

organization and dynamics of the actin cytoskeleton Rho GTPases: molecular switches that control the

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Rho GTPases: molecular switches that control

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The organization and dynamics of the actin
exterkaleter **cytoskeleton**

Alan Hall1,2* **and Catherine D. Nobes**³

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Gover Street London WCIE GRT LIK ratory for Molecular Cell Biology, ¹Cancer Res
ment of Biochemistry, and ³Department of An
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The actin cytoskeleton plays a fundamental role in all eukaryotic cells—it is a major determinant of cell

morphology and polarity and the assembly and disassembly of filamentous actin structures provides a The actin cytoskeleton plays a fundamental role in all eukaryotic cells—it is a major determinant of cell
morphology and polarity and the assembly and disassembly of filamentous actin structures provides a
driving force fo morphology and polarity and the assembly and disassembly of filamentous actin structures provides a
driving force for dynamic processes such as cell motility, phagocytosis, growth cone guidance and
cytokinesis. The ability driving force for dynamic processes such as cell motility, phagocytosis, growth cone guidance and
cytokinesis. The ability to reorganize actin filaments is a fundamental property of embryonic cells during
development; the cytokinesis. The ability to reorganize actin filaments is a fundamental property of embryonic cells during
development; the shape changes accompanying gastrulation and dorsal closure, for example, are
dependent on the plas development; the shape changes accompanying gastrulation and dorsal closure, for example, are dependent on the plasticity of the actin cytoskeleton, while the ability of cells or cell extensions, such as axons, to migrate dependent on the plasticity of the actin cytoskeleton, while the ability of cells or cell extensions, such as
axons, to migrate within the developing embryo requires rapid and spatially organized changes to the
actin cytos axons, to migrate within the developing embryo requires rapid and spatially organized changes to the actin cytoskeleton in response to the external environment. Work in mammalian cells over the last decade has demonstrated actin cytoskeleton in response to the external environment. Work in mammalian cells over the last decade
has demonstrated the central role played by the highly conserved Rho family of small GTPases in signal
transduction p of played by the highly conserved Kho family
k plasma membrane receptors to the organization
Keywords: GTPase; actin; Rho; migration

1. INTRODUCTION

The mammalian Rho family of small GTPases currently comprises eight distinct members: Rho (A, B, C isoforms), The mammalian Rho family of small GTPases currently
comprises eight distinct members: Rho (A, B, C isoforms),
Rac (1, 2, 3 isoforms), Cdc42 (G25K, Cdc42Hs isoforms),
RhoD, RhoG, TCl0, Rnd (Rndl, RhoE/Rnd3, Rnd6) comprises eight distinct members: Rho (A, B, C isoforms),
Rac (1, 2, 3 isoforms), Cdc42 (G25K, Cdc42Hs isoforms),
RhoD, RhoG, TCl0, Rnd (Rnd1, RhoE/Rnd3, Rnd6)
and TTE which share around 50–55% identity to each Rac (1, 2, 3 isoforms), Cdc42 (G25K, Cdc42Hs isoforms),
RhoD, RhoG, TCl0, Rnd (Rndl, RhoE/Rnd3, Rnd6)
and TTF, which share around 50-55% identity to each
other. The consequences of activating Rho, Rac, and RhoD, RhoG, TCl0, Rnd (Rndl, RhoE/Rnd3, Rnd6)
and TTF, which share around 50–55% identity to each
other. The consequences of activating Rho, Rac and
Cdc49 in Swiss 3T3 fibroblasts have been well characterand TTF, which share around 50–55% identity to each
other. The consequences of activating Rho, Rac and
Cdc42 in Swiss 3T3 fibroblasts have been well character-
ized. Rho regulates the assembly of actin stress fibres other. The consequences of activating Rho, Rac and Cdc42 in Swiss 3T3 fibroblasts have been well character-
ized. Rho regulates the assembly of actin stress fibres
(figure 1) Rac regulates the polymerization of actin at th Cdc42 in Swiss 3T3 fibroblasts have been well character-
ized. Rho regulates the assembly of actin stress fibres
(figure 1), Rac regulates the polymerization of actin at the
cell periphery to produce lamellipodia and membr ized. Rho regulates the assembly of actin stress fibres
(figure 1), Rac regulates the polymerization of actin at the
cell periphery to produce lamellipodia and membrane
ruffles while Cdc42 triggers filopodia formation (Rid (figure 1), Rac regulates the polymerization of actin at the cell periphery to produce lamellipodia and membrane ruffles, while Cdc42 triggers filopodia formation (Ridley $\&$ Hall 1992: Ridley $\&$ Hall 1995: cell periphery to produce lamellipodia and membrane
ruffles, while Cdc42 triggers filopodia formation (Ridley
& Hall 1992; Ridley *et al.* 1992; Nobes & Hall 1995; Kozma *et al*. 1995; Puls *et al*. 1999). In addition, all three & Hall 1992; Ridley *et al.* 1992; Nobes & Hall 1995; skeleton along with other cellular processes to promote Kozma *et al.* 1995; Puls *et al.* 1999). In addition, all three coordinated changes in cell behaviour (Hall 199 Kozma *et al.* 1995; Puls *et al.* 1999). In addition, all three GTPases regulate the formation of cell-matrix adhesion sites called focal adhesions, which are intimately associated with the actin structures (Nobes & Hall GTPases regulate the formation of cell–matrix adhesion
sites called focal adhesions, which are intimately
associated with the actin structures (Nobes & Hall 1995;
Hotchin & Hall 1995) Eurthermore the GTPases appear sites called focal adhesions, which are intimately
associated with the actin structures (Nobes & Hall 1995;
Hotchin & Hall 1995). Furthermore, the GTPases appear
to be linked to one another in a cascade: activation of associated with the actin structures (Nobes & Hall 1995;
Hotchin & Hall 1995). Furthermore, the GTPases appear
to be linked to one another in a cascade; activation of
Cdc49 for example leads to rapid localized activation o Hotchin & Hall 1995). Furthermore, the GTPases appear
to be linked to one another in a cascade; activation of
Cdc42, for example, leads to rapid localized activation of Rac (Nobes & Hall 1995). It has been suggested, there-Cdc42, for example, leads to rapid localized activation of Rac (Nobes & Hall 1995). It has been suggested, therefore, that these GTPases may be important regulators of cell movement in response to extracellular signals (Ha Rac (Nobes & Hall 1995). It has been suggested, there-
fore, that these GTPases may be important regulators of
cell movement in response to extracellular signals (Hall
1998: Lauffenburger & Horwitz 1996) fore, that these GTPases may be impor

cell movement in response to extracellu

1998; Lauffenburger & Horwitz 1996).

In addition to regulating the organiz Il movement in response to extracellular signals (Hall
98; Lauffenburger & Horwitz 1996).
In addition to regulating the organization of the actin
toskeleton. Rho-related GTPases regulate gene tran-

1998; Lauffenburger & Horwitz 1996).
In addition to regulating the organization of the actin
cytoskeleton, Rho-related GTPases regulate gene tran-
scription. Rac and Cdc42 have been shown in a variety In addition to regulating the organization of the actin
cytoskeleton, Rho-related GTPases regulate gene tran-
scription. Rac and Cdc42 have been shown, in a variety
of cell types, to activate the INK and p38 MAP kinase cytoskeleton, Rho-related GTPases regulate gene transcription. Rac and Cdc42 have been shown, in a variety of cell types, to activate the JNK and p38 MAP kinase pathways while Rho has been shown to activate the scription. Rac and Cdc42 have been shown, in a variety
of cell types, to activate the JNK and p38 MAP kinase
pathways, while Rho has been shown to activate the pathways, while Rho has been shown to activate the
*Author for correspondence (alan.hall@ucl.ac.uk).

serum response factor, through an unknown signal trans-
duction pathway (Coso et al. 1995: Minden et al. 1995 duction pathway (Coso *et al*. 1995; Minden *et al*. 1995; serum response factor, through an unknown signal trans-
duction pathway (Coso *et al.* 1995; Minden *et al.* 1995;
Hill *et al.* 1995). This dual role of Rho/Rac/Cdc42 in
regulating actin organization and gene transcriptio duction pathway (Coso *et al.* 1995; Minden *et al.* 1995; Hill *et al.* 1995). This dual role of $Rho/Rac/Cdc42$ in regulating actin organization and gene transcription Hill *et al.* 1995). This dual role of Rho/Rac/Cdc42 in regulating actin organization and gene transcription appears to be conserved in yeast and *Drosophila* (Leberer *et al.* 1997: Glise & Noselli 1997). A number of othe regulating actin organization and gene transcription
appears to be conserved in yeast and *Drosophila* (Leberer
et al. 1997; Glise & Noselli 1997). A number of other
cellular activities have been ascribed to these GTPase appears to be conserved in yeast and *Drosophila* (Leberer *et al.* 1997; Glise & Noselli 1997). A number of other cellular activities have been ascribed to these GTPases; R ho and R ac activity are required for the assem *et al.* 1997; Glise & Noselli 1997). A number of other cellular activities have been ascribed to these GTPases;
Rho and Rac activity are required for the assembly of cadherin-based cell-cell innetions in human enithelial cellular activities have been ascribed to these GTPases;
Rho and Rac activity are required for the assembly of
cadherin-based, cell–cell junctions in human epithelial
cells, while Rac requisites the NADPH oxidase enzyme Rho and Rac activity are required for the assembly of cadherin-based, cell-cell junctions in human epithelial cells, while Rac regulates the NADPH oxidase enzyme complex in phagocytic cells (Braga et al. 1997: Abo et al. cells, while Rac regulates the NADPH oxidase enzyme complex in phagocytic cells (Braga *et al.* 1997; Abo *et al.* 1991; Diekmann *et al.* 1994). It appears, therefore, that R ho GTPases regulate the organization of the ac complex in phagocytic cells (Braga *et al.* 1997; Abo *et al.* 1991; Diekmann *et al.* 1994). It appears, therefore, that Rho GTPases regulate the organization of the actin cyto-
skeleton along with other cellular process 1991; Diekmann *et al.* 1994). It appears, therefore, that Rho GTPases regulate the organization of the actin cytocoordinated changes in cell behaviour (Hall 1998).

2. RHO GTPASE SIGNALLING PATHWAYS

2. RHO GTPASE SIGNALLING PATHWAYS
Signalling through Rho GTPases can be initiated by
tivation of many different types of plasma membrane 2. KHO GTPASE SIGNALLING PATHWATS
Signalling through Rho GTPases can be initiated by
activation of many different types of plasma membrane
receptor including typesine kinase. G-protein-counled Signalling through Rho GTPases can be initiated by
activation of many different types of plasma membrane
receptor, including tyrosine kinase, G-protein-coupled
and cytokine receptors. It is believed that receptors are activation of many different types of plasma membrane
receptor, including tyrosine kinase, G-protein-coupled
and cytokine receptors. It is believed that receptors are
able to activate guanine nucleotide exchange factors receptor, including tyrosine kinase, G-protein-coupled
and cytokine receptors. It is believed that receptors are
able to activate guanine nucleotide exchange factors
(GEFs) that catalyze loss of GDP and allow hinding of and cytokine receptors. It is believed that receptors are
able to activate guanine nucleotide exchange factors
(GEFs) that catalyse loss of GDP and allow binding of
GTP but the biochemical mechanisms involved are still able to activate guanine nucleotide exchange factors (GEFs) that catalyse loss of GDP and allow binding of GTP, but the biochemical mechanisms involved are still (GEFs) that catalyse loss of GDP and allow binding of GTP, but the biochemical mechanisms involved are still poorly understood. More than 30 mammalian Rho GEFs have been identified to date and all are characterized by GTP, but the biochemical mechanisms involved are still
poorly understood. More than 30 mammalian Rho GEFs
have been identified to date and all are characterized by
the presence of a Dbl homology domain, which catalyses poorly understood. More than 30 mammalian Rho GEFs
have been identified to date and all are characterized by
the presence of a Dbl homology domain, which catalyses
the exchange reaction, followed immediately by a pleckhave been identified to date and all are characterized by the presence of a Dbl homology domain, which catalyses the exchange reaction, followed immediately by a pleckthe presence of a Dbl homology domain, which catalyses
the exchange reaction, followed immediately by a pleck-
strin homology domain. GEFs often contain other
protein-protein interaction motifs that vary between the exchange reaction, followed immediately by a pleck-
strin homology domain. GEFs often contain other
protein–protein interaction motifs that vary between
different family members (Cerione & Zheng 1996) strin homology domain. GEFs often contain other
protein–protein interaction motifs that vary between
different family members (Cerione & Zheng 1996).

Figure 1. Rho regulates the assembly of actin stress fibres induced by lysophosphatidic acid (LPA). (a) Quiescent, serum-starved
Swiss 3T3 fibroblasts have very little organized actin cytoskeleton as visualized by rhodamin Figure 1. Rho regulates the assembly of actin stress fibres induced by lysophosphatidic acid (LPA). *(a)* Quiescent, serum-s
Swiss 3T3 fibroblasts have very little organized actin cytoskeleton as visualized by rhodamine ph Swiss 3T3 fibroblasts have very little organized actin cytoskeleton as visualized by rhodamine phalloidin, but treatment with serum or LPA (*b*) induces actin:myosin stress fibres within 15 min. If cells are microinjected serum or LPA (b) induces actin:myosin stress fibres within 15 min. If cells are microinjected with the Rho inhibitor, C3
transferase, before LPA addition (c), stress fibre assembly is inhibited. This suggests that the Rho transferase, before LPA addition (c) , stress fibre assembly is inhibited. This suggests that the Rho GTPase regulates a signal
transduction pathway in these fibroblasts linking the LPA receptor to the assembly of actin s transduction pathway in these fibroblasts linking the LPA receptor to the assembly of actin stress fibres (Ridley & Hall 1992).
Using similar approaches (data not shown), it was established that platelet-derived growth fac Using similar approaches (data not shown), it was established that platelet-derived growth factor or insulin activates the Rac
GTPase in quiescent Swiss cells and that this leads to the assembly of actin filaments at the c ō and membrane ruffles (Ridley *et al.* 1992). Bradykinin, tumour necrosis factor- α and interleukin-1 activate Cdc42 in these cells leading to actin filament assembly at the periphery to produce filopodia (Nobes & Hall 19

Figure 2. Actin filament assembly. Monomeric (G-)actin is
sequestered in cells by monomer-sequestering proteins such as
thymosin and profilin. New actin filaments can be induced Figure 2. Actin ¢lament assembly. Monomeric (G-)actin is Figure 2. Actin filament assembly. Monomeric $(G-)$ actin is
sequestered in cells by monomer-sequestering proteins such as
thymosin and profilin. New actin filaments can be induced
 de new by nucleation of actin monomers or *de sequestered in cells by monomer-sequestering proteins such thymosin and profilin. New actin filaments can be induced the novo by nucleation of actin monomers or pre-exisiting filaments can be extended by uncapping of f* thymosin and profilin. New actin filaments can be induced $de novo$ by nucleation of actin monomers or pre-exisiting filaments can be extended by uncapping of filament ends or *de novo* by nucleation of actin monomers or pre-exisiting
filaments can be extended by uncapping of filament ends or
by creating new ends by severing. These activities appear to
occur at membranes and may be facilitated b filaments can be extended by uncapping of filament ends or
by creating new ends by severing. These activities appear to
occur at membranes and may be facilitated by lipids such as
phosphatidylinositol-4.5-bisphosphate. Act occur at membranes and may be facilitated by lipids such as phosphatidylinositol-4,5-bisphosphate. Actin filaments can be occur at membranes and may be facilitated by lipids suc
phosphatidylinositol-4,5-bisphosphate. Actin filaments corganized into higher-order structures by bundling or
cross-linking proteins. Cross-linking induced by myosin. phosphatidylinositol-4,5-bisphosphate. Actin filaments can b
organized into higher-order structures by bundling or
cross-linking proteins. Cross-linking induced by myosin II,
leads to the formation of contractile filaments organized into higher-order structures by bundling or
cross-linking proteins. Cross-linking induced by myosin II,
leads to the formation of contractile filaments that can affect
cell shape. It is possible that R ho GTPases cross-linking proteins. Cross-linking induced by myosin II,
leads to the formation of contractile filaments that can affect
cell shape. It is possible that Rho GTPases can regulate one,
or more likely several, of these ste \mathcal{L} cell shape. It is possible that Rho GTPases can regulate one,

Exchange assays done *in vitro* and *in vivo* suggest that some GEFs can potentially act on multiple GTPases, e.g. Vav on Cdc42, Rac and Rho (Olson *et al*. 1996), while others GEFs can potentially act on multiple GTPases, e.g. Vav
on Cdc42, Rac and Rho (Olson *et al.* 1996), while others
are more specific, e.g. lbc and p115RhoGEF act on Rho
(Hart *et al.* 1996). Tiam-1 acts on Rac (Michiels *et* on Cdc42, Rac and Rho (Olson *et al.* 1996), while others
are more specific, e.g. lbc and pl15RhoGEF act on Rho
(Hart *et al.* 1996), Tiam-1 acts on Rac (Michiels *et al.*
1995) and FGD1 acts on Cdc42 (Olson *et al.* 1996) (Hart *et al.* 1996), Tiam-1 acts on Rac (Michiels *et al.* 1995) and FGDI acts on Cdc42 (Olson *et al.* 1996). Once activated, Rho GTPases mediate their cellular

1995) and FGDI acts on Cdc42 (Olson *et al.* 1996).
Once activated, Rho GTPases mediate their cellular effects through interaction with target proteins and one of the surprises of the last few years has been the large Once activated, Rho GTPases mediate their cellular effects through interaction with target proteins and one of the surprises of the last few years has been the large number (over 20) of targets identified for Rho Rac an effects through interaction with target proteins and one of a response. In the example shown, Cdc42 is required to
the surprises of the last few years has been the large establish a polarized bud and Rho then acts to cause

Cdc42. Targets are defined as proteins that interact
directly with the GTP-bound but not the GDP-bound Cdc42. Targets are defined as proteins that interact
directly with the GTP-bound, but not the GDP-bound,
form of a GTPase and so far these include tyrosine Cdc42. Targets are defined as proteins that interact
directly with the GTP-bound, but not the GDP-bound,
form of a GTPase and so far these include tyrosine
kinases serine or threonine kinases phospholinases linid directly with the GTP-bound, but not the GDP-bound,
form of a GTPase and so far these include tyrosine
kinases, serine or threonine kinases, phospholipases, lipid
kinases and many scaffold-like or structural proteins form of a GTPase and so far these include tyrosine
kinases, serine or threonine kinases, phospholipases, lipid
kinases and many scaffold-like or structural proteins.
While there are data linking some of these molecules to kinases, serine or threonine kinases, phospholipases, lipid
kinases and many scaffold-like or structural proteins.
While there are data linking some of these molecules to kinases and many scaffold-like or structural proteins.
While there are data linking some of these molecules to
changes in the actin cytoskeleton (see figure 2), the roles
of many are unknown There are many excellent review While there are data linking some of these molecules to changes in the actin cytoskeleton (see figure 2), the roles of many are unknown. There are many excellent reviews on this tonic (e.g. Van Aelst & D'Souza-Schorey 199 changes in the actin cytoskeleton (see figure 2), the rol
of many are unknown. There are many excellent revies
on this topic (e.g. Van Aelst & D'Souza-Schorey 1997). **3. CELLULAR RESPONSES CONTROLLED BY RHO**

GTPASES

GELLULAR RESPONSES CONTROLLED BY RHO
GTPASES
Genetic analysis in yeast has revealed the complexity of
tivities associated with these proteins and while the GIFASES
Genetic analysis in yeast has revealed the complexity of
activities associated with these proteins and while the
hiochemical details of their function in yeast and Genetic analysis in yeast has revealed the complexity of
activities associated with these proteins and while the
biochemical details of their function in yeast and
mammalian cells may have diverged some of the general activities associated with these proteins and while the
biochemical details of their function in yeast and
mammalian cells may have diverged, some of the general
lessons learnt in yeast (see figure 3) are likely to be usef biochemical details of their function in yeast and
mammalian cells may have diverged, some of the general
lessons learnt in yeast (see figure 3) are likely to be useful
in thinking about Rho GTPase function in higher eukar mammalian cells may have diverged, some of the general
lessons learnt in yeast (see figure 3) are likely to be useful
in thinking about Rho GTPase function in higher eukary-
otes. The first is that a single GTPase can regu lessons learnt in yeast (see figure 3) are likely to be useful
in thinking about Rho GTPase function in higher eukary-
otes. The first is that a single GTPase can regulate in thinking about Rho GTPase function in higher eukary-
otes. The first is that a single GTPase can regulate
multiple pathways to promote a coordinated biological
response. In this case Rholn in weast has been shown to otes. The first is that a single GTPase can regulate
multiple pathways to promote a coordinated biological
response. In this case Rholp in yeast has been shown to
control the activity of at least three proteins each of multiple pathways to promote a coordinated biological
response. In this case Rholp in yeast has been shown to
control the activity of at least three proteins each of
which is required for bud growth. The large number of response. In this case Rholp in yeast has been shown to control the activity of at least three proteins each of which is required for bud growth. The large number of target proteins and functional activities reported for mammalian Rho GTPases suggests that this is also likely which is required for bud growth. The large number of target proteins and functional activities reported for mammalian Rho GTPases suggests that this is also likely to be true in higher eukaryotes, but to date there are fe target proteins and functional activities reported for mammalian Rho GTPases suggests that this is also likely
to be true in higher eukaryotes, but to date there are few,
if any, examples in a mammalian cell where a single Rho
GTPase has been shown to control two or more activ to be true in higher eukaryotes, but to date there are few,
if any, examples in a mammalian cell where a single Rho
GTPase has been shown to control two or more activities
that together contribute to a particular biologica if any, examples in a mammalian cell where a single Rho
GTPase has been shown to control two or more activities
that together contribute to a particular biological
response. The second lesson from yeast is that members of GTPase has been shown to control two or more activities
that together contribute to a particular biological
response. The second lesson from yeast is that members of that together contribute to a particular biological response. The second lesson from yeast is that members of
the Rho family can cooperate with each other to promote
a response. In the example shown, Cdc42 is required to
establish a polarized bud and Rho then acts to cause the Rho family can cooperate with each other to promote
a response. In the example shown, Cdc42 is required to
establish a polarized bud and Rho then acts to cause
growth of the bud Finally Ras and Rho GTPases growth of the bud. Finally, Ras and Rho GTPases

THE ROYA

e.g. (bud site selection) $\text{Bud1} + \text{Cdc42}$ (bud establishment)
Figure 3. General features of Rho GTPases in yeast. Genetic
analysis of Rho Land Cdc42 in yeast has led to a reasonably Figure 3. General features of Rho GTPases in yeast. Genetic
analysis of Rho1 and Cdc42 in yeast has led to a reasonably
clear picture of at least some of their biological roles. Three Figure 3. General features of Rho GTPases in yeast. Genetic
analysis of Rho1 and Cdc42 in yeast has led to a reasonably
clear picture of at least some of their biological roles. Three
general lessons have emerged that have analysis of Rho1 and Cdc42 in yeast has led to a reasonably
clear picture of at least some of their biological roles. Three
general lessons have emerged that have shaped some of our
thinking about the possible roles of the clear picture of at least some of their biological roles. Three
general lessons have emerged that have shaped some of our
thinking about the possible roles of these proteins in higher
eukaryotes: (i) Rho GTPases can regula thinking about the possible roles of these proteins in higher thinking about the possible roles of these proteins in higher
eukaryotes: (i) Rho GTPases can regulate multiple pathways
to promote a coordinated biological response. In the example
shown Rhol, which is required for bud gr eukaryotes: (i) Rho GTPases can regulate multiple pathway
to promote a coordinated biological response. In the exampl
shown, Rho1, which is required for bud growth, regulates
three pathways, a pkc1-dependent MAP kinase pat shown, Rho1, which is required for bud growth, regulates three pathways, a pkc1-dependent MAP kinase pathway
affecting gene transcription, an enzyme required for cell v
synthesis and a structural protein required for the correct affecting gene transcription, an enzyme required for cell wall affecting gene transcription, an enzyme required for cell wal
synthesis and a structural protein required for the correct
organization of the actin cytoskeleton. (ii) Different Rho
GTPases can cooperate with each other to synthesis and a structural protein required for the correc
organization of the actin cytoskeleton. (ii) Different Rho
GTPases can cooperate with each other to produce a
coordinated response. In the example shown, Cdc42 is organization of the actin cytoskeleton. (ii) Different Rho
GTPases can cooperate with each other to produce a
coordinated response. In the example shown, Cdc42 is
required for initiation of bud assembly at the correct site GTPases can cooperate with each other to produce a
coordinated response. In the example shown, Cdc42 is
required for initiation of bud assembly at the correct site,
while Rhol is then required for continued growth of the coordinated response. In the example shown, Cdc42 is
required for initiation of bud assembly at the correct site,
while Rho1 is then required for continued growth of the bud.
(iii) R ho GTPases can cooperate with R as GTPa required for initiation of bud assembly at the correct site,
while Rho1 is then required for continued growth of the bud.
(iii) Rho GTPases can cooperate with Ras GTPases. In the
example shown, Bud1, a Ras-like GTPase, is while Rho1 is then required for continued growth of the bud.
(iii) Rho GTPases can cooperate with Ras GTPases. In the
example shown, Bud1, a Ras-like GTPase, is required for the
selection of the bud site on the yeast surfa (iii) Rho GTPases can cooperate with Ras GTPases. In the example shown, Bud1, a Ras-like GTPase, is required for the selection of the bud site on the yeast surface. This then defines the sites at which Cdc42 subsequently a selection of the bud site on the yeast surface. This then defines
the sites at which Cdc42 subsequently acts.

cooperate with each other and again in the example of
cooperate with each other and again in the example of
hud formation. Budl (a Ras-like GTPase) is required for cooperate with each other and again in the example of
bud formation, Bud1 (a Ras-like GTPase) is required for
bud site selection, and this then defines the site at which cooperate with each other and again in the example of
bud formation, Budl (a Ras-like GTPase) is required for
bud site selection, and this then defines the site at which
Cdc42 acts. One of the aims of our laboratories is t bud formation, Budl (a Ras-like GTPase) is required for
bud site selection, and this then defines the site at which
Cdc42 acts. One of the aims of our laboratories is to
explore these possibilities in the context of a mamm bud site selection, and this then defines the site at which
Cdc42 acts. One of the aims of our laboratories is to
explore these possibilities in the context of a mammalian
cell cell. plore these possibilities in the context of a mammalian
II.
Since Rho GTPases clearly play an important role in
rulating, actin, assembly, and, disassembly, we have

rell.
Since Rho GTPases clearly play an important role in
regulating actin assembly and disassembly, we have
concentrated our efforts on processes where actin is Since Rho GTPases clearly play an important role in
regulating actin assembly and disassembly, we have
concentrated our efforts on processes where actin is
known to play an important role Table I lists some of the regulating actin assembly and disassembly, we have
concentrated our efforts on processes where actin is
known to play an important role. Table 1 lists some of the important roles of the actin cytoskeleton in eukaryotic known to play an important role. Table 1 lists some of the
important roles of the actin cytoskeleton in eukaryotic
cells. We have so far concentrated our efforts on events
that are dependent on dynamic changes to the actin important roles of the actin cytoskeleton in eukaryotic
cells. We have so far concentrated our efforts on events
that are dependent on dynamic changes to the actin
cytoskeleton and in particular cell movement axon cells. We have so far concentrated our efforts on events
that are dependent on dynamic changes to the actin
cytoskeleton and in particular cell movement, axon
guidance and phagocytosis. Here we will describe our that are dependent on dynamic changes to the actin
cytoskeleton and in particular cell movement, axon
guidance and phagocytosis. Here we will describe our
results so far on the role of Ras and Rho GTPases in cell cytoskeleton and in particular cell movement, axon
guidance and phagocytosis. Here we will describe our
results so far on the role of Ras and Rho GTPases in cell
movement movement.

movement.
 4. THE ROLES OF RAS AND RHO GTPASES IN CELL (c) The r (c) The r **MIGRATION**

(a) *The migration assay*

To analyse the role of Rho GTPases in a simple cell migration assay, we have introduced scratch wounds To analyse the role of Rho GTPases in a simple cell
migration assay, we have introduced scratch wounds
(made with a microinjection needle) into confluent mono-
layers of primary rat embryo fibroblasts (RFFs) The migration assay, we have introduced scratch wounds
(made with a microinjection needle) into confluent mono-
layers of primary rat embryo fibroblasts (REFs). The
wounds are around three to four cell diameters in width (made with a microinjection needle) into confluent mono-layers of primary rat embryo fibroblasts (REFs). The wounds are around three to four cell diameters in width *Phil. Trans. R. Soc. Lond.* B (2000)

Table 1. *Some aspects of cell behaviour that are dependent on the actin cytoskeleton*

and closure of control cells takes 5^6 h in serum. Close and closure of control cells takes 5–6 h in serum. Close
inspection of wound edge cells using time-lapse video
microscopy reveals that cells fill the space by migration and closure of control cells takes 5–6 h in serum. Close
inspection of wound edge cells using time-lapse video
microscopy reveals that cells fill the space by migration
and not simply by spreading, and that on average a ce inspection of wound edge cells using time-lapse video
microscopy reveals that cells fill the space by migration
and not simply by spreading, and that on average, a cell
at the wound edge moves a distance of around $15-2$ c microscopy reveals that cells fill the space by migration
and not simply by spreading, and that on average, a cell
at the wound edge moves a distance of around 1.5–2 cell
diameters Addition of cyclobesimide has no effect o and not simply by spreading, and that on average, a cell
at the wound edge moves a distance of around 1.5–2 cell
diameters. Addition of cycloheximide has no effect on
migration in these short-term assays suggesting that no at the wound edge moves a distance of around 1.5–2 cell
diameters. Addition of cycloheximide has no effect on
migration in these short-term assays, suggesting that no
new gene transcription is required. Finally it is clear diameters. Addition of cycloheximide has no effect on
migration in these short-term assays, suggesting that no
new gene transcription is required. Finally, it is clear
from the video recordings (figure 4a) that cells move migration in these short-term assays, suggesting that no
new gene transcription is required. Finally, it is clear
from the video recordings (figure 4*a*) that cells move
coordinately as a sheet and not as individuals. Cell new gene transcription is required. Finally, it is clear
from the video recordings (figure 4a) that cells move
coordinately as a sheet and not as individuals. Cells at
the wound margin have a highly dynamic leading edge from the video recordings (figure $4a$) that cells move
coordinately as a sheet and not as individuals. Cells at
the wound margin have a highly dynamic leading edge coordinately as a sheet and not as individuals. Cells at
the wound margin have a highly dynamic leading edge
showing lamellipodia, filopodia and membrane ruffles,
while cell borders in contact with neighbouring cells are the wound margin have a highly dynamic leading edge
showing lamellipodia, filopodia and membrane ruffles,
while cell borders in contact with neighbouring cells are
quiescent and show little dynamic activity. The cells have showing lamellipodia, filopodia and membrane ruffles,
while cell borders in contact with neighbouring cells are
quiescent and show little dynamic activity. The cells have,
therefore, a polarized morphology during migration while cell borders in contact with neighbouring cells
quiescent and show little dynamic activity. The cells has
therefore, a polarized morphology during migration.
To analyse the role of GTPases, we have microinied iescent and show little dynamic activity. The cells have,
erefore, a polarized morphology during migration.
To analyse the role of GTPases, we have microinjected
lls (a patch of around 50 on either side of a wound) with

therefore, a polarized morphology during migration.
To analyse the role of GTPases, we have microinjected
cells (a patch of around 50 on either side of a wound) with inhibitors of (i) Rho (C3 transferase), (ii) Rac (dominant negative, N17Rac protein prepared in *Escherichia coli*), inhibitors of (i) Rho (C3 transferase), (ii) Rac (dominant
negative, N17Rac protein prepared in *Escherichia coli*),
(iii) Cdc42 (dominant negative, N17Cdc42 protein
expressed in *E coli* or a Cdc42-binding fragment deriv negative, N17Rac protein prepared in *Escherichia coli*),
(iii) Cdc42 (dominant negative, N17Cdc42 protein
expressed in *E. coli*, or a Cdc42-binding fragment derived
from WASP) or (iv) Ras (a neutralizing monoclonal anti-(iii) Cdc42 (dominant negative, N17Cdc42 protein
expressed in E. coli, or a Cdc42-binding fragment derived
from WASP), or (iv) Ras (a neutralizing monoclonal anti-
hody $V13-959$) $body, Y13-259$).

(b) *The role of Rac*

(b) *The role of Rac*
As shown in figure $4d$, microinjection of fluorescent
wtran as a control has no effect on the migratory (b) **The role of Rac**
As shown in figure 4d, microinjection of fluorescent
dextran, as a control, has no effect on the migratory
properties of the cells. However figure 4b shows that inhi-As shown in figure 4*d*, microinjection of fluorescent dextran, as a control, has no effect on the migratory properties of the cells. However, figure 4*b* shows that inhi-
bition of Rac completely prevents cell movement. C dextran, as a control, has no effect on the migratory
properties of the cells. However, figure $4b$ shows that inhi-
bition of Rac completely prevents cell movement. Closer
inspection of the cells reveals that the dynamic properties of the cells. However, figure $4b$ shows that inhibition of Rac completely prevents cell movement. Closer inspection of the cells reveals that the dynamic activity of the leading edge seen in control cells is c bition of Rac completely prevents cell movement. Closer
inspection of the cells reveals that the dynamic activity of
the leading edge seen in control cells is completely
suppressed though a few filopodia may still remain W inspection of the cells reveals that the dynamic activity of
the leading edge seen in control cells is completely
suppressed, though a few filopodia may still remain. We
conclude that Rac, presumably through its ability to the leading edge seen in control cells is completely
suppressed, though a few filopodia may still remain. We
conclude that Rac, presumably through its ability to
promote actin polymerization at the cell periphery suppressed, though a few filopodia may still remain. We conclude that Rac, presumably through its ability to promote actin polymerization at the cell periphery, provides the driving force for the protrusive activity conclude that Rac, presumably through its ability to
promote actin polymerization at the cell periphery,
provides the driving force for the protrusive activity
required for cell migration promote actin polymerization at the cell periphery, provides the driving force for the protrusive activity

(c) *The role of Cdc42*

Inhibition of Cdc42, using either of two distinct (c) **The role of Cdc42**
Inhibitors (a dominant negative version of Cdc42 or a
fragment derived from a Cdc42 target protein) inhibits Inhibition of Cdc42, using either of two distinct
inhibitors (a dominant negative version of Cdc42 or a
fragment derived from a Cdc42 target protein), inhibits
cell migration by α 50%. Closer inspection of the cells inhibitors (a dominant negative version of $Cdc42$ or a fragment derived from a $Cdc42$ target protein), inhibits cell migration by *ca.* 50%. Closer inspection of the cells reveals that they have lost their polarity. Inste fragment derived from a Cdc42 target protein), inhibits
cell migration by $ca. 50\%$. Closer inspection of the cells
reveals that they have lost their polarity. Instead of
making protrusions only at the leading edge cells cell migration by $ca. 50\%$. Closer inspection of the cells
reveals that they have lost their polarity. Instead of
making protrusions only at the leading edge, cells are
now seen to have protrusions all around the cell pe reveals that they have lost their polarity. Instead of making protrusions only at the leading edge, cells are
now seen to have protrusions all around the cell periphery
and pushing underneath neighbouring cells making protrusions only at the leading edge, cells are
now seen to have protrusions all around the cell periphery
and pushing underneath neighbouring cells.

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Figure 4. A wound-healing assay using primary REFs. To analyse the role of Ras and Rho GTPases, inhibitors were Figure 4. A wound-healing assay using primary REFs. To analyse the role of Ras and Rho GTPases, inhibitors were
microinjected into a patch of *ca*. 50 cells on either side of a wounded monolayer. The time taken for subsequ Figure 4. A wound-healing assay using primary REFs. To analyse the role of Ras and Rho GTPases, inhibitors were
microinjected into a patch of *ca*. 50 cells on either side of a wounded monolayer. The time taken for subsequ microinjected into a patch of *ca*. 50 cells on either side of a wounded monolayer. The time taken for subsequent wound closure compared with that of control, marker-injected cells. (*a*) In phase contrast, polarized cell compared with that of control, marker-injected cells. (*a*) In phase contrast, polarized cells at the wound margin, with highly
dynamic leading edges (lamellipodia, ruffles and filopodia) and quiescent borders where cells dynamic leading edges (lamellipodia, ruffles and filopodia) and quiescent borders where cells are in contact with their neighbours. (*d*, *e*) Cells around a wound were injected with fluorescent dextran (*d*) and then lef fluorescence that the injection procedure does not affect migration and wound closure (e) . (b, c) Cells were injected with domi-

.
During cell migration, the Golgi, which normally
cupies a position extending over about one-third of the During cell migration, the Golgi, which normally
occupies a position extending over about one-third of the
nuclear perimeter reorientates in the direction of During cell migration, the Golgi, which normally
occupies a position extending over about one-third of the
nuclear perimeter, reorientates in the direction of
migration Reorientation of the Golgi to face the wound occupies a position extending over about one-third of the nuclear perimeter, reorientates in the direction of migration. Reorientation of the Golgi to face the wound is a rather slow process increasing from 33% to ca nuclear perimeter, reorientates in the direction of
migration. Reorientation of the Golgi to face the wound
is a rather slow process increasing from 33% to *ca.* 70⁻²⁰⁰% over a period of around 6 b. It is unlikely theref migration. Reorientation of the Golgi to face the wound
is a rather slow process increasing from 33% to ca . $70-80\%$ over a period of around 6 h. It is unlikely, therefore, that Golgi reorientation is a driving force for movement, 80% over a period of around 6 h. It is unlikely, therefore,
that Golgi reorientation is a driving force for movement,
but it presumably facilitates migration by delivering new
membranes to the leading edge (Schliwa et al. that Golgi reorientation is a driving force for movement,
but it presumably facilitates migration by delivering new
membranes to the leading edge (Schliwa *et al.* 1999). Inhi-
bition of Cdc42 also prevents Golgi reorganiz but it presumably facilitates migration by delivering new
membranes to the leading edge (Schliwa *et al.* 1999). Inhi-
bition of Cdc42 also prevents Golgi reorganization. We *Phil. Trans. R. Soc. Lond.* B (2000)

conclude that Cdc42 is required to define the polarity of
the migrating cell and that this is reflected in the localizathe migrating cell and that this is reflected in the polarity of the migrating cell and that this is reflected in the localiza-
tion of Rac-dependent protrusive activity to the leading conclude that Cdc42 is required to define the polarity of
the migrating cell and that this is reflected in the localiza-
tion of Rac-dependent protrusive activity to the leading
edge and in the reorganization of the Golgi, the migrating cell and that this is reflected in the localization of Rac-dependent protrusive activity to the leading
edge and in the reorganization of the Golgi, presumably
in a microtubule-dependent manner, towards the l tion of Rac-dependent protrusive activity to the leading
edge and in the reorganization of the Golgi, presumably
in a microtubule-dependent manner, towards the leading
edge edge.

(d) *The role of Rho*

REFs have very prominent stress fibres and focal adhesion complexes and these are maintained during

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cell migration during a fibroblast wound healing assay.
migration initiated by wounding of the monolayer. As Figure 5. At least four small GTPases cooperate to promote Figure 5. At least four small GTPases cooperate to prom
cell migration during a fibroblast wound healing assay.

recently described by Smilenov *et al*. (1999), cells appear migration initiated by wounding of the monolayer. As
recently described by Smilenov *et al.* (1999), cells appear
to migrate through static focal adhesions and when these
adhesive structures reach the rear of the migrating recently described by Smilenov *et al.* (1999), cells appear
to migrate through static focal adhesions and when these
adhesive structures reach the rear of the migrating cell
they dissinate. To determine whether focal adhe to migrate through static focal adhesions and when these
adhesive structures reach the rear of the migrating cell
they dissipate. To determine whether focal adhesions
nevertheless slow down cell migration, we have removed adhesive structures reach the rear of the migrating cell
they dissipate. To determine whether focal adhesions
nevertheless slow down cell migration, we have removed
focal adhesions and stress fibres in the migrating cells they dissipate. To determine whether focal adhesions nevertheless slow down cell migration, we have removed focal adhesions and stress fibres in the migrating cells by nevertheless slow down cell migration, we have removed
focal adhesions and stress fibres in the migrating cells by
addition of the compound Y27632, an inhibitor of
pl60R ho kinase, an essential downstream target of R ho focal adhesions and stress fibres in the migrating cells by
addition of the compound Y27632, an inhibitor of
p160Rho kinase, an essential downstream target of Rho
required for stress fibre assembly (Watanabe *et al.* 1999 addition of the compound Y27632, an inhibitor of pl60Rho kinase, an essential downstream target of Rho required for stress fibre assembly (Watanabe *et al.* 1999).
Inder these conditions cells move $c\ell$ 50% faster than p160Rho kinase, an essential downstream target of Rho
required for stress fibre assembly (Watanabe *et al.* 1999).
Under these conditions, cells move *ca.* 50% faster than
non-treated cells and we conclude that although ce required for stress fibre assembly (Watanabe *et al.* 1999).
Under these conditions, cells move *ca*. 50% faster than
non-treated cells and we conclude that although cells can
regulate turnover of focal adhesions during mi Under these conditions, cells move ca . 50% faster than non-treated cells and we conclude that although cells can regulate turnover of focal adhesions during migration, non-treated cells and we conclude that although cells can
regulate turnover of focal adhesions during migration,
cells move faster without them. It also shows that actin:-
myosin filaments, at least in the form of stress f regulate turnover of focal adhesions during migration,
cells move faster without them. It also shows that actin:-
myosin filaments, at least in the form of stress fibres, are
not required for migration cells move faster without the
myosin filaments, at least in
not required for migration.
We also attempted to repea Fragment and the form of stress fibres, are
t required for migration.
We also attempted to repeat this experiment by directly
pibiting. Rho, by, microiniection, of, cells, with. C3

not required for migration.
We also attempted to repeat this experiment by directly
inhibiting Rho by microinjection of cells with C3
transferase We found that at concentrations of C3 just We also attempted to repeat this experiment by directly
inhibiting Rho by microinjection of cells with C3
transferase. We found that at concentrations of C3 just
sufficient to remove all visible focal adhesions cells moved inhibiting Rho by microinjection of cells with C3
transferase. We found that at concentrations of C3 just
sufficient to remove all visible focal adhesions cells moved
similar to control cells, confirming that stress fibres transferase. We found that at concentrations of C3 just
sufficient to remove all visible focal adhesions cells moved
similar to control cells, confirming that stress fibres are
not needed for migration. However, injection sufficient to remove all visible focal adhesions cells moved
similar to control cells, confirming that stress fibres are
not needed for migration. However, injection of C3
transferase, at threefold higher concentrations ca not needed for migration. However, injection of C3 controlled by Ras. The molecular link between Ras and
transferase at threefold higher concentrations caused focal adhesions is unclear; it could be that Ras affects
signif transferase at threefold higher concentrations caused transferase at threefold higher concentrations caused
significant rounding up and detachment of cells, and cell
migration was inhibited. This experiment strongly
suggests that Rho has other activities distinct from stress significant rounding up and detachment of cells, and cell
migration was inhibited. This experiment strongly
suggests that Rho has other activities, distinct from stress
fibre and focal adhesion formation, and related to migration was inhibited. This experiment strongly
suggests that Rho has other activities, distinct from stress
fibre and focal adhesion formation, and related to
adhesion and spreading suggests that Rho has oth
fibre and focal adhesio
adhesion and spreading. adhesion and spreading.

(e) *The role of Ras*

Several groups have previously described

(e) The role of Ras
Several groups have previously described the
portance of Ras in cell migration (Fox et al. 1994) (e) **The role of Ras**
Several groups have previously described the
importance of Ras in cell migration (Fox *et al.* 1994).
Immunofluorescence analysis using an antibody specific Several groups have previously described the importance of Ras in cell migration (Fox *et al.* 1994). Immunofluorescence analysis using an antibody specific importance of Ras in cell migration (Fox *et al.* 1994).
Immunofluorescence analysis using an antibody specific
to dually phosphorylated ERK (a marker for Ras activa-
tion) revealed that ERK and Ras are activated 5 min Immunofluorescence analysis using an antibody specific
to dually phosphorylated ERK (a marker for Ras activation), revealed that ERK and Ras are activated 5 min
after wounding, though ERK fluorescence returns to to dually phosphorylated ERK (a marker for Ras activation), revealed that ERK and Ras are activated 5 min
after wounding, though ERK fluorescence returns to
background levels within an hour Interestingly phosphotion), revealed that ERK and Ras are activated 5 min after wounding, though ERK fluorescence returns to background levels within an hour. Interestingly, phospho-ERK is detected up to eight cells back from the wound after wounding, though ERK fluorescence returns to the cell body does not translocate) and we are, therefore, background levels within an hour. Interestingly, phospho- also interested in exploring the roles of Rho family a background levels within an hour. Interestingly, phospho-
ERK is detected up to eight cells back from the wound
margin—we do not know the mechanism by which cells
distant from the wound are activated ERK is detected up to eight cells back
margin—we do not know the mechani
distant from the wound are activated.
To determine whether Ras is required. Example 1 are do not know the mechanism by which cells
tant from the wound are activated.
To determine whether Ras is required for migration,
lls were microiniected with a Ras-neutralizing mono-

distant from the wound are activated.
To determine whether Ras is required for migration,
cells were microinjected with a Ras-neutralizing mono-

clonal antibody. Under these conditions cell movement is clonal antibody. Under these conditions cell movement is
almost completely blocked. The reasons for this were not
entirely clear at first, since cells still have a polarized and almost completely blocked. The reasons for this were not entirely clear at first, since cells still have a polarized and almost completely blocked. The reasons for this were not
entirely clear at first, since cells still have a polarized and
dynamic leading edge. We did notice, however, some
thickening of stress fibres and focal adhesions in entirely clear at first, since cells still have a polarized and
dynamic leading edge. We did notice, however, some
thickening of stress fibres and focal adhesions in injected
cells and wondered if there might be a function dynamic leading edge. We did notice, however, some
thickening of stress fibres and focal adhesions in injected
cells and wondered if there might be a functional
relationship between Ras and Rho To test this idea cells thickening of stress fibres and focal adhesions in injected
cells and wondered if there might be a functional
relationship between Ras and Rho. To test this idea, cells
were microinjected with the neutralizing Ras antibody cells and wondered if there might be a functional
relationship between Ras and Rho. To test this idea, cells
were microinjected with the neutralizing Ras antibody
and at the same time treated with Y27632 to remove focal relationship between Ras and Rho. To test this idea, cells
were microinjected with the neutralizing Ras antibody
and at the same time treated with Y27632 to remove focal
adhesions. Under, these conditions, cells, moved, at were microinjected with the neutralizing Ras antibody
and at the same time treated with Y27632 to remove focal
adhesions. Under these conditions cells moved at a
similar speed to control cells. Our interpretation of this and at the same time treated with Y27632 to remove focal
adhesions. Under these conditions cells moved at a
similar speed to control cells. Our interpretation of this
result is that Ras is required for focal adhesion turno similar speed to control cells. Our interpretation of this result is that Ras is required for focal adhesion turnover. similar speed to control cells. Our interpretation of this
result is that Ras is required for focal adhesion turnover.
Addition of a MEK inhibitor (PD98059) or a PI 3-kinase
inhibitor (wortmannin) had only minor effects on result is that Ras is required for focal adhesion turnover.
Addition of a MEK inhibitor (PD98059) or a PI 3-kinase
inhibitor (wortmannin) had only minor effects on cell
migration migration.

5. CONCLUSIONS

Figure 5 summarizes our conclusions concerning the **EXECUSSIONS**
Figure 5 summarizes our conclusions concerning the
role of four small GTPases (Rho, Rac, Cdc42 and Ras)
in the migration of primary embryo fibroblasts (Nobes & Figure 5 summarizes our conclusions concerning the
role of four small GTPases (Rho, Rac, Cdc42 and Ras)
in the migration of primary embryo fibroblasts (Nobes &
Hall 1999). Rac, is required to induce the protrusive in the migration of primary embryo fibroblasts (Nobes & Hall 1999). Rac is required to induce the protrusive activity at the front of the cell that we believe is the Hall 1999). Rac is required to induce the protrusive activity at the front of the cell that we believe is the driving force for movement. $Cdc42$ is required to maintain the polarity of the migrating cell: it ensures that activity at the front of the cell that we believe is the driving force for movement. Cdc42 is required to maintain the polarity of the migrating cell; it ensures that Rac activity is restricted to the leading edge and that driving force for movement. Cdc42 is required to
maintain the polarity of the migrating cell; it ensures that
Rac activity is restricted to the leading edge and that
microtubules and Golgi reorientates in the direction of maintain the polarity of the migrating cell; it ensures that
Rac activity is restricted to the leading edge and that
microtubules and Golgi reorientates in the direction of Rac activity is restricted to the leading edge and that
microtubules and Golgi reorientates in the direction of
migration. This nicely demonstrates the cooperation
between these two GTPases to promote directed migramicrotubules and Golgi reorientates in the direction of
migration. This nicely demonstrates the cooperation
between these two GTPases to promote directed migra-
tion. A similar conclusion has been reached by others in migration. This nicely demonstrates the cooperation
between these two GTPases to promote directed migra-
tion. A similar conclusion has been reached by others in
experiments analysing chemotaxis of macrophages in a between these two GTPases to promote directed migration. A similar conclusion has been reached by others in experiments analysing chemotaxis of macrophages in a gradient of CSF-1 (Allen *et al* 1998). The molecular tion. A similar conclusion has been reached by others in experiments analysing chemotaxis of macrophages in a gradient of CSF-1 (Allen *et al.* 1998). The molecular mechanisms by which Cdc42 controls cell polarity are not experiments analysing chemotaxis of macrophages in a
gradient of CSF-1 (Allen *et al.* 1998). The molecular
mechanisms by which Cdc42 controls cell polarity are not
clear: perhaps it localizes Rac, or a critical Rac target gradient of CSF-1 (Allen *et al.* 1998). The molecular mechanisms by which Cdc42 controls cell polarity are not clear; perhaps it localizes Rac, or a critical Rac target, to the leading edge. Alternatively, it may inhibit mechanisms by which Cdc42 controls cell polarity are not
clear; perhaps it localizes Rac, or a critical Rac target, to
the leading edge. Alternatively, it may inhibit Rac
activity at sites of cell–cell contact clear; perhaps it localizes Rac, or a c
the leading edge. Alternatively, i
activity at sites of cell–cell contact.
Rho appears to be active both in Exercise expectively, it may inhibit Ractivity at sites of cell-cell contact.
Rho appears to be active both in confluent monolayers
d in migrating cells since cells have prominent stress

activity at sites of cell–cell contact.
Rho appears to be active both in confluent monolayers
and in migrating cells, since cells have prominent stress Rho appears to be active both in confluent monolayers
and in migrating cells, since cells have prominent stress
fibres and focal adhesions. To move, however, matrix
adhesions must be turned over and it appears that this and in migrating cells, since cells have prominent stress
fibres and focal adhesions. To move, however, matrix
adhesions must be turned over and it appears that this
occurs predominantly at the rear of the migrating cell fibres and focal adhesions. To move, however, matrix
adhesions must be turned over and it appears that this
occurs predominantly at the rear of the migrating cell.
Our data suggest that in these cells at least, this is adhesions must be turned over and it appears that this occurs predominantly at the rear of the migrating cell.
Our data suggest that in these cells at least, this is occurs predominantly at the rear of the migrating cell.
Our data suggest that in these cells at least, this is
controlled by Ras. The molecular link between Ras and
focal adhesions is unclear: it could be that Ras affects Our data suggest that in these cells at least, this is
controlled by Ras. The molecular link between Ras and
focal adhesions is unclear; it could be that Ras affects
components of the focal adhesion itself or that Ras controlled by Ras. The molecular link between Ras and
focal adhesions is unclear; it could be that Ras affects
components of the focal adhesion itself, or that Ras
affects the activity of Rho. One interesting possibility i focal adhesions is unclear; it could be that Ras affects
components of the focal adhesion itself, or that Ras
affects the activity of Rho. One interesting possibility is
that the Ras effect is mediated by pl20RasGAP which components of the focal adhesion itself, or that Ras
affects the activity of Rho. One interesting possibility is
that the Ras effect is mediated by p120RasGAP, which is
known to form a complex with p190RboGAP a downaffects the activity of Rho. One interesting possibility is that the Ras effect is mediated by pl20RasGAP, which is known to form a complex with pl90RhoGAP, a down-regulator of Rho. known to form a complex with pl90RhoGAP, a down-

Future work will focus on the molecular pathways that regulator of Rho.
Future work will focus on the molecular pathways that
are regulated by Rho and Ras GTPases and that contri-
bute to movement. We are also interested in analysing Future work will focus on the molecular pathways that
are regulated by Rho and Ras GTPases and that contri-
bute to movement. We are also interested in analysing
movement in other cell types to see whether cells can are regulated by Rho and Ras GTPases and that contribute to movement. We are also interested in analysing
movement in other cell types to see whether cells can
move in different wave and make different uses of bute to movement. We are also interested in analysing
movement in other cell types to see whether cells can
move in different ways and make different uses of
GTPases Growth cone guidance of axons can be thought movement in other cell types to see whether cells can
move in different ways and make different uses of
GTPases. Growth cone guidance of axons can be thought
of as a bighly specialized form of cell migration (where move in different ways and make different uses of GTPases. Growth cone guidance of axons can be thought GTPases. Growth cone guidance of axons can be thought
of as a highly specialized form of cell migration (where
the cell body does not translocate) and we are, therefore,
also interested in exploring the roles of Rho family of as a highly specialized form of cell migration (where
the cell body does not translocate) and we are, therefore,
also interested in exploring the roles of Rho family and
other small GTPases in this process. Finally a cr the cell body does not translocate) and we are, therefore,
also interested in exploring the roles of Rho family and
other small GTPases in this process. Finally, a crucial
component of directed migration is the spatial org also interested in exploring the roles of Rho family and
other small GTPases in this process. Finally, a crucial
component of directed migration is the spatial organiza-
tion of these and other signalling molecules We curr other small GTPases in this process. Finally, a crucial component of directed migration is the spatial organization of these and other signalling molecules. We currently have no idea how this is determined and the early wo component of directed migration is the spatial organization of these and other signalling molecules. We currently have no idea how this is determined and the early work on the activation of signalling pathways by addition of

factors to cells in culture is unlikely to be able to address
this question. Relatively simple assays, such as the one factors to cells in culture is unlikely to be able to address
this question. Relatively simple assays, such as the one
described here will be essential if the spatial organization factors to cells in culture is unlikely to be able to address
this question. Relatively simple assays, such as the one
described here, will be essential if the spatial organization
of GTPase-controlled signals are to be ad this question. Relatively simple assays, such as the described here, will be essential if the spatial organ of GTPase-controlled signals are to be addressed.

of GTPase-controlled signals are to be addressed.
This work was generously supported by a programme grant
from the Cancer Research Campaign to A.H. and by a Lister This work was generously supported by a programme grant
from the Cancer Research Campaign to A.H. and by a Lister
Institute Research Fellowship to C.D.N This work was generously supported b
from the Cancer Research Campaign to
Institute Research Fellowship to C.D.N.

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involved in the activation of the phagocyte NADPH oxidase involved in the activation of the phagocyte NADPH oxidase.
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aga, V. M. M., Machesky, L. M., Hall, A. & Hotchin, N. 1997
The small GTPases Rho and Rac are required for the
establishment of cadherin-dependent cell-cell contacts $\tilde{\tau}$ Cell aga, V. M. M., Machesky, L. M., Hall, A. & Hotchin, N. 1997
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