The Physarum polycephalum actin network: formalisation, topology and morphological correlates with computational ability

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ABSTRACT

The plasmodial form of slime mould *Physarum polycephalum* is a macroscopic acellular organism that is capable of apparently intelligent behaviour, yet it lacks any features usually associated with intelligence. In this investigation, we study the morphology of the plasmodial actin cytoskeleton and formalise its network topology in efforts to correlate cytoskeletal morphology with slime mould computational abilities. The plasmodial actin network is a highly abundant, complex structure which links the functional components of the cell, whose topology may be approximated with a range of proximity graphs, depending on the physiological and environmental conditions within the plasmodium. Its topology is highly dynamical and is likely to rapidly alter in response to environmental stimuli to maximise network efficiency. We conclude by discussing the nature of the computational process in organic networks.

Categories and Subject Descriptors

J.3 [**LIFE AND MEDICAL SCIENCES**]: Biology and Genetics
; C.1.4 [**Parallel Architecture**]:
; C.2.1 [**Network Architecture and Design**]: Network Topology, Network Communications

Keywords

Slime mould, *Physarum polycephalum*, Cytoskeleton, Unconventional computing, Proximity graph

1. INTRODUCTION

The cytoskeleton is a scaffold of proteins within a cell whose functions are myriad and essential to the maintenance of life. These include maintaining a cell's structural integrity, providing a network for trafficking of cytoplasmic molecules and organelles, facilitating cell division and participating in muscle contraction for cellular locomotion. The cytoskeleton is formed from three groups of proteins: tubulin microtubules, actin microfilaments and a range of intermediate filaments (IFs) whose type depends upon the function of the cell. For example, several varieties of human epithelial cell possess keratin IFs for added structural integrity, whereas muscle cells contain desmin IFs, which are thought to contribute to muscle activity. The cytoskeleton is ubiquitous amongst all eukaryotic (and some prokaryotic) cells and many disease states are associated with CSK abnormalities [7, 19].

The cytoskeleton is also thought to be heavily implicated in a range of cellular signalling events between cell surface receptors (and hence, the cell's environment), the nucleus and other organelles. Several authors have advanced evidence suggesting that the cytoskeleton is able to store and transmit electrical potential, mechanical stress, biomolecules and even quantum events such as Davydov solitons in response to intra- and extracellular input (i.e. stimulation) [5, 6, 17, 26]. These discoveries have led to a growing consensus throughout a wide range of scientific disciplines that the cytoskeleton is a self-assembling cellular data network which coordinates a vast amount of cellular processes: consequently, many now support the hypothesis that coherent, emergent behaviours observed in living organisms such as intelligence, learning and memory arise from cytoskeletal signalling events [10, 12, 22]. From a computing perspective, these signalling events may be regarded as a form of computation wherein input comprises the sum of environmental stimuli (mechanical stress, chemical, light etc.) and the output is the cellular response evoked; this encompasses events such as mobilisation of intracellular ions and alterations in gene expression, which lead to a consequent alteration in the cell's behaviour. For example, microtubules have been modelled as data buses in which data are represented by transitions in protein conformational state which undergo Boolean logical operations implemented by intermediate microtubule-associated proteins: recent experimental evidence has been found to support this hypothesis [4, 15]. Whilst it should be noted that no model has been widely accepted regarding the computational role of the cytoskeleton, or indeed, the linkage between molecular and organismal processes, it is nevertheless clear that the cytoskeleton is involved in a range of cellular signalling events which may be interpreted as computation.

In this investigation, we study the actin-component network

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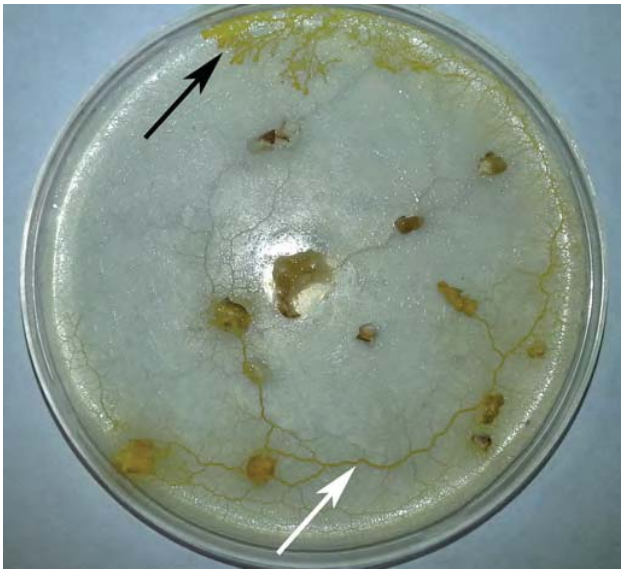
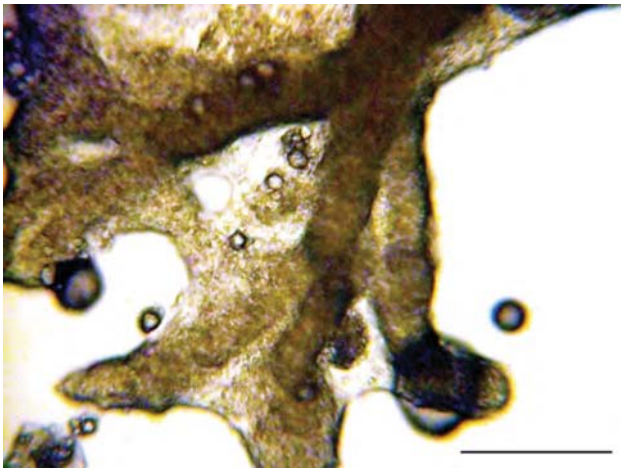
of slime mould *Physarum polycephalum*, formalise its structure and attempt to correlate cytoskeletal morphology with slime mould computational abilities. *P. polycephalum* is a 'true', or 'acellular', slime mould which exists as a macroscopic, multinucleate (containing many thousands of nuclei) cell in its plasmodial (vegetative) life cycle stage [24] (Fig. 1). Essentially a giant single eukaryotic cell, the *P. polycephalum* plasmodium is capable of computing a range of complex tasks ranging from logic to computational geometry, e.g. solving maze puzzles, optimizing nutrient harvesting networks [1, 21]. Slime moulds do not seem to possess any easily identifiable components which have been associated with facilitating such (apparently) intelligent behaviour, however. This study focuses upon exploring the role of the plasmodial cytoskeleton in facilitating emergent behaviour — the generation of complex behaviour patterns which cannot be derived from the characteristics of a system's individual components/inputs — to provide a theoretical basis for how complexity (and hence, 'intelligence') can arise in slime mould.

The actin-component cytoskeletal network is composed interlinked filamentous (f-)actin strands, which are comprised of globular (g-)actin monomers bound in a double helix, as in Fig. 2. In mammalian cells, the actin network spans the entire cell but is most concentrated in a dense cortical region about the cell's periphery, where it articulates onto membrane-bound proteins such as integrins, cadherins and trans-membrane ion channels [27]. Longer, more sturdy 'stress fibres' link the cortical region network to more central organelles, such as the nucleus and other cytoskeletal components [20]. The functions of f-actin include participating in muscle contraction (via integral tropomyosin units), maintaining structural rigidity, driving pseudopodic movement via rapid directional tip growth and transducing a range of environmental stimuli into signals comprehensible by the cell [12]. Of these environmental stimuli, the ability of actin to compress and transmit mechanical stress (either directly or via cell surface mechanoreceptors conjugated to actin filaments) is arguably the best characterised, but signalling events may also arise from stimulation of other varieties of surface receptor or intracellular signalling events. There is also evidence to suggest that actin may also transduce cell-surface stimulation via electrical, molecular and quantum events (e.g. Davydov solitons) [3, 5, 10, 12, 23]. The majority of environmental data is assumed to be transmitted to the nucleus, although the 'controller region' of the cell is likely to be decentralised. The cytoskeleton is also likely to be involved in coordinating cellular responses to these stimuli, e.g. nucleus-instigated signalling my alter surface receptor activity via cytoskeletal innervation.

By interpreting these biophysical processes in terms of computation, we may speculate that the cytoskeleton is a sensorimotor data network. By extension, this implies that the apparently intelligent behaviour we observe in slime mould is facilitated by the cytoskeleton. This would appear to be logical, as it is commonly considered that an intelligent entity requires a means for structuring (i.e. transducing and transmitting in a repeatable and unambiguous manner) sensorimotor data streams [8, 11]. This paradigm applies to both artificial and natural systems; indeed, the mammalian brain is an extremely complex network which fits this de-



(a)



(b)

Figure 1: Plasmodium of slime mould *P. polycephalum* growing on an agar plate. (a) Macroscopic appearance, black arrow indicates 'fan-shaped' advancing anterior margin, white arrow indicates plasmodial vein. (b) Microscopic view of anterior margin showing the confluence of several plasmodial pseudopodia into an amorphous leading edge. Scale bar: 500μm.

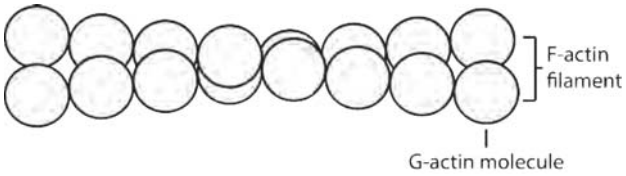


Figure 2: Schematic diagram of an actin filament as a double helix structure composed of individual G-actin monomers.

scription.

Although the *P. polycephalum* is a eukaryotic cell, its cytoskeleton is significantly less well characterised than its mammalian counterpart and fundamental differences in cellular physiology — multinuclearity, massive scale etc. — disallow any direct comparisons between the two. Thus, a main aim of this investigation was to determine the topology of the plasmodial actin network; this is addressed in section 2. As the actin network topology is not homogenous throughout the interior of a cell, both of the two major anatomical locations of the plasmodium were sampled — the tube-like plasmodial veins which form the body of the plasmodium, and the 'fan-shaped' advancing anterior margin (see Fig 1). We proceed to formalise the actin network and model its topology with a range of graph theoretical techniques in section 3. Finally, the nature of the computational process in such a network is discussed.

2. MICROSCOPICAL EXAMINATION OF THE PLASMODIAL ACTIN NETWORK

The plasmodial actin network was visualised with confocal laser scanning microscopy (CLSM). See section 6 for details of specimen preparation.

The plasmodial actin network was observed to exist as an extremely dense, complex network at the anterior margin (Fig. 3). As the advancing tip of the plasmodium is essentially the confluence of several pseudopodia, this would suggest that slime mould motility is achieved via actin-driven tip growth in a manner similar to amoebae, albeit on a giant scale. Actin filaments appear to articulate onto each nucleus in several locations.

The actin network was still present but less abundant and significantly less chaotic in plasmodial veins, being concentrated mostly at the peripheral regions (forming what appears to be a typical cortical zone) of the tubes and around vesicles. Interestingly, the association of actin and nuclei is less apparent in plasmodial veins, although this may be due to their comparative under-abundance in veins rendering them invisible with the techniques used. Nuclei were also more abundant at the anterior margin.

If it is assumed that each actin strand is capable of transducing and transmitting information, the computational ability of a cell is, by extension, proportional to the abundance of actin and the efficiency of its network (where efficiency here encompasses the informational capacity of the network, speed/efficiency of communication, processor/controller availability etc.). The plasmodial cytoskeleton is arguablly a more complex network when compared with the actin network in mammalian cells, and furthermore it is significantly more abundant; as such, the *P. polycephalum* plasmodium may possibly have superior informational capacity than a single mammalian cell.

3. FORMALISATION OF THE ACTIN NETWORK

The topology of plasmodial actin communications network can be derived from proximity graphs if nuclei are assumed to be nodes and edges the actin cytoskeleton.

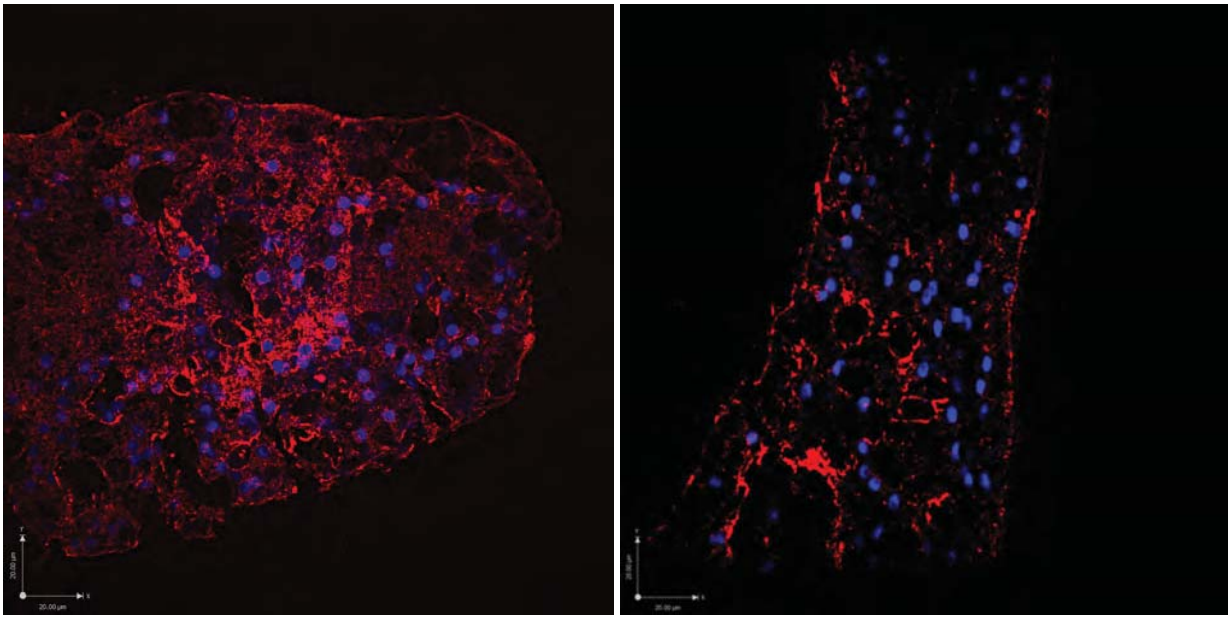
A planar graph consists of nodes which are points of the Euclidean plane and edges which are straight segments connecting the points. A planar proximity graph is a planar graph where two points are connected by an edge if they are close in some sense. A pair of points is assigned a certain neighbourhood, and points of the pair are connected by an edge if their neighbourhood is empty. Here we consider the most common proximity graph as follows.

- **GG**: Points a and b are connected by an edge in the Gabriel Graph **GG** if disc with diameter $dist(a, b)$ centred in middle of the segment ab is empty [9, 18].
- **RNG**: Points a and b are connected by an edge in the Relative Neighbourhood Graph **RNG** if no other point c is closer to a and b than $dist(a, b)$ [25].
- **MST**: The Euclidean minimum spanning tree (MST) is a connected acyclic graph which has minimum possible sum of edges' lengths.

In general, the graphs relate as **MST** $\subseteq$ **RNG** $\subseteq$ **GG** [13, 18, 25]; this is called Toussaint hierarchy.

As shown in Fig. 4, and as spanning trees (acyclic proximity graphs), shown in Fig. 5. The spanning tree is a subgraph of the relative neighbourhood graph, which in turn is a subgraph of the Gabriel graph [13, 18, 25], and represents the most efficient variety of network topology, i.e. the minimum total edge length to link all nodes. These graphs depict only nucleus-nucleus network connections as it is assumed that plasmodial nuclei are components which receive and act upon (coordinate responses to) environmental data perceived from surface receptors — note that labelling nuclei as 'processors' is a contentious issue which is not discussed here — and that the activities of each nucleus are likely synchronised to produce coherent behaviour patterns at the organismal level.

Thus, we can speculate that depending on momentary actual demands of the slime mould's physiology, as well as changes in its environmental conditions, the topology of its communication network can rapidly change between a proximity graph with a high number of connections between nodes (Gabriel graph), the cyclic graph with minimal number of links (relative neighbourhood graph), and the acyclic proximity graph (spanning tree). The topological transformations between the proximity graphs could be also expected



(a) (b)

Figure 3: Confocal micrographs showing 12μm plasmodial sections stained for actin (red) and nucleic acids (blue). (a) Transverse section through advancing anterior margin. North corresponds to the plasmodial dorsal margin. The actin network is extremely complex and abundant. (b) Longditudinal section through plasmodial vein. Note how actin is less abundant.

during morphological transformations of the slime mould's active growing zones.

Another way to formalise transformations of the actin networks would be to use β-skeletons (Fig. 6). Given a set $\mathbf{V}$ of planar points, for any two points p and q we define a β-neighbourhood $U_\beta(p,q)$ as the intersection of two discs with radius $\beta|p-q|/2$ centered at points $((1-\frac{\beta}{2})p, \frac{\beta}{2}q)$ and $(\frac{\beta}{2}p, (1-\frac{\beta}{2})q)$, $\beta \geq 1$ [13, 14]. Nodes p and q are connected by an edge in β-skeleton if the pair's β-neighbourhood contains no other nodes from $\mathbf{V}$. In the hypothetical scenario shown in Fig. 6 we imitate transformation of a centrifugal network by tuning the neighbourhood parameter β from 1 to 50; see details of the algorithm to grow β-skeletons in [2]. The transformation is implemented via pruning of redundant links and gradual formation of the acyclic graphs. The pruning starts at the peripheral parts of the graph, see e.g. Fig. 6de, and propagates towards the central core of the graph, see e.g. Fig. 6fg, until the whole graph is transformed into an —almost — acyclic graph, as shown in Fig. 6l.

4. DISCUSSION

Speculatively, the ability to switch network topology allows the *P. polycephalum* plasmodium to process details of its environment whilst conserving energy by always utilising the most efficient morphology; this would appear to make perfect evolutionary sense. To delineate, the Gabriel graph could, for example, be used for majority voting [25] as a means for synchronising the processes in each of the plasmodium's nuclei into a coherent pattern of behaviour, whereas the spanning tree conserves resources by only using the bare minimum skeleton network required to continue linking each node. This state could also occur when the organism is in an unstimulated state, where the informational capacity requirement of the organism is low.

The plasmodium is often described as an amorphous computing substrate [1], meaning that it a massively-parallel, architecture-less processing device. When modelled as a proximity graph, we can derive how generalised signalling events may propagate through the interconnected cytoskeletal network and innervate multiple controllers; thus, parallelism is achieved without the need for synchronisation. The computational process is therefore outsourced to the entity's morphology to some degree, further reducing the energy cost of the process whilst promoting the development of complexity. These observations are complimentary to recent advances in the field of morphological computation and entity embodiment [11, 16].

We conclude that *P. polycephalum's* extraordinary processing capabilities may be a product of its cytoskeletal topology. The occurrence of apparently intelligent behaviour occurring in a unicellular organism's cytoskeletal network would seem to imply that emergent behaviour is a product the physical properties of is data network; this has important implications for our understanding of human intelligence, which is commonly regarded as a product (in part) of neural network morphology and the characteristics of the juctions (synapses) therein. Further work will involve elucidating the morphology and computational role of the tubulin and intermediate filament components of the plasmodium.

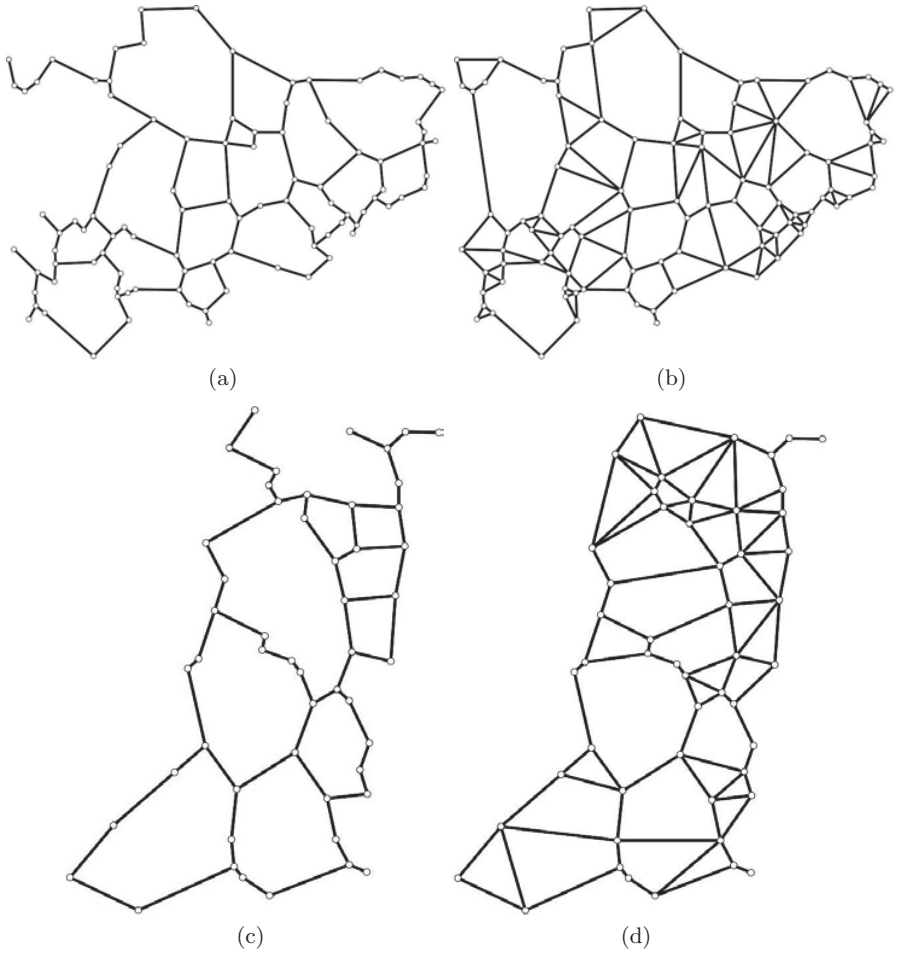


Figure 4: Proximity graphs as a potential formalisation of the communication and actuation network. (ac) Relative neighbourhood graph. (bd) Gabriel graph. Graphs (ab) are derived from Fig. 2a and graphs (cd) from Fig. 2b, assuming that nuclei are nodes.

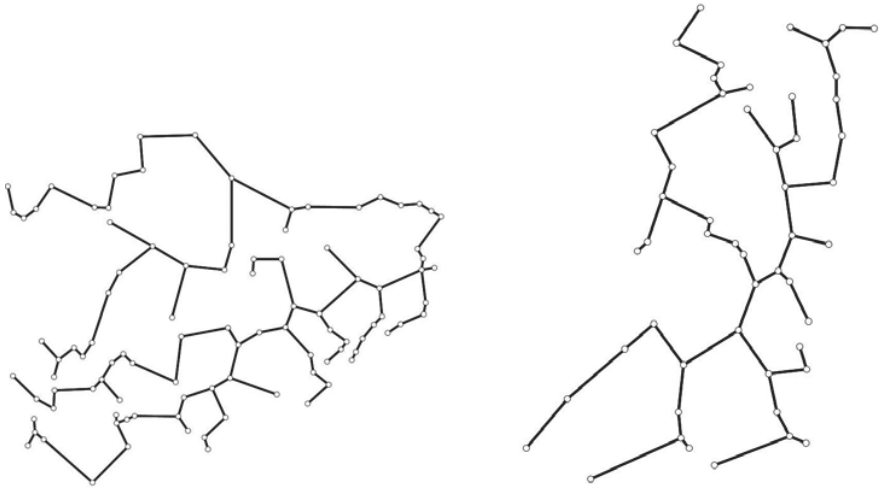
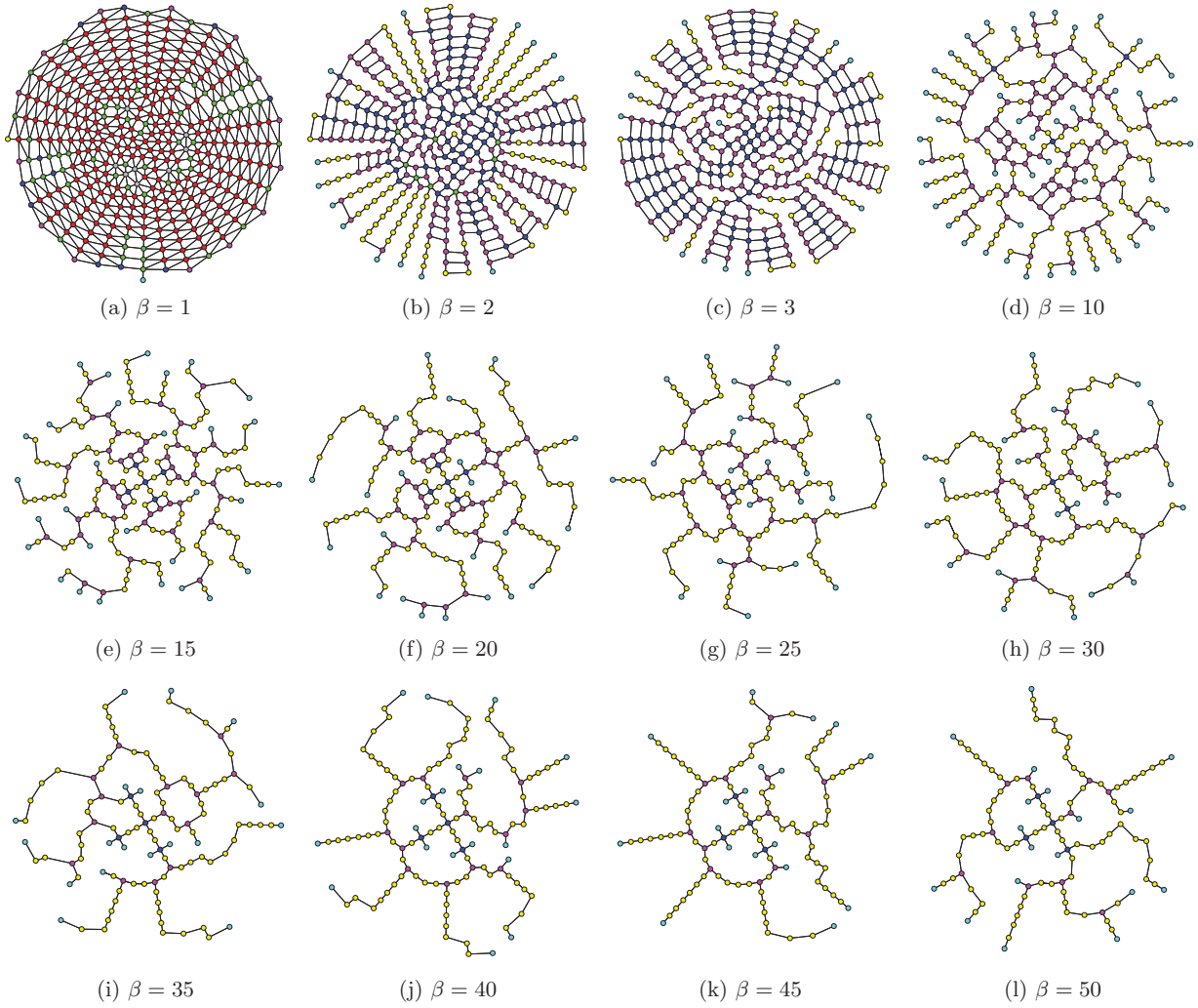


Figure 5: Spanning tree derived from (a) Fig. 3a and Fig. 3b where nuclei are represented by nodes.



(a) $\beta = 1$ (b) $\beta = 2$ (c) $\beta = 3$ (d) $\beta = 10$

(e) $\beta = 15$ (f) $\beta = 20$ (g) $\beta = 25$ (h) $\beta = 30$

(i) $\beta = 35$ (j) $\beta = 40$ (k) $\beta = 45$ (l) $\beta = 50$

Figure 6: A hypothetical scenario of topological transformations of actin-based communication/contractile network in *P. polycephalum*. The networks are represented by β-skeletons. The network evolution is controlled by neighbourhood parameter β. Colour, or grey level, of nodes codes their degrees. See details in [2].

5. SUMMARY

In this investigation, we have demonstrated the *P. polycephalum* actin network to be an abundant organelle whose distribution and topology differ throughout different anatomical regions of the organism. The actin-component network is likely to link cell surface proteins, nuclei and other organelles into one interconnected graph whose topology may be formalised with a range of proximity graphs. We speculate that, due to the plasmodium's requirement to constantly alter its cytoskeletal network topology due to tip-growth and changing environmental and physiological conditions, it is able to rearrange its graph topology to a more appropriate proximity graph. The role and physical associations of the plasmodial actin network are likely concomitant with those of the other cytoskeletal components (tubulin and intermediate filaments).

6. METHODOLOGY

6.1 Slime mould culture & tissue processing

Plasmodia of *P. polycephalum* were cultured on 2% non-nutrient agar plates in the dark at room temperature. They were fed porridge oats and were sub-cultured every 3-4 days. Approximately 10mm^2 samples were removed from plates (including their agar base) and fixed in 2% paraformaldehyde in pH 7.2 potassium phosphate buffer for 1 hour. After 3x 15 minute rinses in the same buffer, the samples were dehydrated in a graduated series of ethanol (30% $\rightarrow$ 50% $\rightarrow$ 70% $\rightarrow$ 90% $\rightarrow$ [100%x2], 15 minutes each) and cleared in 3x 15 minute changes of limonene. Clearing was found to render the tissue segments stiff, which were consequently detached from their agar bases at this stage. They were then infiltrated with 3x 15 minute changes of paraffin wax and embedded. Sections were cut at 12μm on a Leica RM2235 microtome.

6.2 Confocal microscopy

Antigen retrieval, endogenous antigen blocking and permeabalisation were found to be unnecessary. Tubulin was visualised via indirect immunofluorescence with monoclonal antibody KMX-1 (β-tubulin) (Abcam, UK), which was applied at a concentration of 2μg/ml for one hour. Actin was visualised with ACTN05(C4) (pan-actin) (Abcam, UK) which was applied at 4μg/ml for one hour. After three rinses in pH 7.2 phosphate-buffered saline solution, secondary antibodies bound to fluorophores (Alexa Fluor 488 or 568) (Molecular Probes, USA) were applied for 1 hour. All sections were mounted in a solution containing DAPI (4',6-Diamidino-2-phenylindole dilacetate) with an anti-fade reagent (Molecular Probes, USA). Confocal microscopy was performed with a Perkin Elmer UltraView ERS FRET-H spinning disk laser confocal microscope. For notes on image post-processing, please see Appendix B.

APPENDIX

A. CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

B. IMAGE PROCESSING

Photographs were taken with a FujiFilm AX650 digital camera. Photomicrographs were taken with a Nikon DS-U1 digital sight camera via NIS-elements software, which automatically adjusted exposure times and white balance.

Confocal micrographs were digitally post-processed in Volocity (Improvision, UK), and received colour assignment, deconvolution and brightness and colour adjustments.

Unprocessed image files will be made available on request.

C. ACKNOWLEDGEMENTS

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