PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

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Phil. Trans. R. Soc. Lond. B 2000 **355**, 873-877
doi: 10.1098/rstb.2000.0622

THE ROYAL SOCIETY

doi 10.1098/rstb.2000.0622

New concepts in fission yeast morphogenesis

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The ability to generate spatial form is a fundamental characteristic of all living organisms, which has been much studied by successive generations of developmental biologists. In recent years increasing numbers of cell biologists have turned their attention to the mechanisms by which cells generate their spatial form. These include the mechanisms that position components in different places within the cell, that specify the position of these components, and that generate the overall shape of these components. These problems are entirely analogous to those studied by developmental biologists, although usually at the level of the whole organism, organ or tissue. Because the organization of all cells is basically similar, it is possible that the concepts and the underlying molecular mechanisms of cell morphogenesis may be highly conserved. In this article we consider the generation of spatial form within the fission yeast cell, focusing on emerging new concepts, which may be applicable to the morphogenesis of other cells.

Keywords: fission yeast; cell morphogenesis; cell polarity; microtubules

1. FISSION YEAST CELL MORPHOGENESIS

The fission yeast is a simple single-celled eukaryote. It is cylindrical in form, 3–4 μm in diameter, and 8–15 μm in length, depending on how far the cell has proceeded through the cell cycle. Cell diameter remains approximately constant during the cell cycle and so most growth occurs as a consequence of cell elongation, with newly born cells being the shortest and cells just about to divide being the longest. The two cell ends or tips grow apart in a precisely opposed manner such that cell elongation of the cylinder occurs in a straight line. A newly born cell begins growth at only one of its ends, the end that already existed in the mother cell before its division. Because this is the end that pre-existed in the mother cell it is termed the old end. Some way into the cell cycle the cell shifts from being monopolar to bipolar in growth mode by activating growth at the new end. This transition, termed new-end take-off (NETO), occurs around the time of S-phase when the cell attains a certain minimal cell length. During mitosis and cytokinesis cell elongation ceases, and a septum is formed in the middle of the cell. Splitting of the septum forms two new cells each with an old end derived from the mother cell and a new end formed from the septum. It is these basic growth characteristics that account for the overall form of the fission yeast cell (Mata & Nurse 1998).

Coincident with these morphogenetic changes during the cell cycle are changes in the organization of both the actin and microtubular cytoskeletons (figure 1). Actin dots are found concentrated in the region of growing cell ends. During unipolar growth, these dots are found mostly at the sole growing end, and during bipolar growth they are found at both cell ends (Marks & Hyams 1985). During both unipolar and bipolar growth, a cytoplasmic microtubular cytoskeleton extends throughout the length of the cell forming a cage around the nucleus. The microtubules appear to be initiated from the region of the nuclear surface with their tips often terminating at the cellular ends. At mitosis and cytokinesis, both the actin and microtubular cytoskeletons undergo major changes. Most of the actin relocates from the cell ends to the middle of the cell where the septum is to be formed. At the completion of septation, the actin relocates from the septum to the old ends and cell elongation recommences. The cytoplasmic mictrotubular cage disappears at mitosis and an intranuclear spindle is formed. The spindle elongates, the nucleus divides, and the two daughter nuclei move apart. At this stage cytoplasmic microtubules emanating from the spindle pole body appear and these could contribute to nuclear separation. As mitosis is completed, the intranuclear spindle disappears and new cytoplasmic microtubules form (Hagan 1998).

2. LONG-RANGE SPATIAL ORGANIZATION IN THE CELL

The ability of the fission yeast cell to grow in a straight line with the two growing ends precisely opposed to each other must reflect some sort of long-range spatial organization extending throughout the cell. To study this problem, mutations or drug treatments producing cells that are no longer able to maintain growth in a straight line, but which can still form a properly organized growth zone, should be revealing. Such cells would be expected to position the growth site in the wrong place in the cell and as a consequence to grow bent or even branched.

Cold-sensitive tubulin mutants (Toda *et al*. 1983) and the addition of the microtubule inhibitor thiabendazole (TBZ) (Walker 1982) produce bent and branched cells at a low frequency, implicating microtubules in long-range spatial organization. A more systematic study was made possible when it was discovered that the release of a

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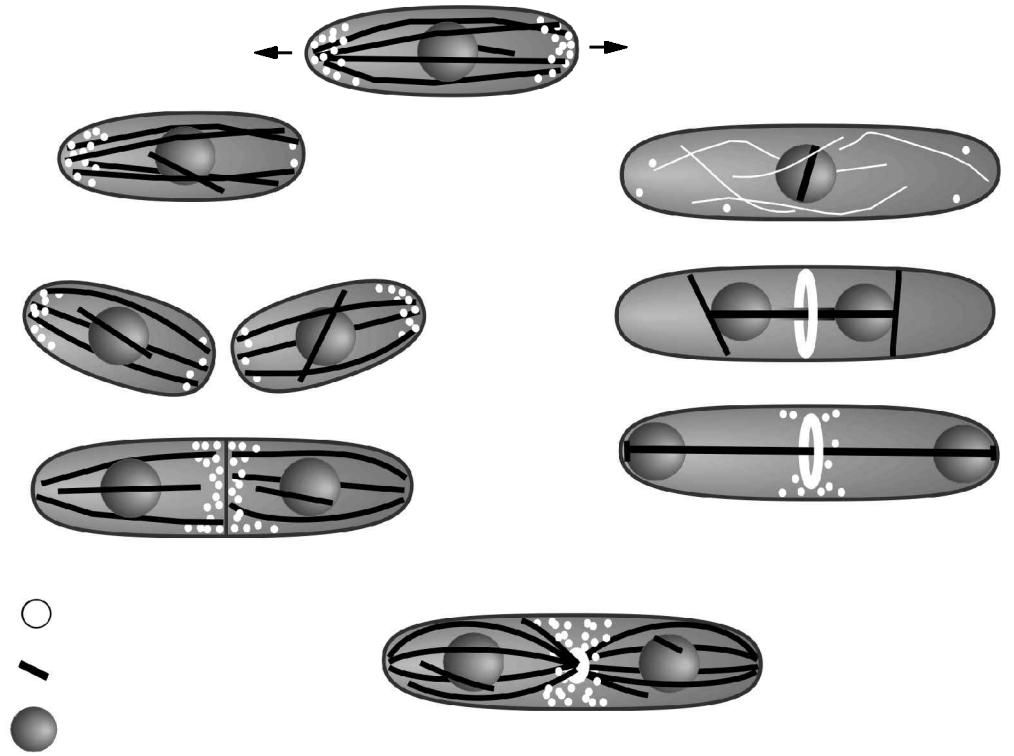

Figure 1. A schematic drawing of stages of the cell cycle shown in a clockwise orientation. Multiple changes in the organization of both the actin and microtubular cytoskeletons occur during the cell cycle. During interphase, actin dots accumulate at the growing ends (arrows). In cells before NETO this is at the old end, which existed in the previous cell cycle, and in post-NETO cells it is at both the old and the new ends. At the onset of mitosis, actin relocates to the cell centre where it forms a contractile ring before septum formation. During ring contraction actin dots accumulate on both sides of the growing septum where the new cell walls are synthesized. After the completion of cytokinesis, actin dots in both daughter cells relocate to the old ends that existed before division. Interphase microtubules originate from multiple locations in the nuclear periphery and grow into both ends of the cell. These microtubules disappear at the beginning of mitosis whilst an intranuclear spindle forms, originating from the two spindle pole bodies. While the spindle elongates, new cytoplasmic microtubules originate from the spindle pole bodies. When the nuclei have reached the cell tips, the spindle breaks down and cytoplasmic microtubules nucleate from the cell centre and from the two daughter nuclei.

cdc10ts cell-cycle mutant from its G1 block in the presence of TBZ generated high levels of branched cells (Sawin & Nurse 1998). These high levels of branching are the consequence of cells undergoing NETO in the absence of microtubules. Instead of activating growth at the new end opposed to the old end, it is activated off-axis or in the middle of the cell to generate bent or branched cells, respectively. In the latter case actin dots now accumulate as a patch in the middle region of the cell where the new growing tip will be formed. Therefore, an intact cytoplasmic microtubular cytoskeleton is required to properly place a new growing end exactly opposed to the old end.

Further insight into the role of microtubules in long-range spatial order has come from analysis of a *tea1* mutant, which generates bent and branched cells (figure 2) (Mata & Nurse 1997). Tealp is found at both cell tips whether the cells have undergone NETO or not and so appears to be a marker of cell geometry rather than of cell growth (figure 2). This makes tealp a potential marker for cellular ends. Within minutes of disrupting the microtubular cytoskeleton with TBZ, tealp disappears from the cell tips and is instead found distributed throughout the cytoplasm. When TBZ is removed, tealp is found at the distal ends of the microtubules, which are growing back towards the cell tips. Tealp is observed to accumulate once again at the cell ends as soon as microtubules have reached them. In the absence of tealp, newly forming growth zones are located incorrectly even in the presence of microtubules.

These results suggest a model whereby the microtubule-dependent transport of the cell end marker tealp underlies the long-range spatial organization that precisely opposes cell ends. Tealp is involved in properly placing locally organized growth zones at the opposite or antipodal ends of the cell. Importantly, growth zones can still be formed in the absence of tealp, but they are incorrectly placed with respect to the overall spatial geometry of the cell. Microtubules extending through the length of the cell identify the antipodes at the extremes of the cellular long axis. Tealp is transported along the microtubules and accumulates in the cell tips where most microtubules terminate. The major difficulty with this model is understanding how microtubules accurately identify the antipodes at the extremes of the cellular axis. One possibility is that a mechanism exists which leads to a transient stabilization of those microtubules that happen to reach the cell ends. This would consequently lead to a majority of microtubules extending the length of the cell between the antipodes. An alternative possibility is that microtubules have a tendency to extend along the long axis as a consequence of them becoming deflected when they grow in middle regions of the cell cortex.

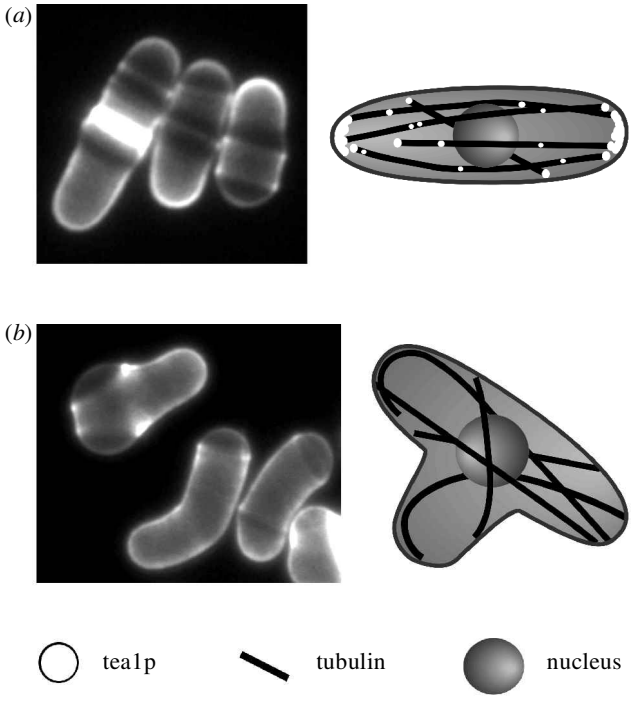

Figure 2. (*a*, *b*) Photographs of cells stained with the fluorescent dye calcofluor and the schematic drawings illustrate the distribution of tea1p and microtubules in (*a*) wild-type and (*b*) *Δtea1* cells. (*a*) Cytoplasmic microtubules grow into the longitudinal axis of the cylindrical cells and terminate in the cell tips. Tea1p accumulates at the cell ends but is also found on the distal tips of microtubules and in dots along the microtubules. (*b*) Cells deleted for *tea1* are unable to correctly position their growth site and therefore are bent or branched. In the absence of tea1p, the microtubules do not always terminate growth when they reach the cell tips and instead can curl around the end.

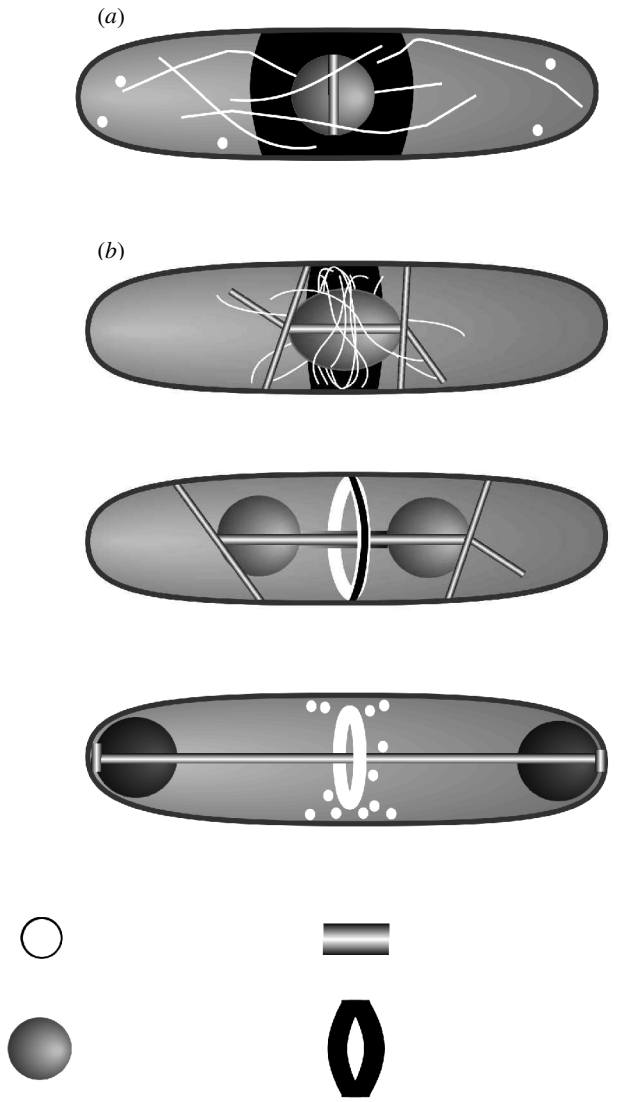

Figure 3. Schematic drawing showing cells at different stages of mitosis. (*a*, *b*) At the onset of mitosis, mid1p exits from the nucleus and accumulates in a band at the cell cortex. This outlines an area within which actin filaments organize into a central ring. (*c*, *d*) During septum formation, the actin ring contracts and mid1p disappears from the ring whilst accumulating again in the nucleus.

Because most microtubules are initiated from the region of the nucleus in the cell centre, they will be deflected towards the cell ends if their growth occurs at an oblique angle. The subsequent arrest of microtubule growth in the cell tips could be simply due to further growth becoming impossible when the microtubule tip is fully surrounded by cortex or, if the microtubule is too stiff to bend away when it approaches the cortex almost at right angles, as will be the case at the cell tips. There is, however, some evidence that tea1p itself might influence the behaviour of the microtubules in the cell tips. When *tea1* is deleted, at a certain frequency, microtubules do not stop at the cell ends but continue to grow to form U-shaped structures extending back into the body of the cell. This observation suggests that if tea1p is not present in the cell ends, microtubules can continue to grow beyond these regions. The molecular mechanisms underlying this are not clear but their significance is that the location of tea1p influencing the direction of its own transport provides a positive feedback system. In other words, if the presence of tea1p attracts microtubule ends, which in turn deliver more tea1p, then more microtubule ends will become attracted.

Microtubular instability might have additional implications for long-range spatial order in the cell. Because microtubules are highly dynamic, turning over every 1–2 min, the mechanism that results in microtubules extending along the length of the cell could be quite crude if some sort of integrating mechanism was operative. What is meant by this is that each individual microtubule only imprecisely locates the cell antipodes, but the attempt to do so by 100 microtubules will produce over time a distribution of microtubular tip locations spread across the end of the cell. The mean of this distribution will define the precise antipodes of the cell. Delivery of tea1p along microtubules could obviously provide such an integration mechanism because tea1p will be deposited at the region of the cell cortex adjacent to the microtubular tips, providing a historical record of microtubular tip locations.

What concepts of cell morphogenesis have emerged from this work? One concept to emerge is the potential for microtubules to establish long-range spatial order within cells. This provides an alternative to the mechanism that was found to operate in budding yeast,

where microtubules are not involved in the positioning of the growth zone (Chant 1996). Linear polarized polymers like microtubules can extend over long distances and can transport molecules in a controlled way allowing communication between different regions of the cell. Their relative straightness is particularly appropriate to define a linear axis, a characteristic that is obvious in a cylindrical fission yeast cell but which may be a general characteristic of many polarized cells. The dynamic behaviour of microtubules combined with the transport of a morphogenetic marker like tea1p allows an imprecise positioning mechanism to be constantly repeated and then averaged resulting in a more precise process. This may well be an important general concept of spatial organization whereby reiteration of an inaccurate process generates a much more accurate one. If this is combined with a positive feedback loop, such as may operate in the case of microtubules and tea1p, then this will result in the emergence of a mechanism of considerable precision. Such a mechanism could in principle also allow a linear axis to be established *de novo*, by a stochastic build up of tea1p somewhere on the cell cortex. Such self-organization may occur in apolar cells like germinating spores or regenerating protoplasts.

Another important feature of the microtubule–tea1p mechanism is the fact that the long-range spatial order of a cell is being constantly evaluated. It does not involve the setting up of cellular markers that remain fixed, as in the case of growth-site selection in budding yeast where spatial organization is dependent upon the previous history of the cell. The microtubule–tea1p mechanism provides more flexibility since the position of the cellular antipodes is under constant surveillance, so should an error occur this can be efficiently corrected. This may be an important concept in understanding how an accurate cellular shape is specified.

3. SPECIFYING POSITION

Monitoring the location of a green fluorescent protein (GFP)-corset fusion protein has revealed that different regions of the cortical membrane have changed properties (Sawin *et al*. 1999). This protein is located in the central region of the cell closely associated with the cell membrane just under the cell wall. It is excluded from the ends of the cell, and when a cell divides there is a clearing of GFP-corset from the new end that is formed at division. Thus GFP-corset appears to be a marker of the cortex in the central region of a cell. There are various mutants that only grow from one end. In most of these mutants, GFP-corset is still excluded from both ends of the cell. However, mutations in *shk1*/*orb2*, a gene which encodes a p21-activated kinase (PAK) kinase, have a different GFP-corset distribution. In this mutant, the fusion protein is now also found at the non-growing end. These observations suggest that there is something different about the cortical regions in the middle of the cell compared with those at the end which have the potential to grow. GPF-corset can occupy the middle but not the end cortical regions. The regions are specified differently and this specification requires the PAK kinase.

The idea of specification of a region is a very familiar concept to developmental biologists. Its application to cells implies that spatially distinct regions within cells have different specifications with significance to understanding cell morphogenesis.

4. SEPTUM POSITIONING

The fission yeast septum is generally placed very close to the middle of the cell. Mutations in two genes, *mid1*/*dmf1* and *plo1* result in the septum being misplaced away from the middle (Chang *et al*. 1996; Sohrmann *et al*. 1996; Bähler *et al*. 1998). In these mutants the septum can be formed at right angles anywhere in the cell, creating two unequally sized cells, or in an oblique angle in any position, or in a disorganized way throughout the cell. In the former two situations, a reasonably well-formed septum is made suggesting that the ability to make a correctly organized septum is intact in these mutants, whilst the global positioning mechanism is defective.

Mid1p, which has a nuclear localization signal and a pleckstrin homology domain, is found within the nucleus during interphase and in a tight medial ring during mitosis (Sohrmann *et al*. 1996). This ring forms by the coalescence of an initially broad cortical band of mid1p (figure 3). (Bähler *et al*. 1998). Exit of mid1p from the nucleus requires the plo1p protein kinase. In a *plo1* mutant, mid1p remains in the nucleus and the septum is misplaced (Bähler *et al*. 1998). These observations suggest that the medial position of the septum is determined by the position of the nucleus. At the onset of mitosis, mid1p is driven out of the nucleus by plo1p and relocates into a broad cortical band around the nucleus. Upon nuclear exit, mid1p presumably diffuses out and associates with the nearest cortical membrane, which will result in a broad mid1p band around the nucleus. The band then tightens up into a ring, which co-localizes with the contractile actin ring in the cell centre (figure 3). The actin itself might play a role in concentrating mid1p into the ring. It is important to realize that in the absence of mid1p, the actin ring and septum can still be formed although they are mislocalized. So, similar to tea1p in interphase cells, the role of mid1p is to properly locate but not organize the growth machinery during mitosis.

This model obviously restates the septum-positioning mechanism in terms of what positions the nucleus in the middle of the cell. The major clue to nuclear positioning is that again microtubules appear to have a role. In the absence of a normal cytoplasmic microtubular cytoskeleton the nucleus is no longer centrally located in the cell (Umesono *et al*. 1983). Various models have been proposed by which microtubules lead to the nucleus being placed centrally in the cell (Reinsch & Gonczy 1998). One of them is that the nucleus is attached to microtubules, which extend into the cell and where they are themselves attached to fixed structures within the cytoplasm by microtubule motor proteins. The position of the nucleus is then determined by the opposing forces generated by the microtubular motors attached to the fixed sites in the cytoplasm. Assuming that the fixed sites are distributed homogeneously throughout the cell a consequence of this mechanism is that a nucleus will become centrally located within the cytoplasmic volume of the

cell. So if a cell is asymmetrically shaped, like a pear for example, then the nucleus will be placed midway between equivalent cytoplasmic volumes rather than midway between the cell ends. Fission yeast morphological mutants with asymmetrical shapes tend to behave like this, and the cytoplasmic microtubules are closely associated with the nucleus, making this model an attractive one to test.

Defining the position of septation by the position of the early mitotic nucleus clearly makes sense as it means that cytokinesis will be initiated in the region of the dividing nucleus, maximizing the chance that the two newly formed nuclei will be separated into the two new cells. It also makes sense to position the nucleus and therefore the division site, such that both daughter cells obtain an equal cell volume independently of the overall cell shape of the mother cell. Again cytoplasmic microtubules may have a central role also in performing the task of splitting the fission yeast cell and its contents into two halves during mitosis. A combination of extended microtubular polymers anchored within the cytoplasm by polarized motors provides an elegant mechanism to position organelles such as the nucleus. These possibilities have already been recognized by those studying the mitotic spindle (Hyman & Karsenti 1996), but have not yet been generally applied to problems of cell morphogenesis.

A second general concept illustrated by this work is the idea of approximation as an intermediate step in a positioning mechanism. Formation of a septum requires a number of components including actin to form a very precise, thin ring (Gould & Simanis 1997). Generating a ring from a single point that can extend around the cell and then precisely meet the other end to close the circle is a difficult task to perform. In contrast, establishing a broad cortical band by exporting midlp from the nucleus is straightforward. This broad band then needs to tighten up to produce the thin ring. How such a tightening up may occur is not understood, but it could involve yet again some form of positive feedback whereby an increase in concentration of a component in the central region of a broad band leads to a further increase by drawing in components from the lower concentration found in the peripheral regions of the broad band. An alternative possibility is a role for actin–myosin in transporting components locally within the broad band. Such transport could properly concentrate components if the actin was polarized towards the central region of the broad band. A further concept to emerge from these results is that temporal controls can contribute to spatial order. Triggering the export of midlp from the nucleus at a particular time of the cell cycle, that is the onset of mitosis, has spatial implications for defining the correct positioning of the septum.

A more general point can be made about the roles of the actin and microtubule cytoskeletons in cell morphogenesis. Actin has a very specific distribution within fission yeast cells, where it is predominantly found at the growing cell tips or the septum. It has become apparent that both of these structures can form perfectly, regardless of where they are placed within the cell. This raises the general concept that microtubules may play a role in more global cellular positioning mechanisms whilst actin is used in more local organization. Reverting to the language of developmental biologists: actin-based 'submorphogenetic fields' are organized with respect to each other by a microtubular-based global cellular 'morphogenetic field'.

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