M. J., Madaule, P. & Myers, A. M. (1994). The *Saccharomyces cerevisiae* mutation *elm 4-1* facilitates pseudohyphal differentiation and interacts with a deficiency in phosphoribosyl pyrophosphate synthase activity to cause constitutive pseudohyphal growth. *Mol Cell Biol* **14**, 4671–4681.
- Brawley, S. H. & Robinson, K. R. (1985). Cytochalasin treatment disrupts the endogenous currents associated with cell polarisation in fucoid zygotes. *J Cell Biol* **100**, 1173–1184.
- Brière, C. & Goodwin, B. C. (1988). Geometry and dynamics of tip morphogenesis in *Acetabularia*. *J Theor Biol* **131**, 461–475.
- Brockerhoff, S. E. & Davis, T. N. (1992). Calmodulin concentrates at regions of cell growth in *Saccharomyces cerevisiae*. *J Cell Biol* **118**, 619–629.
- Chant, J. & Pringle, J. R. (1995). Patterns of bud-site selection in the yeast *Saccharomyces cerevisiae*. *J Cell Biol* **129**, 751–765.
- Chant, J. & Stowers, L. (1995). GTPase cascades choreographing cellular behavior: movement, morphogenesis and more. *Cell* **81**, 1–4.
- Chenevert, J., Corrado, K., Bender, A., Pringle, J. & Herskowitz, I. (1992). A yeast gene (BEM1) necessary for cell polarization whose product contains two SH3 domains. *Nature* **356**, 77–79.
- Dickinson, J. R. & Williams, A. S. (1987). The *cdc30* mutation in *Saccharomyces cerevisiae* results in a temperature-sensitive isoenzyme of phosphoglucose isomerase. *J Gen Microbiol* **133**, 135–140.
- Drubin, D. G. (1991). Development of cell polarity in budding yeast. *Cell* **65**, 1093–1096.
- Elorza, M. V., Sentandreu, R. & Ruiz-Herrera, J. (1994). Isolation and characterization of yeast monomorphic mutants of *Candida albicans*. *J Bacteriol* **176**, 2318–2325.
- Flescher, E. G., Madden, K. & Snyder, M. (1993). Components required for cytokinesis are important for bud site selection in yeast. *J Cell Biol* **122**, 373–386.
- Frankel, J. (1989). *Pattern Formation: Ciliate Studies and Models*. New York: Oxford University Press.
- Frankel, J. (1991). The patterning of ciliates. *J Protozool* **38**, 519–525.
- Frankel, J. (1992). Positional information in cells and organisms. *Trends Cell Biol* **2**, 256–260.
- Gabriel, M. & Kopecká, M. (1995). Disruption of the actin cytoskeleton in budding yeast results in the formation of an aberrant cell wall. *Microbiology* **141**, 891–899.
- Garrill, A., Jackson, S. A., Lew, R. R. & Heath, I. B. (1993). Ion channel activity and tip growth: tip-localized stretch-activated channels generate an essential Ca^{2+} gradient in the oomycete *Saprolegnia ferax*. *Eur J Cell Biol* **60**, 358–365.
- Gimeno, C. J., Ljungdahl, P. O., Styles, C. A. & Fink, G. R. (1992). Unipolar cell divisions in the yeast *Saccharomyces cerevisiae* lead to filamentous growth: regulation by starvation and RAS. *Cell* **68**, 1077–1090.
- Goodwin, B. C. (1986). What are the causes of morphogenesis? *Bioessays* **3**, 32–36.
- Goodwin, B. C. & Brière, C. (1992). A mathematical model of cytoskeletal dynamics and morphogenesis in *Acetabularia*. In *The Cytoskeleton of the Algae*, pp. 219–237. Edited by D. Menzel. Boca Raton, FL: CRC Press.
- Goodwin, B. C., Kauffman, S. & Murray, J. D. (1993). Is morphogenesis an intrinsically robust process? *J Theor Biol* **163**, 135–144.
- Govindvan, B., Bowser, R. & Novick, P. (1995). The role of MYO2, a yeast class V myosin, in vesicular transport. *J Cell Biol* **128**, 1055–1068.
- Gow, N. A. R. (1994). Growth and guidance of the fungal hypha. *Microbiology* **140**, 3193–3205.
- Gozalbo, D., Dubon, R., Schwenke, J. & Sentandreu, R. (1987). Characterisation of chitosomes in *Candida albicans* protoplasts. *Exp Mycol* **11**, 331–338.

- Haarer, B. K., Lillie, S. H., Adams, A. E. M., Magdolen, V., Brandlow, W. & Brown, S. S. (1990). Purification of profilin from *Saccharomyces cerevisiae* and analysis of profilin-deficient cells. *J Cell Biol* **110**, 105–114.
- Harold, F. M. (1990). To shape a cell: an inquiry into the causes of morphogenesis of microorganisms. *Microbiol Rev* **54**, 381–431.
- Harold, F. M. (1994). Ionic and electrical dimensions of hyphal growth. In *The Mycota*, vol. 1, pp. 89–109. Edited by J. G. H. Wessels & F. Meinhardt. Berlin & Heidelberg: Springer-Verlag.
- Harold, F. M. & Caldwell, J. H. (1990). Tips and currents: electrophysiology of apical growth. In *Tip Growth in Plant and Fungal Cells*, pp. 59–90. Edited by I. B. Heath. San Diego, CA: Academic Press.
- Harrison, L. G. (1993). *Kinetic Theory of Living Pattern*. London: Cambridge University Press.
- Hartwell, L. H. & Weinert, T. A. (1989). Checkpoints: controls that ensure the order of cell cycle events. *Science* **246**, 629–634.
- Heath, I. B. (editor) (1990). *Tip Growth in Plant and Fungal Cells*. San Diego, CA: Academic Press.
- Heath, I. B. (1994). The cytoskeleton in hyphal growth, organelle movements and mitosis. In *The Mycota*, vol. 1, pp. 43–65. Edited by J. G. H. Wessels & F. Meinhardt. Berlin & Heidelberg: Springer-Verlag.
- Herth, W., Reiss, H.-D. & Hartmann, E. (1990). Role of calcium ions in tip growth of pollen tubes and moss protonema cells. In *Tip Growth in Plant and Fungal Cells*, pp. 91–118. Edited by I. B. Heath. San Diego, CA: Academic Press.
- Huffaker, T. C., Thomas, J. H. & Botstein, D. (1988). Adverse effects of β -tubulin mutations on microtubule formation and function. *J Cell Biol* **106**, 1997–2010.
- Ingber, D. E. (1993). Cellular tensegrity: defining new rules of biological design that govern the cytoskeleton. *J Cell Sci* **104**, 613–627.
- Jackson, S. L. & Heath, I. B. (1993). Roles of calcium ions in hyphal tip growth. *Microbiol Rev* **57**, 367–382.
- Jacob, F. (1973). *The Logic of Life: a History of Heredity*. New York: Pantheon Books.
- Jacobs, C. W., Adams, A. E. M., Szanislo, P. J. & Pringle, J. R. (1988). Functions of microtubules in the cell cycle. *J Cell Biol* **107**, 1409–1426.
- Jaffe, L. F. (1968). Localization in the developing *Fucus* egg and the general role of localizing currents. *Adv Morphog* **7**, 295–328.
- Jaffe, L. F. (1981). The role of ionic currents in establishing developmental patterns. *Philos Trans R Soc Lond Ser B* **295**, 553–566.
- Jaffe, L. F., Robinson, K. R. & Nuccitelli, R. (1974). Local cation entry and self-electrophoresis as an intracellular localization mechanism. *Ann NY Acad Sci* **238**, 372–389.
- Johnston, L. H. (1992). Cell cycle control of gene expression in yeast. *Trends Cell Biol* **2**, 353–357.
- Johnston, G. C., Prendergast, J. A. & Singer, B. A. (1991). The *Saccharomyces cerevisiae* MYO2 gene encodes an essential myosin for vectorial transport of vesicles. *J Cell Biol* **113**, 539–551.
- Kauffman, S. A. (1993). *Origins of Order: Self-Organization and Selection in Evolution*. Oxford: Oxford University Press.
- Kellenberger, E. (1990). Form determination of the heads of bacteriophages. *Eur J Biochem* **190**, 233–248.
- Kim, H. B., Haarer, B. K. & Pringle, J. R. (1991). Cellular morphogenesis in the yeast *Saccharomyces cerevisiae* cell cycle: localization of the CDC3 gene product and the timing of events at the budding site. *J Cell Biol* **112**, 535–544.
- Klis, F. M. (1994). Cell wall assembly in yeast. *Yeast* **10**, 851–869.
- Koch, A. L. (1983). The surface stress theory of microbial morphogenesis. *Adv Microb Physiol* **24**, 301–366.
- Koch, A. L. (1994). The problem of hyphal growth in streptomycetes and fungi. *J Theor Biol* **171**, 137–150.
- Koch, A. L., Higgins, M. L. & Doyle, R. J. (1982). The role of surface stress in the morphology of microbes. *J Gen Microbiol* **128**, 927–945.
- Kopecká, M. & Gabriel, M. (1995). Actin cortical cytoskeleton and cell wall synthesis in regenerating protoplasts of the *Saccharomyces cerevisiae* actin mutant DBY 1693. *Microbiology* **141**, 1289–1299.
- Kropf, D. L. (1992). Establishment and expression of cellular polarity in fucoid zygotes. *Microbiol Rev* **56**, 316–339.
- Kropf, D. L. (1994). Cytoskeletal control of cell polarity in a plant zygote. *Dev Biol* **165**, 361–371.
- Kropf, D. L., Hopkins, R. & Quatrano, R. S. (1989). Protein synthesis and morphogenesis are not tightly linked during embryogenesis in *Fucus*. *Dev Biol* **134**, 452–461.
- Kühtreiber, W. M. & Jaffe, L. F. (1990). Detection of extracellular calcium gradients with a calcium-specific vibrating electrode. *J Cell Biol* **110**, 1565–1573.
- Levin, D. E. & Bartlett-Heubusch, E. (1992). Mutants in the *S. cerevisiae* PKC-1 gene display a cell-cycle specific osmotic stability defect. *J Cell Biol* **116**, 1221–1229.
- Lew, D. J. & Reed, S. I. (1995). A cell cycle checkpoint monitors cell morphogenesis in budding yeast. *J Cell Biol* **129**, 739–749.
- Li, R., Zheng, Y. & Drubin, D. G. (1995). Regulation of cortical actin cytoskeleton assembly during polarized cell growth in budding yeast. *J Cell Biol* **128**, 599–615.
- Liu, H. & Bretscher, A. (1992). Characterization of TPM1 disrupted yeast cells indicates an involvement of tropomyosin in directed vesicular transport. *J Cell Biol* **188**, 285–299.
- Liu, H., Köhler, J. & Fink, A. (1994). Suppression of hyphal formation in *Candida albicans* by mutation of a STE12 homolog. *Science* **266**, 1723–1726.
- Madden, K., Costigan, C. & Snyder, M. (1992). Cell polarity and morphogenesis in *Saccharomyces cerevisiae*. *Trends Cell Biol* **2**, 22–29.
- Maeda, T., Worgler-Murphy, S. & Saito, H. (1994). A two-component system that regulates an osmosensing MAD kinase cascade in yeast. *Nature* **369**, 242–245.
- Merson-Davies, L. A. & Odds, F. C. (1992). Expansion of the *Candida albicans* cell envelope in different morphological forms of the fungus. *J Gen Microbiol* **138**, 461–466.
- Miller, D. D., Callahan, D. A., Gross, D. J. & Hepler, P. K. (1992). Free Ca^{2+} gradient in growing pollen tubes of *Lilium*. *J Cell Sci* **101**, 7–12.
- Money, N. P. & Harold, F. M. (1992). Extension growth of the water mold *Achlya*: interplay of turgor and wall strength. *Proc Natl Acad Sci USA* **89**, 4245–4249.
- Money, N. P. & Harold, F. M. (1993). Two water moulds can grow without measurable turgor pressure. *Planta* **190**, 426–430.
- Mulholland, J., Preuss, D., Moon, A., Wong, A., Drubin, D. & Botstein, D. (1994). Ultrastructure of the yeast actin cytoskeleton and its association with the plasma membrane. *J Cell Biol* **125**, 381–391.
- Nelson, J. W. (1992). Regulation of cell surface polarity from bacteria to mammals. *Science* **258**, 948–955.
- Novick, P. & Botstein, D. (1985). Phenotypic analysis of temperature-sensitive yeast actin mutants. *Cell* **40**, 405–416.
- Odds, F. C. (1985). Morphogenesis in *Candida albicans*. *CRC Crit Rev Microbiol* **12**, 45–93.

- Pringle, J. R. & Hartwell, L. H. (1981).** The *Saccharomyces cerevisiae* cell cycle. In *The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance*, pp. 97–142. Edited by J. N. Strathern, E. W. Jones & J. R. Broach. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Rathore, K. S., Cork, R. J. & Robinson, K. R. (1991).** A cytoplasmic gradient of Ca^{2+} is correlated with the growth of lily pollen tubes. *Dev Biol* **148**, 612–619.
- Riedl, R. (1978).** *Order in Living Organisms*. New York: Wiley.
- St Johnston, D. & Nüsslein-Volhardt, C. (1992).** The origin of pattern and polarity in the *Drosophila* embryo. *Cell* **68**, 201–219.
- Sapp, J. (1987).** *Beyond the Gene: Cytoplasmic Inheritance and the Struggle for Authority in Genetics*. New York & Oxford: Oxford University Press.
- Saunders, P. T. & Trinci, A. P. J. (1979).** Determination of tip shape in fungal hyphae. *J Gen Microbiol* **110**, 469–473.
- Segall, J. A. (1993).** Polarization of yeast cells in spatial gradients of a mating factor. *Proc Natl Acad Sci USA* **90**, 8332–8336.
- Shaw, J. A., Mol, P. C., Bowers, B., Silverman, S. J., Valdivieso, M. H., Duran, A. & Carib, E. (1991).** The functions of chitin synthases 2 and 3 in the *Saccharomyces cerevisiae* cell cycle. *J Cell Biol* **114**, 111–123.
- Sloat, B. F., Adams, A. & Pringle, J. R. (1981).** Roles of the CDC24 gene product in cellular morphogenesis during the *Saccharomyces cerevisiae* cell cycle. *J Cell Biol* **89**, 395–405.
- Soll, D. R. & Sonneborn, D. (1971).** Zoospore germination in *Blastocladiella emersonii*: cell differentiation without protein synthesis. *Proc Natl Acad Sci USA* **68**, 459–463.
- Soll, D. R., Herman, M. A. & Staebell, M. A. (1985).** The involvement of cell wall expansion in the two modes of mycelium formation of *Candida albicans*. *J Gen Microbiol* **131**, 2367–2375.
- Staebell, M. & Soll, D. R. (1985).** Temporal and spatial differences in cell wall expansion during bud and mycelium formation in *Candida albicans*. *J Gen Microbiol* **131**, 1467–1480.
- Stent, G. S. (1978).** *Paradoxes of Progress*. New York: W. H. Freeman.
- Stent, G. S. (1981).** Strength and weakness of the genetic approach to the development of the nervous system. *Annu Rev Neurosci* **4**, 163–194.
- Stent, G. S. (1985).** Thinking in one dimension: the impact of molecular biology on development. *Cell* **40**, 1–2.
- Sun, G. H., Ohya, Y. & Anraku, Y. (1992).** Yeast calmodulin localizes to sites of cell growth. *Protoplasma* **166**, 110–112.
- Thompson, D'A. W. (1961).** *On Growth and Form*, abridged edition. Cambridge: Cambridge University Press.
- Waddington, C. H. (1957).** *The Strategy of the Genes*. London: George, Allen & Unwin.
- Webster, G. & Goodwin, B. C. (1981).** History and structure in biology. *Perspect Biol Med* **25**, 39–62.
- Wessels, J. G. H. (1986).** Cell wall synthesis in apical hyphal growth. *Int Rev Cytol* **104**, 37–79.
- Wessels, J. G. H. (1993).** Wall growth, protein excretion and morphogenesis in fungi. *New Phytol* **123**, 397–413.
- Woodger, J. H. (1929).** *Biological Principles – A Critical Study*. London: Routledge & Kegan Paul (reprinted 1967).
- Yokoyama, K., Kaji, H., Nishimura, K. & Miyaji, M. (1994).** The role of microfilaments and microtubules during pH-regulated morphological transition in *Candida albicans*. *Microbiology* **140**, 281–287.