The Artificial Cytoskeleton for Lifetime Adaptation of Morphology

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Abstract

The Artificial Cytoskeleton (AC) is introduced as a new model for generating adaptive growth of an artificial cell's morphology throughout its lifetime in response to environmental cues. The AC utilizes swarm and cellular automata techniques. It is closely modelled on the eukaryotic cytoskeleton which is responsible for giving the cell dynamic structure and function. The AC is tested in a simple chemotaxis experiment and is shown to effect morphological adaptation during the cell's lifetime.

Introduction

Computational Development (Kumar, 2004) is an umbrella term that subsumes what was previously known as Computational Embryology. This distinction was made to avoid implications that the field only researches *early* development. Development is an ongoing process spanning the entire lifetime of an organism. *Lifetime* adaptation in form is therefore one of the key aims of Computational Development, yet it has not been realized by current models which generate static morphologies only (Sims, 1994; Bongard, 2001; Eggenberger, 1997; Taylor and Massey, 2001).

The unicell exhibits morphological dynamics that surpass those of multicellular organisms. Due to its fluid nature, a unicell can rapidly reorganize its entire inner structure, outer body shape, transport organelles from one side to the other and even split itself in two; all in direct response to environmental changes. All these behaviours are executed by the cell's *cytoskeleton* (Alberts et al., 1994). The cytoskeleton is a complex distribution of proteins which acts as a transport network, contractile muscle and/or structural support. Due to its non-rigidity it can rapidly disassemble and reform in a more advantageous distribution. This far superior, dynamic ability of unicells forms the basis of our investigations. The study of self-organization within an artificial cell illuminates the mechanisms involved in adaptive behaviour for organisms with no neural system. This also provides a novel model for investigating lifetime adaptation in morphology for artificial systems, for example to increase survival chances in unpredictable environments.

The Artificial Cytoskeleton (AC) is a computational model of the eukaryotic cytoskeleton. To focus on the operation of the cytoskeleton, gene expression is omitted; proteins are synthesized at random positions ab initio, there is no Genetic Regulatory Network (GRN) and so protein synthesis is not continual. The AC is tested in a simple experiment based on animal cell chemotaxis and is shown to effect morphological adaptation during a cell's lifetime, thereby increasing survival chances. Animal cell chemotaxis is a well defined example of lifetime morphological adaptation as it involves no obvious locomotory organelle, such as a flagellum. Instead, the cell undergoes transformations in form. In the first identified stage *protrusions* extend forward in a 'leading edge' (see Fig.4(a)). The further stages *attachment* and *traction* involve less well defined biological mechanisms and physics and exceed the scope of this paper, thus only the first stage has been modelled (Alberts et al., 1994; Bray, 2001).

In the next section the AC framework is described and the specific proteins included for the chemotaxis experiment detailed. The experiment is then outlined and the results discussed in terms of the artificial cell's achievement, morphology and self-organizing properties. Finally, conclusions and future work are outlined.

The Artificial Cytoskeleton (AC)

The AC uses swarm techniques combined with Cellular Automata rules to allow proteins to exist and interact with their 26 nearest neighbours (NNs) in a 3D voxellated environment, partially inspired by artificial chemistry modelling techniques (Hutton, 2002). The AC resides within a membrane-bound 'cell' and receptors in the membrane relay external signals to the AC via a pathway of proteins: the Transduction Pathway (TP). The AC constantly responds by reorganising, i.e. altering the cell's internal topography and the membrane morphology.

Each voxel contains one of the following five units: 1) *environment*, which may contain concentrations of chemoattractant *'C'*; 2) *plasmalemma* (PL), which may contain a receptor and/or membrane-associated proteins (WASP and PIP_2 complexes); 3) *cytoplasm*, which may contain concen-

trations of Profilin; 4) *actin*, which may be in the states P1, P2, F-actin or S-actin and which has 2 opposing binding sites ('+','-'); 5) *arp2/3*, which may be switched on or off and has one binding site. Actin and arp2/3 are the agents of the swarm system; their interactions drive the creation, growth and disassociation of structural chains of actin: *actin filaments* (AFs). AF growth forces local PL shape changes, therefore altering the cell's overall shape. For efficiency, the AC and TP comprise only a selection of the various proteins necessary for a particular experiment.

The following general rules govern AC protein behaviour. **Diffusion:** proteins whose direct effects are not structural are represented as concentration gradients which diffuse through cytoplasm units. Diffusion is calculated as in (Glazier and Graner, 1993); each cytoplasm unit has a threshold for given proteins, the excess being evenly distributed to its cytoplasm NN's. **Random Movement:** when an agent is not bound or stuck it moves randomly. It has (arbitrarily) ten tries to randomly pick a cytoplasm NN to move to, otherwise it remains still. The target unit's protein gradients are diffused away and it acquires the agent's identifier; the agent's position data is updated; its previous voxel becomes cytoplasm. **Recruitment:** the biological concept of recruitment of proteins, to a specific protein *S*, is modelled as follows: the agent follows random movement until it encounters an *S* in its NNs. It then can only move such that an *S* is still in its NNs. Recruitment stops if there is no *S* NN.

Plasmalemma (PL)

The eukaryotic PL is a lipid membrane which compartmentalizes the cell from the environment (Alberts et al., 1994). Initially, no PL units contain WASP or PIP_2 but each has a probability (set to '$1/5$') of containing a receptor. **Receptors:** cell surface receptors are protein complexes embedded in the PL, which mediate signals from the external environment to the cell's internal environment. PL units containing receptors sum the concentration of *C* in their environment unit NNs. If the sum exceeds the threshold (set to '0.005') a cascade reaction inside the cell is triggered; the membrane-associated proteins WASP and PIP2 are activated for the unit and for its PL NNs. If the receptor deactivates, WASP and PIP_2 deactivate. See Fig 1.

Transduction Pathway (TP) Selection

External signals are relayed to the AC by the TP protein reactions, triggered by the PL receptors. The TP selection consists of WASP and PIP_2. The **WASP** family are proline-rich proteins which, when activated by a receptor, recruit agents Arp2/3 and actin (in state P1) to the PL. Once recruited, Arp2/3 switches on and P1 changes state to P2 (recruited form). Activated **PIP_2** releases a one-off plume ('0.005') of profilin which diffuses through cytoplasm units (with threshold 0.0005). Deactivated PIP_2 causes *removal* of all profilin in the PL unit's cytoplasm NNs (Holt and Koffer, 2001).

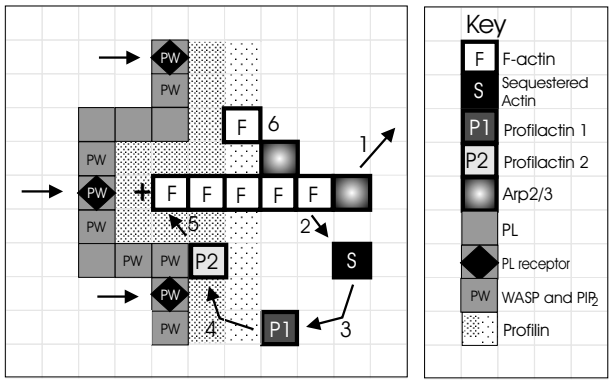

Figure 1: AC interactions. Receptors detect chemoattractant, WASP and PIP_2 activate and cause the cytoskeletal behaviours shown in stages 1 - 6, see text for details.

The AC Selected Proteins

The selection consists of actin, Arp2/3, profilin, thymosin and cofilin; thymosin and cofilin are assumed to be uniformly abundant in the cell and so not modelled directly. **Actin state changes:** initiated in the inactive state S-actin (bound by thymosin); S-actin units sum the concentration of profilin in their NNs, if it exceeds the threshold ('0.008') then it binds to profilin and changes to P1 (profilactin), removing amount *P* ('0.0001') of profilin, see stage 3 Fig. 1. P1 is recruited to active WASP to form P2 (stage 4). After recruited movement, if P2 has an AF '+' site in its NNs, it binds to it, changes state to F-actin (actin in a filament), releases *P* to a cytoplasm NN, and moves to the NN cytoplasm voxel that permits its '-' site to directly abut the AF '+' site (stage 5). **Arp2/3:** activates when recruited by WASP and then can nucleate (start) AFs and set their orientation by binding to a P2 in its NNs (see push-out rule below). If there is a fully bound F-actin NN, then Arp2/3 can 'stick' to it and nucleate a *branch* AF: stage 6 (Bray, 2001).

Actin Filaments (AF): AFs are the key AC mechanism for affecting PL morphology. Fig.1 illustrates the construction process for an AF. Over time Arp2/3 disassociates (and un-sticks) from its AF and deactivates (stage 1). Similarly F-actin loses affinity for the AF allowing cofilin (a severing protein) to disassociate it; it then gets sequestered and becomes S-actin again (stage 2). Disassociation occurs at the AF's '-' end. The actin or Arp2/3 unit disassociates with probability *B*, which increases with time spent in an AF; $B =$ time spent in filament$/(100 \times FTI)$, where FTI is a set interval ('2'). As the '+' end of the AF grows, the '-' end shrinks and the AF, as a higher level entity, moves towards the PL.

Modifying the PL

Morphology Push-out: The AFs push out the PL, affecting morphology. The biological mechanism for this process is unclear (Condeelis, 2001). Our mechanism assumes a gap must exist (or be created) between the AF's '+' end and the

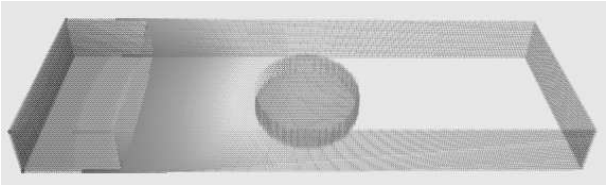

Figure 2: Initial cell in environment, $[C]$ gradient featured left

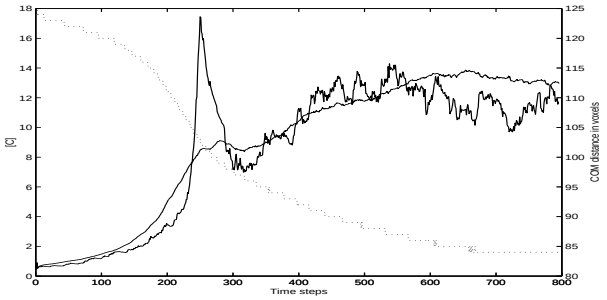

Figure 3: The average distance of the cell's COM from C's plume drop site over time (dotted), the average $[C]$ in all PL voxels NN's over time (thin), the concentration of C ($[C]$) one typical cell (run A) was exposed to (thick, peaked).

PL to allow space for further actin to bind. For F-actin on the AF's '+' end, we therefore 'push-out' all PL NNs by replacing the PLs with cytoplasm and the exposed environment units become the PL (all C in the environment units is diffused away first). As with F-actin, Arp2/3 must also push-out PL to allow room to bind actin, however the biological mechanism for this is again unclear. After implementing the above rule, Arp2/3 would switch off as it would no longer have WASP NNs, so we permit Arp2/3 to remain switched on if any of its 26 NNs or any of their surrounding 98 voxels contain WASP. **Contract-PL:** To keep cytoplasm volume constant, other cytoplasm units are contracted following 'push-out'. The furthest voxel within the cell containing either cytoplasm, actin (but not F-actin) or unbound Arp2/3) from the newly created cytoplasm is replaced with a PL voxel. If it was cytoplasm, any profilin it contained is diffused away first. If it was Arp2/3 or actin it is re-synthesized as a new unit replacing a randomly picked cytoplasm unit, after its contents have been diffused away. **PL thinning:** if a PL unit has no contact with inner cellular units, it is removed; this ensures there are no doubled-up layers of PL. The combination of these three interactions contracts the cell at the opposite side to a leading edge and allows the cell's centre of mass (COM) to move.

Experiment

A cylindrical cell, radius 25, height 10 was placed on the floor in the centre of a 3D environment with dimensions: $75 \times 250 \times 20$. A plume of chemoattractant C ('1000') had been dropped 2000 time steps previously at position

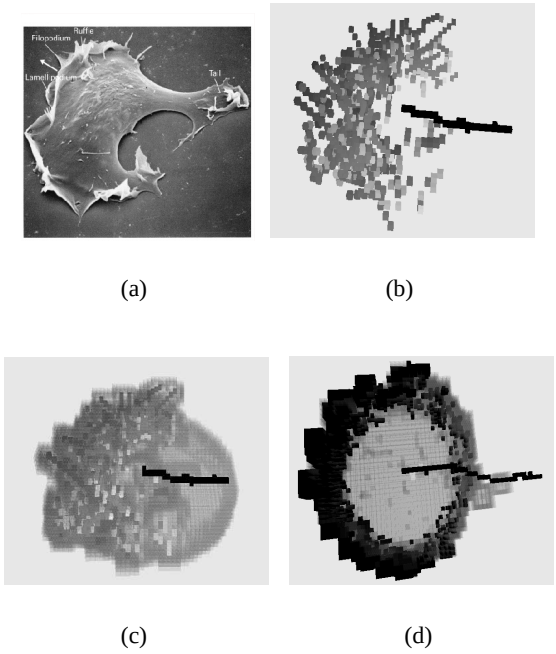

Figure 4: 4(a) A fibroblast cell during chemotaxis (Alberts et al., 1994). Leading edge and lamellipodia (branched, cross-connected AFs) left. Microspikes (parallel AF bundles) visible top left. 4(b) AFs and Arp2/3 form lamellipodia leading edge left, at time step 230 (during accelerated growth), black line shows COM path. 4(c) PL morphology with underlying AFs, timestep 230. 4(d) PL morphology, black units contain WASP, time step 800.

(37,0,0), 125 voxels away, and allowed to diffuse (threshold 0.0001) see Fig.2. For simplicity a GRN was omitted; instead all proteins were synthesized randomly within the cell, with overall concentrations remaining constant (6000 actin, 1500 Arp2/3 units). Similarly, all threshold values were set by hand. The model was run 40 times, each for 800 time steps. **Hypothesis:** the AC/TP selections will allow the cell to adapt its morphology by forming protrusions and gain greater exposure to C by moving the cell's COM towards C.

Results

The mean distance between the cell's COM, the average position of the cell's contents, and the initial plume drop site of C after 800 time steps was 84. The mean distance path over time is shown in Fig.3, the mean variance over the time steps was 3. The cells accelerated around time step 230 and then more gradually crept nearly twice their radius towards the C plume site, showing that a correct leading edge formed. The average sum of $[C]$ in all PL voxels' NNs increased from 0.5 to 12.1, see Fig.3. This was as hypothesized, however, an unexpected peak occurred in every run. As it occurred at varying times, the peak averaged out, but can be clearly seen in Fig.3. The variance around the peak time steps increased from an average 1.85 to 3.99, see Fig. 5 for explanation.

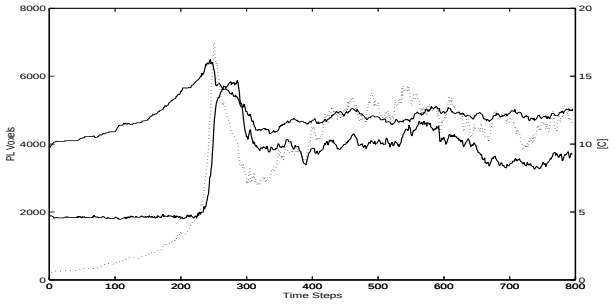

Figure 5: Data from run A. The number of exposed PL units (upper thick line) decreases rapidly just before $[C]$ peaks (dotted line). The no. of PL units touching environment sides (lower thick line) rapidly increases at this point. This shows that vertical protrusions increase the cell's exposed surface area until they abut the environment roof, significantly decreasing the cell's exposed surface area.

The cell had three distinguishable morphologies during a run: the initial cylindrical form; a leading edge lamellipodia with protrusions during accelerated movement; finally a skewed cylinder with short protrusions in most directions. Due to the lack of AF movement, the AFs could not align correctly to form microspikes. However, the small scale of the model led to similar protrusions occurring from the growth of a single AF.

Self-organization and Efficiency

The cytoskeleton self-organizes into beneficial distributions of proteins through cooperative and competitive interactions (Alberts et al., 1994). Cofilin and profilin *cooperate* to recycle actin in AFs: disassociating F-actin from an AF allows it to then form P1 and be sent back to the leading edge, improving efficiency. P2 performing recruited movement near WASP *competes* with the movement of S-actin and explains their differential distribution, leading to large scale polymerization at the cell edge as opposed to centrally. WASP has been described as a 'conveyor belt' delivering 'building blocks' to the AF 'construction site' (Condeelis, 2001); its funnelling of Profilactin and Arp2/3 to receptor localities greatly improves AFs efficiency for forming protrusions which immerse receptors deeper into C, positively reinforcing the process. The cell can affect its environment; the morphology push-out rule allows the cell to locally redistribute C in the environment as the AFs force PL protrusions out, causing trapped pockets of C between protrusions which continually reinforces growth. Internally, profilin similarly becomes trapped, as AF formation further compartmentalizes the cell, also resulting in positive feedback.

Conclusions

The Artificial cytoskeleton (AC) has been introduced as a new model for lifetime adaptation of an artificial cell's morphology, closely modelled on the eukaryotic cytoskeleton. A cell chemotaxis experiment using an AC in basic configuration has demonstrated the ability of the AC to effect lifetime morphological adaptation in an artificial cell.

Work on the AC is continuing in three key areas: (i) further work with biologists to improve the fidelity of the model to the real eukaryotic cytoskeleton, thereby improving understanding of simple organisms such as diatoms; (ii) to extend the model to include evolution of parameters and a GRN, and to identify a minimal set of proteins for useful adaptive response; and (iii) to determine areas of applicability for the AC, such as autonomous software agents and (via concept remapping) networks-on-a-chip.

Acknowledgements

We are indebted to Eileen J. Cox for inspiring our AC work, and to Anna Koffer for her expert advice on cell physiology.

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