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# A Model of Localised Rac1 Activation in Endothelial Cells Due to Fluid Flow

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## Abstract

Endothelial cells respond to fluid flow by elongating in the direction of flow. Cytoskeletal changes and activation of signalling molecules have been extensively studied in this response, including: activation of receptors by mechano-transduction, actin filament alignment in the direction of flow, changes to cell-substratum adhesions, actin-driven lamellipodium extension, and localised activation of Rho GTPases. To study this process we model the force over a single cell and couple this to a model of the Rho GTPases, Rac and Rho, via a Kelvin-body model of mechano-transduction. It is demonstrated that a mechano-transducer that can respond to the normal component of the force is likely to be a necessary component of the signalling network in order to establish polarity. Furthermore, the rate-limiting step of Rac1 activation is predicted to be conversion of Rac-GDP to Rac-GTP, rather than activation of upstream components. Modelling illustrates that the aligned endothelial cell morphology could attenuate the signalling network.

*Key words:* Stokes Flow, Endothelial Cell, Rho GTPases, Src, Shear Stress

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## 1 Introduction

Endothelial cells (ECs) respond to fluid flow by elongating in the direction of flow (Davies, 1995; Ballerman et al., 1998; Wojciak-Stothard and Ridley,

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2003; Tzima et al., 2002; McCue et al., 2006). This response is believed to be crucial for the correct function of the endothelium. Atherosclerotic plaques usually localize in regions of turbulent blood flow (for example, at sites of arterial branching). In these regions the endothelium has increased permeability and ECs take on a rounded morphology. This is in contrast to their ellipsoidal shape in regions of smooth flow (Weinbaum et al., 1985; Ross, 1999). Hence, there is a correlation (although not necessarily causation) between EC morphology and atherosclerotic plaques.

In order to respond to fluid flow, ECs must be able to transduce the mechanical force of the fluid into a biochemical signal. This mechano-transduction is likely to be mediated by activation of proteins via conformational changes due to increased tension or compression. A number of different proteins have been implicated in mechano-transduction, including cell-cell adhesion proteins such as PECAM-1 (Chiu et al., 2008; Tzima et al., 2005), stretch-activated ion channels (Martinac, 2004), and integrin-associated proteins in focal adhesions (FA). Integrins are transmembrane hetero-dimers that anchor the cell to the extracellular matrix (ECM). Hence if the cell is to remain adherent they must experience an increase in tensile force following the onset of fluid flow. This force is transmitted through the cytoskeleton to FAs. Integrins have been shown to respond to fluid flow (Tzima et al., 2001) and to be locally activated at the downstream edge (Goldfinger et al., 2008). However, this may be due to increased integrin-ECM ligation. The role of FAs and integrins is further supported by early FA remodelling (Davies et al., 1994) and fast activation of the integrin-associated tyrosine kinase Src (Wang et al., 2005a). p130Cas, an adaptor protein, is a known mechano-transducer associated with FAs, and can be activated in response to mechanical stretching (Sawada et al., 2006). It is also activated through Src-induced tyrosine phosphorylation (Okud et al., 1999).

Mechano-transduction and cellular mechanics have been extensively modelled previously (Mazzag et al., 2003; Stamenovic et al., 1996; Schwarz et al., 2005; Herant et al., 2003). For example, force transduction through ECs has been modelled using Kelvin bodies in parallel and in series (Mazzag et al., 2003) and focal adhesions have been modelled using a two spring model (Schwarz et al., 2005). Cell structure has been described by a tension-integrity mode, and a tensegrity model has been applied to a discrete system of struts (microtubules) and cables (actin filaments) (Stamenovic et al., 1996). This model demonstrated that stiffness of the model cell increased with pre-stress generated by actin-myosin contraction. Aspects of neutrophil mechanics have been successfully described using a two-phase model of reactive interpenetrating flows (Herant et al., 2003).

The Rho GTPases Rho and Rac regulate both FA formation and endothelial shape changes in response to fluid flow. Rho GTPases regulate cytoskeletal

dynamics and contribute to many cellular responses including cell migration and cytokinesis (Ridley, 2001; Narumiya and Yasuda, 2006; Ridley, 2006; Jaffe and Hall, 2005). Rho GTPases switch between an inactive guanosine diphosphate (GDP) bound form and an active guanosine triphosphate (GTP) bound form which interacts with downstream effectors. Switching between these two forms usually occurs on the membrane and is regulated by guanine nucleotide exchange factors (GEFs), GTPase-activating proteins (GAPs) and guanine dissociation inhibitors (GDIs; see figure 1).

RhoA leads to acto-myosin contraction via Rho kinases (ROCKs) and myosin light chain (MLC) phosphorylation (Riento and Ridley, 2003; Kawano et al., 1999). In response to fluid flow, RhoA has been reported to contribute to cell shape change by transiently inducing cell contraction, subsequently allowing cells to elongate in the direction of flow (Wojciak-Stothard and Ridley, 2003). Rac activation is localised to the downstream edge in ECs responding to fluid flow (Tzima et al., 2002). In response to integrin activation, Src has been shown to activate a Rac GEF, Vav2 (Garrett et al., 2007), and p190RhoGAP (a GAP for Rho) (Arthur et al., 2000). Thus, in this context, activation of Rac and inhibition of Rho is hypothesised to occur. In addition, it is believed that inhibitory crosstalk between Rho and Rac leads to separate zones of activation (Jilkine et al., 2007; Narang, 2006).

In order to investigate how the interplay between mechanical forces and EC morphology can influence downstream signalling, the work presented here models the mechanical force, transduction of the mechanical force into a biochemical signal and the downstream signaling to Rac1. We will describe three coupled computational models of these processes: fluid flow over an EC attached to a planar surface, mechano-transduction on the basal surface and signalling to Rac1. The flow model predicts the force on the surface of the cell. This force is coupled to a biochemical model via a mechano-transduction model.

## 2 The Flow Model

To establish a possible mechanism linking the force on an EC to localised Rac activation, the fluid flow,  $\mathbf{u}$ , is modelled over a single cell attached to a planar surface ( $z = 0$ ) by the Stokes equation:

$$-\nabla P + \mu \nabla^2 \mathbf{u} + \rho \mathbf{b} = 0 \quad (1)$$

where  $P$  is the pressure and  $\mu$  and  $\rho$  are the viscosity and density of the fluid respectively. On the characteristic length and time scales of this problem the Reynolds number is very small. Thus, assuming Newtonian characteristics,

the fluid is well modelled by equation 1. Here the Stokes equation is solved by using a boundary integral representation, which has previously been used in this context (Hazel and Pedley, 2000; Wang and Dimitrakopoulos, 2006a,b). In this representation the fluid flow at  $u_j(\mathbf{x}_0)$  is given by:

$$u_j(\mathbf{x}_0) = u_j^\infty(\mathbf{x}_0) - \frac{1}{8\pi\mu} \int_S f_i(\mathbf{x}_0) G_{ij}(\mathbf{x}, \mathbf{x}_0) dS \quad (2)$$

where  $\mathbf{u}^\infty(\mathbf{x}_0) = (kz, 0, 0)$  is the flow far away from the cell,  $S$  is the cell surface,  $f_i$  is the surface force and  $G_{ij}$  is a Green's function that vanishes if  $\mathbf{x} \in S$  (Pozrikidis, 1992; Blake, 1971; Higdon, 1985). Intuitively equation 2 represents a sum of point forces weighted so that the boundary conditions are satisfied. It is these weights that are the unknowns.

To solve for the unknowns the cell surface is discretised into  $n$  triangles (figure 2). The surface is defined by:

$$z = \begin{cases} H \frac{(R^2 - r^2)}{R^2} & \text{when } r^2 < R^2 \\ 0 & \text{otherwise} \end{cases} \quad (3)$$

where  $H$  is the maximum height of the EC,  $R = 20 \mu$  is the typical radius of an approximately circular (in the  $x - y$  plane) EC and  $r^2 = ax^2 + by^2$ . Unless otherwise noted,  $a = b = 1$ , figure 2. It is supposed that the EC is not adapted to flow, and the height of the EC is taken to be  $H = 6 \mu\text{m}$ . Depending on conditions, and EC type, ECs vary in height from 1-12  $\mu\text{m}$  (Wee et al., 2009; Barbee et al., 1994).

To facilitate the solution of a system of partial differential equations (to model reaction-diffusion), the discretization is chosen such that the projection of the surface nodes onto the plane  $z = 0$  gives a hexagonal lattice, with each hexagon composed of four of the surface element triangles. In the following results the area of the hexagons is  $1.66 \mu\text{m}^2$ , corresponding to a side length of  $0.8 \mu\text{m}$ . Equation 2 is solved by applying the no-slip boundary condition on  $S$ ;  $\mathbf{u}(\mathbf{x}_0) = 0$  if  $\mathbf{x}_0 \in S$ . A linear problem for the unknown surface force can be formed by applying the discrete version of equation 2 at  $n$  points on  $S$ ,  $\mathbf{x}_0$ . The surface force is assumed constant over surface elements. The linear problem for the  $f_i(\mathbf{x}_0)$  is solved by an iterative Krylov subspace method (Saad, 1981). The numerical method was validated on a hemisphere surface (in contrast to the surface described by equation 3) and compared to a known approximate analytical solution for the non-dimensional total force on this surface due to shear flow ( $F = 4.30\pi$ , (Price, 1985)) and was found to be accurate up to an error of 1.7% for 3000 surface elements.

## 2.1 Results

All parameters are given in table 1. Figure 3 gives the pattern of shear stress on the cell surface as a result of flow in a large artery. Under these conditions the maximum shear stress is  $10 \text{ dynes cm}^{-2}$ , which is similar to the wall shear stress calculated in *in vitro* flow chamber experiments (Wojciak-Stothard and Ridley, 2003; Tzima et al., 2001, 2002).

It is evident that the force component normal to the surface of the cell could give rise to the spatial heterogeneity via separate regions of tension and compression (figure 4). For a symmetric cell, the tangential surface component is symmetrical with respect to the upstream and downstream points of the cell (figure 3). Although the surface component could be crucial for signalling via ion channels, this signalling would (assuming uniform distributions of channels) initially lead to global changes in the cell.

## 3 Force Transduction & GTPase signalling

The force on the cell surface is coupled to the network (figure 7), which is modelled below, by treating integrins as a Kelvin body, figure 5. Previously, Ferko et al. (2007) showed that heterogeneity in intra-cellular could play an important role in the sub-cellular localization of mechano-transduction. However, here we make the simplifying assumptions that the cytosol/cytoskeletal medium is uniform and that force is transmitted to the basal surface locally. Both shear and normal forces are borne by cytoskeletal elements and, potentially, could activate mechano-transducers either at the basal surface or at ion channels in the membrane. However, the hypothesis we aim to test is whether a response to the force normal to the surface could be responsible for breaking the symmetry in the upstream and downstream signaling regions. Hence, to test whether the magnitude of the normal force could stretch a mechano-transducer by a biologically relevant amount we coupled the Kelvin body model, figure 5, to the force due to fluid flow.

The equation governing the Kelvin body (Mazzag et al., 2003), given that the force is constant, is

$$\nu \left( 1 + \frac{h_1}{h_2} \right) \frac{dz_e}{dt} = h_1 z_e - F \quad (4)$$

where  $F$  is the force on the body and  $z_e$  is its extension. Only tensile forces are considered and, to simplify the model, a smooth concentration of integrins is assumed; that is, clustering is ignored. Interestingly, Ferko et al. (2007) illustrated that focal adhesions focus and amplify shear forces on the apical surfaces of ECs. Hence, the coarse grained approach applied here provides a

conservative estimate for the force applied at the basal surface. The parameters for modelling integrins as a Kelvin body have been fitted to experimental data (Bausch et al., 1999). The normal force on the cell surface is of the order of pN, leading to an extension of the mechano-transducer in the order of nanometres, figure 6.

This model is coupled to a model of Rho GTPase interaction, figure 7, by up-regulation of Src activation, figure 8. The coupling, between extension ( $z_e$ ) and the biochemical network (figure 7) is via the parameter  $k_+(z_e)$ , which -in the model- is the rate of Src activation. Src has been shown to be activated (either directly or indirectly) by both shear stress and applied mechanical force (Wang et al., 2005b; Jalali et al., 1998; Fleming et al., 2005). Furthermore, there is evidence that stretch activation exhibits behaviour similar to figure 8. For example, in response to mechanical stretching PECAM-1 has switch like, saturating, activation (Chiu et al., 2008). However, for quantitative parameter estimation for the Src activation coupling (figure 8) it was necessary to use a course fitting to other experimental data, see appendix.

In the model, the resulting Src activation activates the Rho GTPase Rac1. Rho GTPases have previously been modelled in in a variety of contexts (Jilkin et al., 2007; Goryachev and Pokhilko, 2006; Sakumura et al., 2005; Marè et al., 2006). Civelekoglu-Scholey et al. (2005) modelled GTPase activation in this context, but with broader scope and less details of the activation of Rho GTPases themselves.

There is no feedback in this system so once activated there is no return to basal values. The reactions governing the network in figure 7 are:



GEFs are specific for either Rho or Rac but not both (Rossman et al., 2005; Tcherkezian and Lamarche-Vane, 2007); in reality this need not be the case. The effect of this additional level of coupling is a direction of future research.

The PDEs were integrated on a 2-D projection of the cell surface mesh. This projection was designed so that the projected mesh defined a hexagonal grid. The PDEs were integrated on this grid by a simple Euler method with the spatial derivative approximated by central differences. On the time-scale of interest it was assumed that the cell was impermeable. Hence, no flux boundary conditions were implemented using reflective conditions at the cell edge.

It was assumed that the transmission of the force on the cell due to fluid flow is fast relative to Src activation. Hence, the mechano-transducer extension (figure 6) is taken to occur instantaneously. This extension (predicted by the flow model coupled with the mechano-transduction model) leads to Src activation and is the input signal to the reaction network, figure 7.

### 3.1 Results

Applying these equations on the cell (which is now a 2-D projection) gives localised Src and Rac activation in the downstream edge, figure 9. Using the derived parameters (see appendix) the time-course of Src activation is comparable to previously reported (Fleming et al., 2005; Jalali et al., 1998). The whole cell response for Rac, figure 9(d), is slightly less than reported (Tzima et al., 2002). However, the model predicts maximal Rac activation within 25 minutes, which is comparable to the observed response (Tzima et al., 2002). Interestingly, the rates of  $\text{GEF}_R$  activation due to active Src reach new steady states relatively quickly, and it is actually the rates of GAP and GEF catalysed GDP-GTP exchange which are the rate limiting ones. The pattern of Rac activation, figure 9(b), is generated by localised Src activation; however it is maintained due to mutual Rho and Rac inhibition (though with removal of this cross-talk a similar, albeit less distinct, pattern as shown in figure 9(b) occurs - not shown) and slow diffusion of the active membrane bound RacGTP. It is also crucial that the RacGDP-GEF intermediary is membrane bound. Figure 10 shows the same equations solved whilst taking this intermediary as diffusing both on the membrane and in the cytosol. With this higher diffusion rate, even of this relatively short-lived intermediary, the Rac activation pattern is destroyed, figure 10(a), whereas the whole cell measure of Rac activation, figure 10(b), is broadly similar to figure 9(d). Whole cell profiles of activation therefore characterise the system poorly, and thus to test and generate hypotheses of EC responses to fluid flow it is necessary to have experimental data that quantifies local activation of proteins.

Previous experimental work has shown activation of Rac and inhibition of Rho returning to basal levels after an extended period of time (Tzima et al., 2001, 2002; Wojciak-Stothard and Ridley, 2003). In this current model there is no feedback, so the new steady states are maintained. However it is not necessarily the case that there must be direct biochemical feedback. It is possible that the change in morphology itself is enough to damp the system back to basal levels, i.e. as the cell polarises the signal from the flow is attenuated. After long term exposure to shear-stress ECs flatten to approximately 50% of their original height, which reduces the total and maximum shear on their surface (Barbee et al. (1994)). This could switch off the signalling network. However a cell elongated (and flattened) in one direction would not align with the flow in a different direction if this was the only effect. The EC orientation may play an important role in modulating the signal. Cells orientated perpendicular ( $a = 1/3$  and  $b = 3$ , equation 3) to the flow have a higher response (as measured by Rac-GTP) than parallel ( $a = 3$  and  $b = 1/3$ , equation 3) and symmetric cells (Figure 11). This effect may be amplified by clustering of integrins: if they are localised in FA then the location of FAs with respect to the up and downstream regions of the cell could be important.

Figure 11(d) shows Rho inhibition across the whole cell: the concentration distribution of Rho-GTP is more homogenous (not shown) than the Rac-GTP distribution because Src activates  $GAP_{\rho}$  which can diffuse relatively quickly through the cell. Some experiments report rapid transient activation of Rho (Wojciak-Stothard and Ridley, 2003), but this could be through a different pathway (either via integrins or a different mechano-transduction mechanism).

#### 4 Discussion

Exactly how ECs interpret the force due to fluid flow and how the components of the process interact remains an open question. Here, coupling models of fluid flow, mechano-transduction and Rho GTPase activation we have tested a plausible mechanism of interpreting the flow by a mechano-transducer and the downstream signal this would induce in the Rho GTPases RhoA and Rac1. The strength of this work is the coupling of the multi-scale models from fluid flow through to localized biochemical signaling.

The hypothesis presented here is that there is a mechano-transducer that is sensitive to the force normal to surface. We suggest that this mechano-transducer is activated in a region localized near the applied force, and tested whether this could lead to spatial localization of Rac1 activity (i.e. given the pattern of normal force and diffusion of some key components of the signalling network, can local activation of Rac1 be initiated). If our hypothesis had failed using this model then we would also expect it to fail on inclusion of a more

complete consideration of cytoskeletal biomechanics and shear force.

The mechano-transduction model is a simplified first approximation and only one component of the force was considered. This model could be developed to allow force in any direction. However, to do so would require a more sophisticated treatment of cytoskeletal mechanics- which was beyond the scope of this work. This would be a particularly interesting future direction; the heterogeneity, and complexity, of cellular structure may lead to unexpected conclusions regarding the transmission of force through ECs. For example, more realistic models of large focal adhesions in discrete locations, rather than the coarse grained continuous adhesions assumed here, could potentially lead to distinct conclusions.

This work implies that the heterogeneity of the force normal to the cell surface could give rise to the polarized signalling response. It has previously been suggested that the normal force could play an important role in EC signalling (Wang and Dimitrakopoulos, 2006a,b). Response to the normal force may be a necessary component of the process of EC polarisation and alignment, although it is probably not sufficient. It is probable that mechano-transduction via other pathways could supplement the model here. For example, it is possible that the ion channels could either respond to the normal component of the force in some manner, perhaps due to cytoskeleton coupling (Barakat et al., 2006), or are pre-oriented asymmetrically. Precisely how ECs transduce and respond to a mechanical force is unclear. However, modelling has also shown if cells respond to the normal force then a change in the morphology of the cell itself may attenuate signalling. In particular, the orientation of cells with respect to the cell can, potentially, lead to very different signalling. This suggests a plausible mechanism for cells sensing the direction of the flow and whether, given their current morphology, they should respond to the flow.

Here, a plausible mechanism for signal transduced is presented. Src is taken as a read-out of FA signalling, but it should be noted that it is likely that there are many intermediaries linking FA signalling and Rho GTPase activity. Hence, further work is required to establish, either computationally or experimentally, the complete nature of FA signalling in response to fluid flow. Components of integrin complexes are known to respond to force. Although the exact nature of the mechano-transduction is unclear.

Src, a tyrosine kinase associated with integrins, was taken as a biochemical measure of EC response to force and hypothesised to link directly to Rac GEF and Rho GAP activation. This hypothesis was tested with a simple model of Rho GTPase cross-talk and cycling (between GTP- and GDP-bound forms). Modelling showed that this hypothesis could lead to GTPase activation comparable to the reported levels for whole cell averages and qualitatively similar to localized regions of Rac activation.

It is evident that the Rho GTPases make excellent candidates for triggering local cytoskeleton events. Acting as switches they can be highly regulated by GEFs and GAPs and, furthermore, their association with the plasma membrane (once activated) means that the activation is kept in locally distinct areas due to slow diffusion. Thus to generate localised patterns of Rho GTPase activation, it seems that either a bistability is established or that it is the GEF that is activated rather than a GAP inactivated. In the latter case the faster diffusing GAP will result in activation being a more global phenomenon, similar to figure 10. The mechanism presented here is similar to a more general suggestion for local activation of GTPases (Bement et al., 2006). It is also demonstrated that measures of activation across the whole cell can not distinguish between different hypotheses; more local measures of protein activity in response to fluid flow are required to make such a determination.

In principle this hypothesis could be valid in an alternative form with a different read out from integrin complex signalling and a different link between GEFs and GAPs and the mechano-transducer. However, it does seem clear that the normal component of the force on the EC must play a role in initiating the heterogeneity in signalling, however the model here could be augmented by different pathways resulting from either integrin, ion channel or cell junction mechano-transduction.

## Appendix

See the table below for the parameters used in this work.

### *Flow Parameters*

The shear rate is estimated by modelling the flow for a steady Newtonian fluid in a large cylinder as a Poiseuille flow:

$$\mathbf{u}(r) = V(r)\hat{\mathbf{x}}, \quad V(r) = \frac{2Q}{\pi a^4}(a^2 - r^2) \quad (6)$$

where  $\mathbf{u}(r)$  is the flow velocity at  $r$ ,  $\hat{\mathbf{x}}$  is a unit vector along the cylinder,  $a$  is the radius of the cylinder and  $r$  is the distance from the centre of the cylinder and  $Q$  is the volumetric flow rate. The mean volumetric flow rate through the common carotid artery (CCA) has been measured as approximately  $6 \text{ ml s}^{-1}$  (Marshall et al., 2004), and the inner radius as  $3 \text{ mm}$  (Polak et al., 1996). Equation 6 is used to estimate  $\mathbf{u}$  at  $6 \mu\text{m}$ ; approximately the height of an endothelial cell. The shear rate  $k$  is found by comparing this solution with a simpler model for the flow,  $\mathbf{u}(\mathbf{x}) = kz\hat{\mathbf{x}}$ , simple shear flow, which we assume

is a good approximation near the arterial wall. The viscosity of the fluid was taken to be 1cP.

### *GTPase Model Parameters*

Parameters were found from (in order of preference) experimental data, from previous modelling work or were estimated.

For the force activation of Src, reaction 5, the parameters were estimated by comparison with an experimental time course (Jalali et al., 1998) for Src activation due to shear stress. The form of  $k_+(z_e)$  was taken as  $k_+(z_e) = k_+ + k_m/(1 + e^{10-z_e})$ . To estimate  $k_+$ ,  $k_m$  and  $k_-$  the level of Src was assumed to be in a steady state  $S$  (in the absence of diffusion):

$$S = \frac{k_+ + \frac{k_m}{1+e^{10-z_e}}}{k_+ + \frac{k_m}{1+e^{10-z_e}} + k_-}$$

By integrating this function over a square (as an approximation to the cell) and assuming a linear relationship (in the downstream half of the cell) between  $z_e$  and the distance from the centre of the cell an expression for the fold increase in Src can be reached. Comparing this with the experimental observations (Jalali et al., 1998; Fleming et al., 2005) of a characteristic 2-5 fold increase in activated Src leads to a relationship between  $k_+$ ,  $k_m$  and  $k_-$ . A second relationship can be drawn by comparing the order of activation of Src in the model with experimental data. Solving the ODE for reaction 5, gives a characteristic time for Src to be maximally activated; the solution has a  $-D.exp(-(k_+ + k_m + k_-)t)$  term, so as  $t \rightarrow \infty$  the activated Src level approaches its maximum. This maximum is observed within 4-10 minutes, hence  $(k_+ + k_m + k_-)t$  must be large for  $t$  in this range. To finalise the parameter values a third relationship is assumed, following previous authors (Fuß et al., 2007): initially the steady state level of activated Src is  $\approx 1\%$  of the total, so  $100k_+ = k_-$ . These relationships are sufficient to estimate  $k_+ = 0.00006s^{-1}$ ,  $k_- = 0.006s^{-1}$  and  $k_m = 0.0147s^{-1}$ .

The parameters for the Kelvin body, figure 5, were taken from previously reported modelling and experimental work (Mazzag et al., 2003; Bausch et al., 1999). Note that these parameters were derived by attaching a microbead to the cell surface, with an estimated area of approximately  $6 \mu m^2$  attached to the cell (Bausch et al., 1998). Hence, to correctly apply these parameters to the Kelvin body the  $f_i$  (note that this is a force per area, equation 2) have to be scaled by  $6 \mu m^2$ .

It was found that the GAP and GEF activation rates were not the rate limiting steps in network, figure 7. However, the rate-limiting step of conversion from

the GDP- to GTP-bound form of Rac is dependent on GEF activity. Hence, provided the GEF activation rate was sufficiently fast then the rate-limiting Rac activation could be initiated early enough so that it occurred on a similar time scale to observed (Tzima et al., 2002). Hence, it was necessary to have the Rac GEF activating quickly, on the order of a minute. Similarly, the Rho GAP was also required to become activated quickly. These criterion determined the rates for the Rho GAP, and Rac GEF. Similar rates were taken for the corresponding Rac GAP and Rho GEF. However, they were altered slightly so that there were similar basal levels of the active form (otherwise the basal level of Src gives a bias to the Rho GAP and the Rac GEF). Rates were finally fixed by demanding a low ratio initially of active to inactive forms, and by (following the onset of flow) demanding the new level of active GAP or GEF was significantly less than the total (to avoid saturation). Initial total concentration of Rac and Rho (either active, inactive or bound in intermediary complexes) was taken to be  $10 \mu M$  of each, which is a typical cellular amount (Jilkin et al., 2007; Goryachev and Pokhilko, 2006). Total concentration of RacGAP and RhoGAP, RhoGEF and RacGEF was taken to be  $1 \mu M$ ,  $1 \mu M$ ,  $2 \mu M$  and  $2 \mu M$ ; with corresponding (initial) active concentrations  $0.46 \mu M$ ,  $0.75 \mu M$ ,  $0.41 \mu M$  and  $0.24 \mu M$ .

The parameters governing GTPase cycling between GTP and GDP bound states were found to be the most significant in governing the response; fortunately experimental data was available for this data (Goryachev and Pokhilko, 2006; Zhang et al., 1997, 2000).

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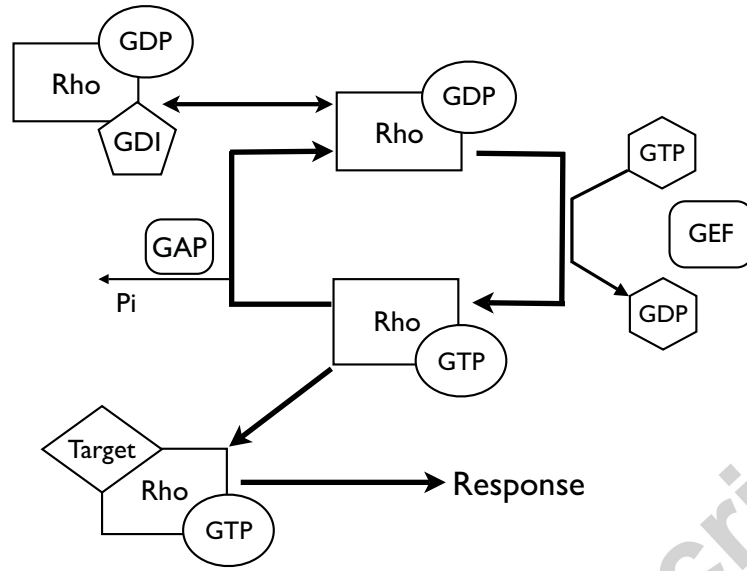


Fig. 1. Rho GTPase regulation by GAPs, GEFs and GDIs. In the inactive state (GDP bound) the GTPase interaction with the plasma membrane is inhibited by interaction with a GDI, which in turn prevents activation. The GEF removes GDP from the GTPase. Subsequent binding of GTP occurs quickly (because of the high ratio of GTP to GDP). The rate of hydrolysis of RhoGTP is increased by binding of a GAP (DerMardirossian and Bokoch, 2005; Bos et al., 2007).

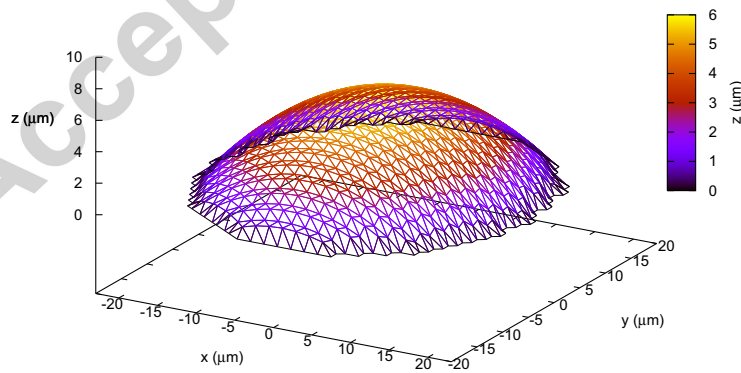


Fig. 2. An individual cell's (attached to a plane at  $z = 0$ ) surface discretised into a triangle mesh, such that the projection on the  $x - y$  plane of the mesh nodes gives the vertices of hexagons. The fluid flow is in the positive  $x$  direction:  $\mathbf{u} = (kz, 0, 0)$ ,  $z \geq 0$ .

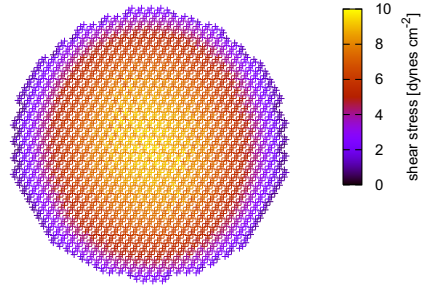


Fig. 3. Shear stress on the cell in figure 2 (projected on the plane  $z = 0$ ) and subjected to laminar fluid flow (left to right). Note that the shear is symmetrical with respect to the upstream and downstream edges.

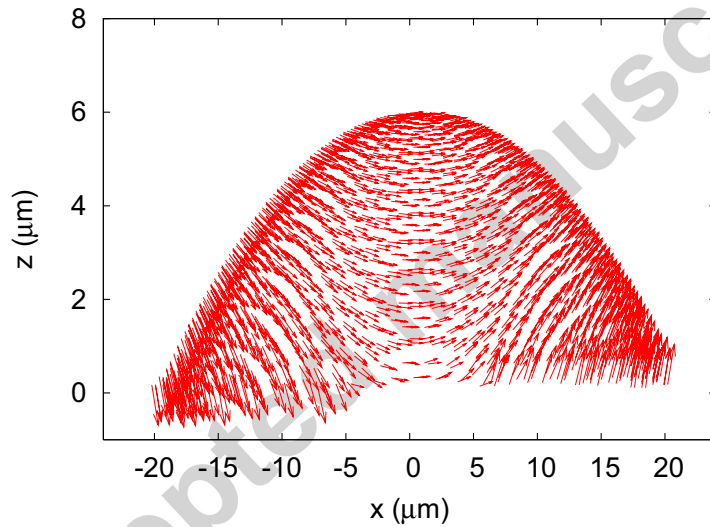


Fig. 4. Unit force,  $\hat{f}$ , on the surface of the cell (as in figures 2 and 3) due to laminar fluid flow (left to right). Note that the upstream edge of the cell is in compression and the downstream edge is under tension.

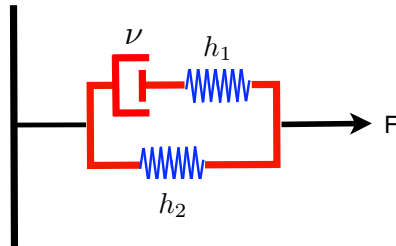


Fig. 5. Kelvin body model of integrin deformation under a force  $F$ , with spring constants  $h_1$  and  $h_2$  and dash-pot viscosity  $\mu$ .



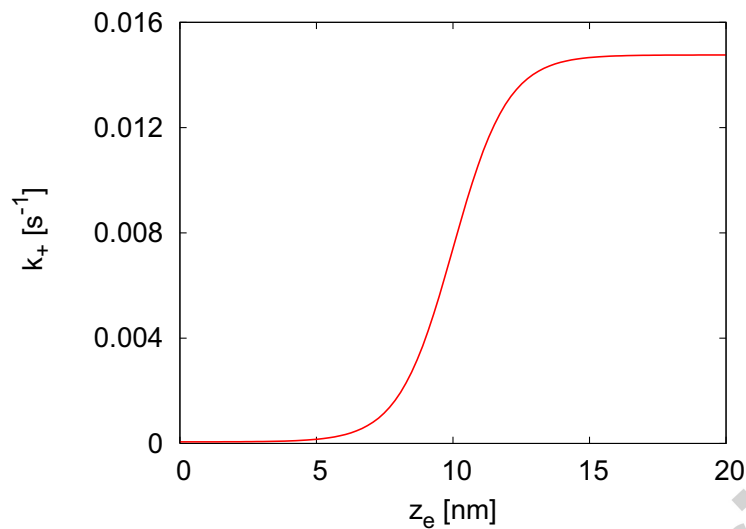


Fig. 8. Up-regulation of the forward Src activation rate  $k_+(z_e)$ , equation 5, as a function of mechano-transducer extension  $z_e$ :  $k_+(z_e) = 0.0006 + 0.0147 / (1 + e^{(k_e - z_e)})$ , where  $k_e = 10$  nm.

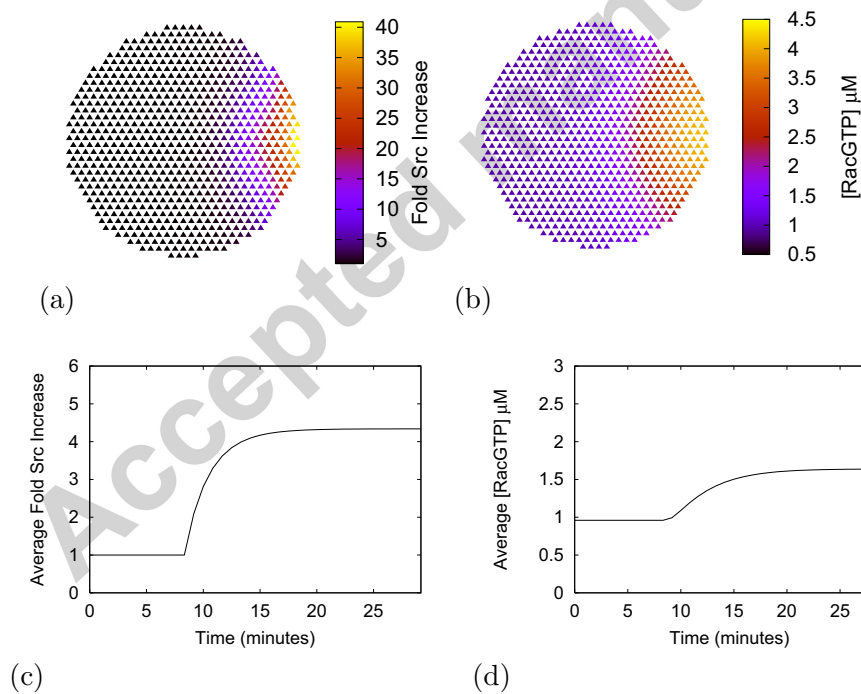


Fig. 9. Src and Rac activation due to fluid flow (left to right) as generated by the model equations (5). (a) Src activation on a 2-D projection of the cell (figure 2) according to the Kelvin body model coupled to reaction 5 via figure 8. (b) Subsequent RacGTP concentration distribution in the cell. (c) Fold Src increase over the whole cell. This is fixed by parameter estimation (Appendix) to give a similar response as found experimentally (Fleming et al., 2005; Jalali et al., 1998). (d) Average RacGTP concentration over the whole cell.

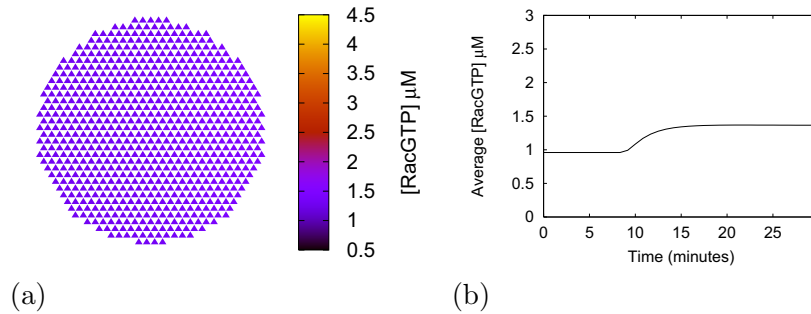


Fig. 10. The effect of allowing the RacGDP-GEF intermediary (equations 5) to diffuse in the cytosol and on the membrane as opposed to just on the membrane as in figure 9(b). A localised pattern of activation is not generated, though the average [RacGTP] is similar as in 9(d). (a) Concentration distribution of RacGTP, (b) Average RacGTP concentration over the whole cell.

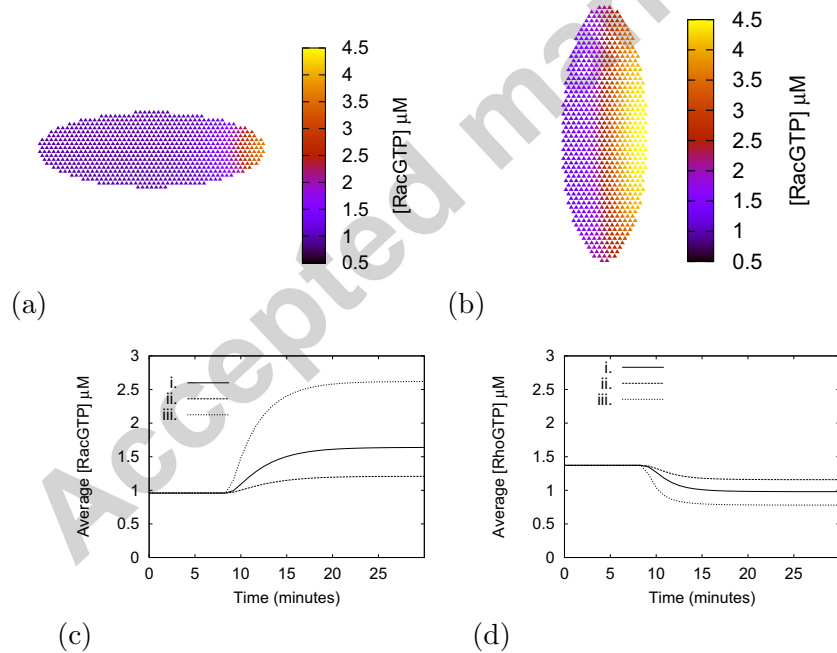


Fig. 11. Rac activation due to fluid flow (left to right in (a) and (b)) as generated by the reactions (5) for different cell morphologies with the same volume and maximum height as figure 9(b). (a) RacGTP concentration for a cell already aligned with the flow. (b) RacGTP concentration distribution for a cell aligned perpendicular to the flow. (c) and (d) mean RacGTP and RhoGTP concentrations (respectively) over the whole cells: i. cell shape as in 9(b), ii. 11(a), iii. 11(b)

Parameter	Description	Value	Units	Source
$D_m$	Membrane diffusion	0.1	$\mu m^2 s^{-1}$	Marèe 2006
$D_{mc}$	Membrane and cytosol diffusion	10	$\mu m^2 s^{-1}$	Marèe 2006
$k$	Shear rate	280	$s^{-1}$	See Appendix
$\mu$	Fluid viscosity	$1 \times 10^{-6}$	$g \mu m^{-1} s^{-1}$	See Appendix
$\nu$	Dashpot viscosity	$6.33 \times 10^{-5}$	Pa m s	Mazzag 2003
$h_1$	Spring constant	$1.25 \times 10^{-3}$	Pa m	Mazzag 2003
$h_2$	Spring constant	$1.61 \times 10^{-3}$	Pa m	Mazzag 2003
$k_+(z_e)$	Extension dependent Src activation	fig 8	$s^{-1}$	See Appendix
$k_-$	Src inactivation rate	0.006	$s^{-1}$	See Appendix
$k_1$	GEF activation	0.83	$\mu M^{-1} s^{-1}$	See Appendix
$k_2$	GEF inactivation	0	$\mu M^{-1} s^{-1}$	See Appendix
$k_3$	Rac GEF binding rate	0.0034	$\mu M^{-1} s^{-1}$	B. Zhang, 2000
$k_4$	Rac GEF unbinding rate	$1.1 \times 10^{-6}$	$s^{-1}$	B. Zhang, 2000
$k_5$	Rac GDP disassociation	0.094	$s^{-1}$	B. Zhang, 2000
$k_6$ ,	Rac GAP binding	0.017	$\mu M^{-1} s^{-1}$	Goraychev, 2006
$k_7$	Rac GAP unbinding	0.05	$s^{-1}$	Goraychev, 2006
$k_8$	RacGTP GAP hydrolysis	8.3	$s^{-1}$	Goraychev, 2006
$k_9$	Rac GAP activation	0.001	$\mu M^{-1} s^{-1}$	See Appendix
$k_{10}$	Rac GAP inactivation	0	$\mu M^{-1} s^{-1}$	See Appendix
$k_{11}$	Rho GAP activation	0.8	$\mu M^{-1} s^{-1}$	See Appendix
$k_{12}$	Rho GAP inactivation	0	$\mu M^{-1} s^{-1}$	See Appendix
$k_{13}$	Rho GAP binding	0.017	$\mu M^{-1} s^{-1}$	= $k_6$
$k_{14}$	Rho GAP unbinding	0.05	$\mu s^{-1}$	= $k_7$
$k_{15}$	RhoGTP GAP hydrolysis	8.3	$\mu s^{-1}$	= $k_8$
$k_{16}$	Rho GEF binding rate	0.0034	$\mu M^{-1} s^{-1}$	= $k_3$
$k_{17}$	Rho GEF unbinding rate	$1.1 \times 10^{-6}$	$s^{-1}$	= $k_4$
$k_{18}$	Rho GDP disassociation rate	0.094	$s^{-1}$	= $k_5$
$k_{19}$	Rho GEF activation	0	$\mu M^{-1} s^{-1}$	See Appendix
$k_{20}$	Rho GEF inactivation	0.0001	$\mu M^{-1} s^{-1}$	See Appendix
$k_{21}$	basal Rho GAP activation	0.002	$s^{-1}$	See Appendix
$k_{22}$	basal Rho GAP inactivation	0.009	$s^{-1}$	See Appendix
$k_{23}$	basal Rac GEF activation	0.00045	$s^{-1}$	See Appendix
$k_{24}$	basal Rac GEF inactivation	0.09	$s^{-1}$	See Appendix
$k_{25}$	basal Rac GAP activation	0.002	$s^{-1}$	See Appendix
$k_{26}$	basal Rac GAP inactivation	0.004	$s^{-1}$	See Appendix
$k_{27}$	basal Rho GEF activation	0.00045	$s^{-1}$	See Appendix
$k_{28}$	basal Rho GEF inactivation	0.015	$s^{-1}$	See Appendix

Table 1

Model Parameters. See references for accuracy in parameter values. Parameters estimated in the appendix are determined up to an order of magnitude.

## Highlights

1. We develop a multi-scale model of the endothelial response to fluid flow.
2. We couple models of fluid flow, mechano-transduction and Rho GTPase signaling.
3. We hypothesize that the normal force on the cell surface establishes signaling polarity.
4. We show that signaling can be attenuated by cell morphologies aligned with the direction of fluid flow.
5. Conversion of Rac-GDP to Rac-GTP is likely to be the rate limiting step in Rac activation.

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