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Constitutive active GTPases Rac and Cdc42 are associated with endoreplication in PAE cells

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Abstract

The Rho-like guanine triphosphate (GTP)ases become activated by extracellular ligands and regulate a wide variety of biological processes, including cell motility, spreading of cells, cytoskeletal organisation and transcriptional activity. We studied the effect of expression of WtRac and Cdc42 and of their constitutive active V12 variants on cell cycle transition using the isopropylthiogalactoside (IPTG) inducible Rac and Cdc42 transfectants of porcine aortic endothelial (PAE) cells. Expression of V12Rac or V12Cdc42 resulted initially in an enrichment of cells in G2/M, followed by the appearance of multinucleated cells with some of the nuclei still being able to incorporate bromodeoxyuridine (BrdU). By fluorescent activated cell sorter (FACS) analysis, these cells appeared as polyploid cells. Prolonged activation of V12Rac or V12Cdc42 resulted in genomic instability and these cells finally detached from the culture plate. These findings indicate that induction of the constitutive active V12 forms of Rac and Cdc42 results in 'mitotic slippage', where endoreplication takes place irrespective of the exit from cytokinesis.

Keywords: Rho-GTPases; Cytokinesis; Cell cycle arrest; V12Rac; V12Cdc42

1. Introduction

The Rho family of guanine triphosphate (GTP)ases are members of the Ras superfamily and include Cdc42, Rac and Rho. The Rho-like GTPases can be activated by extracellular ligands and regulate a wide range of biological processes, including cytoskeletal organisation, cell motility, cell morphology, cell adhesion, cell growth, transcriptional activation, membrane trafficking and development. Their activity is regulated by a combination of GTPase-activating proteins (GAPs), guanine nucleotide exchange factors (GEFs) and guanine nucleotide dissociation inhibitors (GDIs) [1]. The Rho-like GTPases are important regulators of the cytoskeletal organisation, where Rac induces cell spreading and membrane ruffles, Cdc42 governs the extension of filopodia and RhoA regulates the assembly of stress fibres [1]. A hierarchy among these GTPases was established, in which activated Cdc42 activates Rac, which in its turn activates RhoA [2]. However, Rac and Cdc42 oppose the action of RhoA during cell movement, most likely by inactivation of the myosin light chain kinase, which when activated by Rho-kinase, mediates myosin activation and stress fibre formation [3].

A final target of the Rho-like GTPases associated signalling is activation of cell cycle progression, since microinjection of RhoA, Rac and Cdc42 in quiescent fibroblasts stimulated cell cycle progression through the G1 and S phases [4]. Furthermore, constitutive active mutants of Rac and Cdc42 induce E2F transcriptional activity and accumulation of cyclin D1 and pRb phosphorylation in serum-deprived NIH 3T3 fibroblasts and rat-1 cells [5].

We examined long-term (4 days) effects of activation of Rac and Cdc42 in order to investigate the role of these regulators on cell cycle progression in cells with a conditional overexpression of Rac or Cdc42 [6].

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2. Materials and methods

2.1. Cell culture

The inducible Rac and Cdc42 PAE cell lines [6] were kindly provided by Leonard Stephens (Babraham Institute, Cambridge, UK). The inducible Rac and Cdc42 PAE cell lines were cultured in F12 nutrient mixture (Ham F12; Gibco BRL, Life Technologies, Inc.) containing 9% heat-inactivated FBS and 0.3 μg/ml puromycin and 60 μg/ml hygromycin at 37 °C in a 5% CO_2 humidified atmosphere. The PAE cell lines were induced for Rac and Cdc42 expression by addition of 2 mM IPTG to the medium.

2.2. GTP-binding activity of Rac and Cdc42

We employed the glutathione-S-transferase (GST)-PAK binding assay as described by van Leeuwen and colleagues in Ref. [7] to measure the GTP-binding activity of Rac or Cdc42. In brief, cells were washed in ice cold phosphate-buffered saline (PBS), containing 1 mM $MgCl_2$ and 0.5 mM $CaCl_2$, incubated for 5 min in lysis buffer (50 mM Tris–HCl, pH 7.4; 2 mM $MgCl_2$; 1% Nonidet (N)P-40; 10% glycerol; 1 mM benzamide; 1 μg/ml leupeptin; 1 μg/ml pepstatin; 1 μg/ml aprotinin) and centrifuged for 5 min at 21 000*g* at 4 °C. Aliquots were taken to measure the protein amount. The supernatant was then incubated with a bacterial-derived GST-PAK-CD fusion protein [7], bound to glutathione-coupled sepharose beads for 60 min at 4 °C. The beads and proteins bound to the fusion protein were washed three times with lysis buffer, eluted with Laemmli buffer, and analysed for bound Rac or Cdc42 by Western blotting, using monoclonal antibodies against Rac (Transduction Laboratories) or Cdc42 (Sc-87, Santa Cruz).

2.3. FACS analysis

Cells were harvested by PBS/ethylene diamine tetra acetic acid (EDTA) and trypsin treatment. The single cell suspension was washed twice with PBS, resuspended in 0.5 ml PBS and then fixed with 7.5 ml 70% ethanol and incubated overnight at 4 °C. Cells were washed with PBS containing 1% FBS, resuspended in 200 μl PBS/1% FBS containing EtBr (20 μg/ml) and RNAse A (100 μg/ml) and incubated for 30 min at 37 °C. Cells were analysed in a fluorescent activated cell sorter (FACS).

2.4. Immunofluorescence

Cells were cultured on coverslips and washed twice with PBS (containing 0.5 mM $MgCl_2$ and 1 mM $CaCl_2$) and fixed with 3.7% formaldehyde in PBS (containing 0.5 mM $MgCl_2$ and 1 mM $CaCl_2$). Cells were washed twice with PBS, treated for 3 min with 0.5% Triton/PBS and blocked for 30 min with 1% Bovine Serum Albumin (BSA)/PBS. Cells were incubated with TRITC-Phalloidin (Sigma; 1:2500) and TO-PRO-3 (1:500) (Molecular Probes, Leiden, The Netherlands) for 1 h at 37 °C and then washed six times with PBS. The coverslips were sealed with Vectashield onto a microscope slide and analysed with confocal microscopy.

For BrdU-labelling experiments, cells were cultured for the indicated period of time with or without isopropyl-thiogalactoside (IPTG), the last 24 h in the presence of 1 μM BrdU, then fixed in ice-cold methanol, briefly dipped in acetone, and prepared for immunohistochemistry. The analysis consisted of an acid denaturation step (2 N HCl for 20 min at room temperature), incubation with a BrdU-DNA specific mouse antibody (Central Laboratory Blood Transfusion, Amsterdam, The Netherlands), staining with a fluorescein-isothiocyanate labelled goat-anti mouse antibody, whereas the total DNA was stained with ethidium bromide.

For staining of Microtubule Organising Centres (MTOCs), PAE cells were cultured in the presence or absence of IPTG, fixed with methanol, and incubated with an anti β-tubulin antibody (T8535, Sigma) and stained with a FITC labelled second antibody and for total DNA with 4,6 diamidine-2-phenylindole-dihydrochloride (DAPI). The antibodies were used in the concentrations recommended by the suppliers.

2.5. Metaphase spreads

The PAE cell lines were cultured as subconfluent monolayers, with or without 2 mM IPTG for 4 days. The cells were then treated with 50 μg /ml colcemide for 6 h and cells were harvested. Cells were washed twice with PBS and treated with 1% sodium citrate for 20 min at room temperature. An equal amount of fixative (methanol/acetic acid (3:1)) was added and cells were harvested at 1000 rpm by 14 °C during 8 min. Cells were washed three times with fixative and were dropped on a thin water layer containing 0.1 μg/ml DAPI on a microscope slide and analysed by ultraviolet (UV) microscopy.

2.6. Thymidine incorporation

Cells were plated in 24 wells (10 000 cells/well) and cultured in medium containing 9% fetal bovine serum (FBS). The next day the medium was replaced by medium containing 0.1, 1, 3 or 9% FBS with or without 2 mM IPTG and cells were incubated for 4 days. The cells were pulse-labelled with tritium-thymidine (2 μCi/well) for 1 h at 37 °C. The reaction was stopped by addition of 1 M ascorbic acid for 1 h at 4 °C. The cells were washed twice with PBS, treated with 5% trichloroacetic acid (TCA) for 15 min at 4 °C. After one wash with 5% TCA, the TCA was removed completely and 300 μl 0.1 N NaOH was added to each well, the cells were incubated

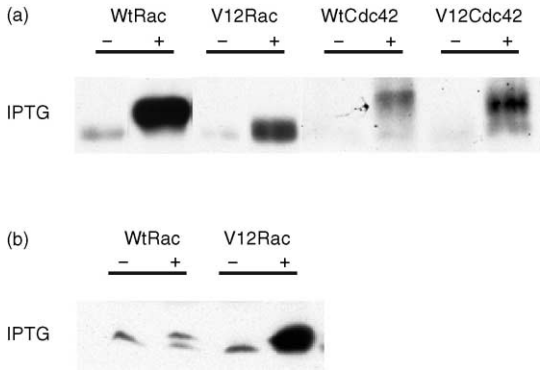

Fig. 1. (a) Expression of endogenous and induced exogenous WtRac and WtCdc42 proteins and of their V12 forms in porcine aortic endothelial (PAE) cells. WtRac or WtCdc42 and V12Rac or V12Cdc42 PAE cells were cultured for 3 days in the absence (−) or presence (+) of 2 mM IPTG and assayed by western blotting for the protein levels of these Rho-(GTP)ases. (b) GTP-binding activity of exogenous WtRac and V12Rac. Cells were cultured for 6 h in the presence of IPTG. GTP-binding activity was measured by the ability of the guanine triphosphate (GTP)-binding Rac proteins to bind to glutathione-5-transferase (GST)-PAK as described in Materials and methods.

for 30 min, harvested and radioactivity was counted in a liquid scintillation counter.

3. Results

3.1. V12Rac and V12Cdc42 affect proliferation of PAE cells

To address the effect of the overexpression of Rac and Cdc42 on cell proliferation, we used stably transfected PAE cells with IPTG-inducible wild-type Rac (WtRac), constitutive active Rac (V12Rac), wild-type Cdc42 (WtCdc42) or constitutive active Cdc42 (V12Cdc42) as described by Welch and colleagues in Ref. [6]. Induction by IPTG resulted in elevated levels of exogenous Wt and V12 Rac and Cdc42 proteins (Fig. 1a) that migrated slower because of the EE-tag epitope present in these constructs [6]. IPTG induction resulted, however, in a small increase of GTP-binding activity of exogenous WtRac, whereas V12Rac GTP-binding activity was greatly enhanced (Fig. 1b), which was measured by the ability of activated, GTP-binding form of Rac to bind to its substrate, PAK [7]. A similar increase in GTP-binding activity of V12Cdc42 over WtCdc42 activity was observed upon IPTG induction (data not shown).

The effect of prolonged induction on proliferation was studied by culturing the cells in medium containing different percentages of sera and measuring thymidine incorporation after 4 days. The tritium-thymidine incorporation of cells expressing V12Rac or V12Cdc42 proteins was reduced compared with the non-induced PAE cells. This was independent of the serum concentration and was most pronounced in cells growing in medium containing 9% serum (see Fig. 2). A slight reduction was also observed in WtRac-expressing cells, but not in WtCdc42 expressing cells.

3.2. V12Rac- and V12Cdc42-expressing cells become multinucleated

The effect of prolonged expression of V12Rac or V12Cdc42 proteins on cell cycle transition was examined by FACS analysis (Fig. 3). Non-induced PAE cells gave a normal DNA profile representing asynchronously growing cells. No major change in the DNA profile was detected in PAE cells which overexpress the wild-type version of exogenous Rac or Cdc42 protein after 1 or 3 days. However, already after 1 day, PAE cells expressing V12Rac or V12Cdc42 protein started to accumulate at G2/M. After 3 days, an even larger portion of the cells had accumulated in G2/M and on top of that a significant portion of the cells became polyploid. To investigate the morphology of these cells in detail, we employed confocal microscopy (see Fig. 4). After 1 day of overexpression of exogenous WtRac, lamellipodia and membrane ruffles became visible at the leading edge of the cells. In general, these cells have one nucleus and the morphology of these cells did not change very much after 2 days. In cells overexpressing exogenous WtCdc42, filopodia were induced and most of the cells contained one single nucleus, as was seen in WtRac-overexpressing cells. V12Rac-expressing PAE cells, however, became flat and showed big lamellipodia at multiple leading edges. We noticed that part of the cells were stretched into different directions and that the cells were sometimes pulled out into thin cytoplasm bridges. After 1 day of V12Rac induction, a few cells were detected with more than one nucleus. Up to 60% of the cells became multinucleated after 2 days of induction of V12Rac, as well as those induced for V12Cdc42-expression (Fig. 4). This corresponds with the fraction of polyploid cells observed in the FACS analysis (Fig. 3). Thereafter, these cells started to detach from the culture plate, which hampered an accurate estimation of the fraction of polyploid cells. Multinucleated and polyploid cells, however, were only observed in V12Rac and V12Cdc42 expressing cells, and not in the WtRac and WtCdc42 cells or in the non-transfected control cells.

3.3. DNA synthesis in multinucleated V12Rac and V12Cdc42 PAE cells

Cells were labelled for 24 h with BrdU at the fourth day after induction of either WtRac/Cdc42 or their V12 forms and examined by immunofluorescence for incorporation of BrdU and thus for the ability of cells to recently pass through S phase (see Fig. 5). BrdU incorporation still occurred in some of the multinucleated

cells, which arose as result of induction of V12Rac or V12Cdc42 protein. Note also that only a part of the V12Rac or V12Cdc42 cells turned into multinucleated cells, as was also observed in Fig. 4 and that only a part of those multinucleated cells showed BrdU incorporation. Detachment of V12Rac- and V12Cdc42-expressing cells from the culture plate that started after 3 days of IPTG exposure, hampered an accurate estimation of the fraction of the multinucleated cells incorporating BrdU. However, incorporation of BrdU in multinucleated cells only occurred in V12Rac- and V12Cdc42-expressing cells, and not in exogenous WtRac- or WtCdc42-expressing cells, nor in the non-transfected control cells. These data indicated that induction of V12Rac or V12Cdc42 in PAE cells resulted in multinucleated cells with some of the nuclei still being capable of replicating their DNA.

The endoreplication of nuclei went along with the increased number of MTOCs. Non-induced cells, and cells with induction of WtRac or WtCdc42 proteins showed either one or two MTOCs, whereas V12Rac or V12Cdc42 protein induction resulted in multiple MTOCs in multinucleated cells (see Fig. 6) upon paclitaxel-induced G2/M arrest. The karyotype of non-induced PAE cells or PAE cells expressing WtCdc42 showed an average number of 35 chromosomes. In PAE cells over-expressing WtRac, the majority of the cells had a normal chromosome number, whereas some cells contained a

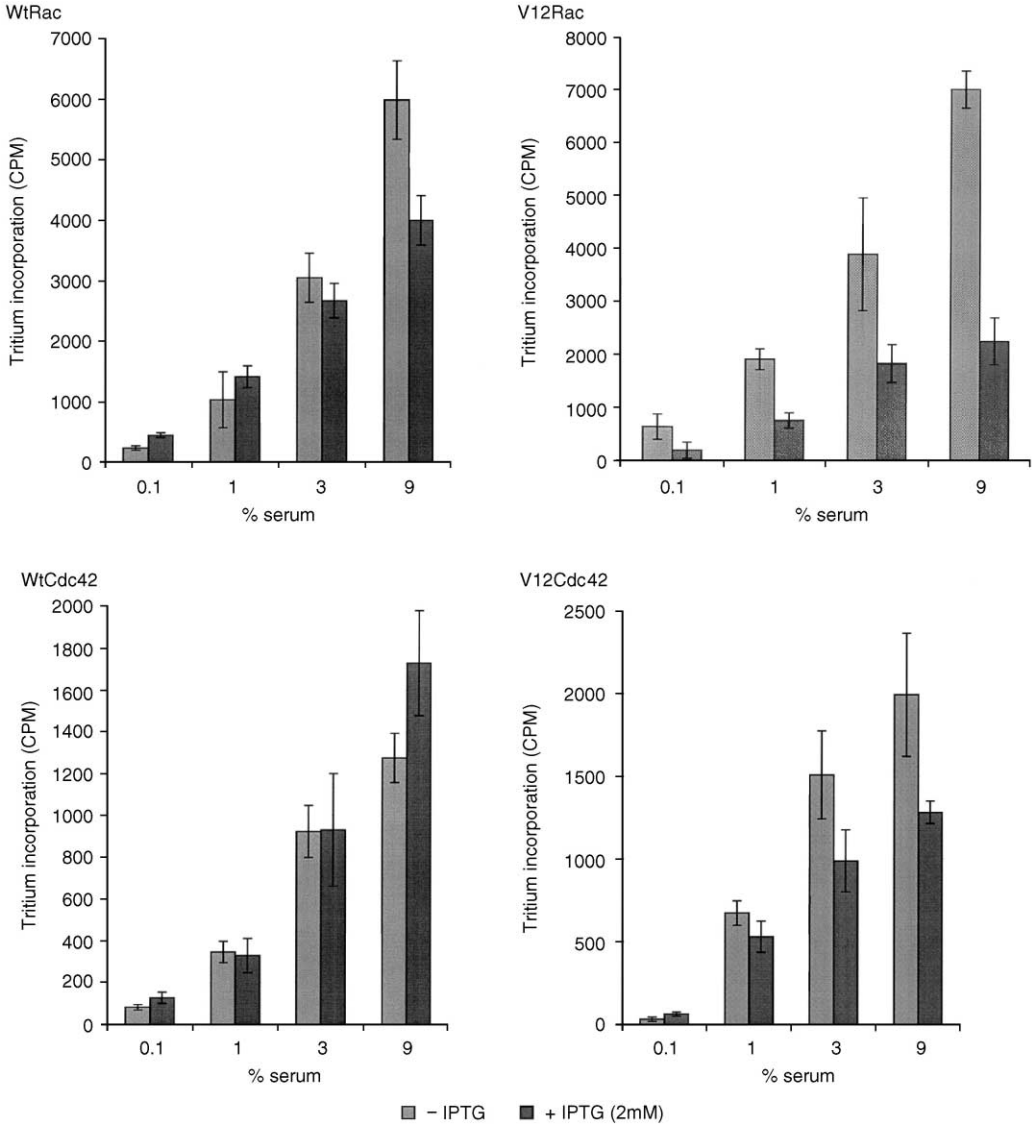

Fig. 2. Thymidine incorporation in porcine aortic endothelial (PAE) cells. 10 000 cells were seeded in triplicate in a 24-well plate and cultured in various concentrations of fetal bovine serum (FBS) in the presence (+) or absence (−) of 2 mM IPTG. After 4 days, cells were assayed for tritium-thymidine incorporation as described in Materials and methods. This experiment was repeated twice, whereas cells were seeded in triplicate, with no differences between these two experiments. The results of one such an experiment is presented with standard deviations (S.D.). CPM, counts per minute.

slightly elevated chromosome number (see Fig. 7). However, metaphase spreads of V12Rac and V12Cdc42 PAE cells showed a significant proportion of cells displaying an increased number of chromosomes (see Fig. 7).

4. Discussion

This study indicates that sustained expression of V12Rac or V12Cdc42 protein in PAE cells leads to multinucleated cells with some of the nuclei still being able to replicate their DNA in the absence of cytokinesis. This absence of cytokinesis does not arrest the nuclei in a mitotic state, since some BrdU incorporation still takes place (Fig. 5). These features indicate that V12Rac and V12Cdc42 activity leads to 'mitotic slippage' in these cells. It should be noted here that not all of the cells ended up as multinucleated cells, suggesting that V12Rac and V12Cdc42 activity affected some delicately balanced process which links nuclear replication to cytokinesis. This uncoupling of nuclear replication from cytokinesis was only observed upon induction of the V12 forms and not upon induction of the WtRac and Cdc42 forms. This is most likely due to a sustained high GTP-binding activity associated with the V12 Rac/Cdc42

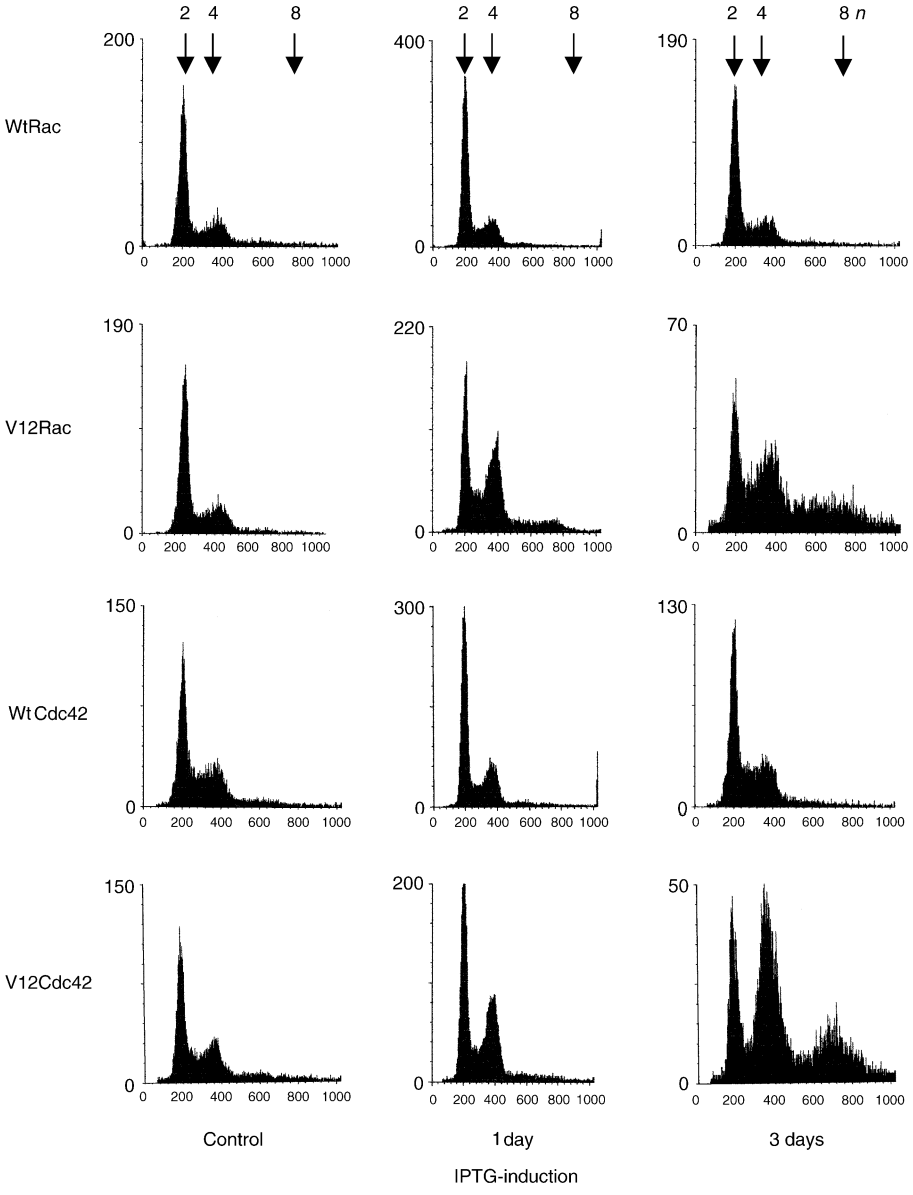

Fig. 3. FACSCAN analysis of WtRac or WtCdc42 and of V12Rac or V12Cdc42 porcine aortic endothelial (PAE) cells cultured for 0, 1 and 3 days in the presence of 2 mM IPTG. FACSCAN analysis was performed as described in Materials and methods. Standard 10 000 cells were analysed by fluorescent activated cell sorter (FACS). The experiment was performed twice, with no substantial difference between the two experiments. Arrows on top indicate the DNA content.

forms which is lacking in the Wt forms, despite a similar induction of proteins by the IPTG treatment (see Fig. 1). The relatively low GTP-binding activity despite the excess of exogenous WtRac hints furthermore, to a rapid inactivation of GTP-binding activity of WtRac.

Our study indicates that PAE cells expressing V12Rac are more flat than the non-induced or WtRac expressing PAE cells (Fig. 4). Normally, cells are flat during the interphase of the cell cycle and remain flat during the first part of mitosis until metaphase. Then, cells start to round up in order to enter mitosis [8]. The Rho-like GTPases Rac and Cdc42 are involved in cell spreading. Micro-injection of dominant-negative N17Rac1 or N17Cdc42 mutants into growing cells not only leads to the loss of lamellipodia and filopodia, respectively, but also leads to the rapid loss of their focal complexes resulting in cell rounding [9,10]. We, and others [1], found the opposite, initially an increased spreading of the cells, when constitutive active V12Rac and V12Cdc42 were induced. Upon prolonged induction of these proteins, cells started to detach from their substratum. With respect to cell rounding and cell spreading, it is assumed that RhoA plays an opposing role in cell division compared with Rac and Cdc42. RhoA is likely involved in cell rounding and cleavage furrow formation, which is induced by the contractile ring consisting of actinomyosin [11]. Activated RhoA induces phosphorylation of myosin light chains, which is directly associated with cleavage furrow induction [12]. P21-activating kinase (PAK), which is activated by either Rac or Cdc42, blocks the phosphorylation of myosin light chains induced by RhoA. This results in decreased myosin activity, a reduction in contractility, and in the disassembly of stress fibres [3,13]. Expression of dominant-negative Rho kinase, the target of Rho GTPase, impaired segregation of the cleavage furrow-glial filaments resulting in multinucleated cells [14]. Although we did not investigate the effects of RhoA in this study, our data clearly demonstrate that expression of active V12Rac or V12Cdc42 mimics the effect of dominant-negative Rho: expression of V12Rac or

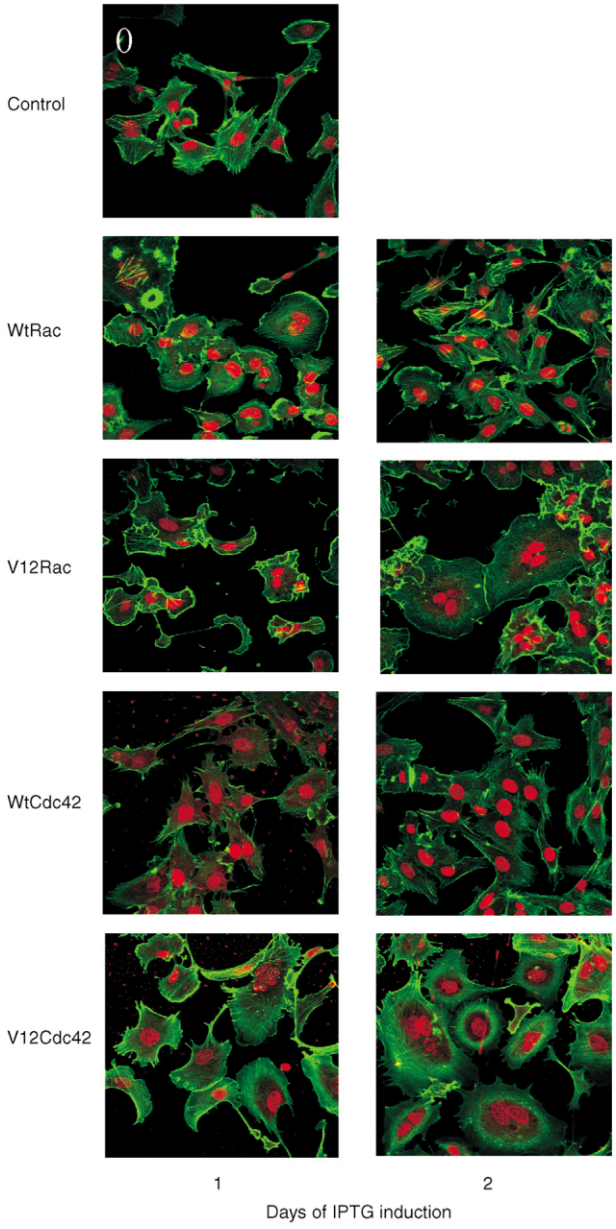

Fig. 4. Effect of Rac or Cdc42 activation on the morphology of porcine aortic endothelial (PAE) cells. WtRac or WtCdc42 and V12Rac or V12Cdc42 PAE cells were cultured for 0, 1 and 2 days in the presence of 2 mM IPTG and were after fixation, stained for actin by TRITC-Phalloidin and for nuclei by TO-PRO-3 staining as described in Materials and methods. 300 000 cells were seeded in a six-well plate and cells were followed for 2 days in the presence of IPTG. The experiment was performed three times with similar results. Representative pictures are presented.

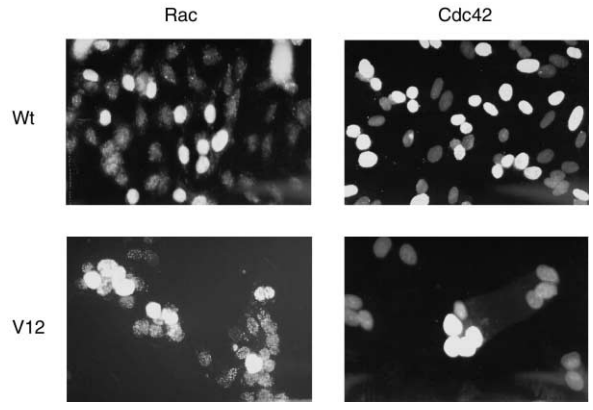

Fig. 5. BrdU incorporation in WtRac, WtCdc42 cells and their V12 variants after various periods of Rac/Cdc42 induction. Cells were cultured for 4 days with 2 mM IPTG, the last 24 h including 1 μM BrdU, and analysed for BrdU incorporation as described in Materials and methods. BrdU incorporating nuclei stain yellow and appear grey on the photo, nuclei were stained with EtBr and appear bright on the photo. Note the multinucleated cells in the V12 variants after 4 days of induction, with not all of the nuclei incorporating BrdU.

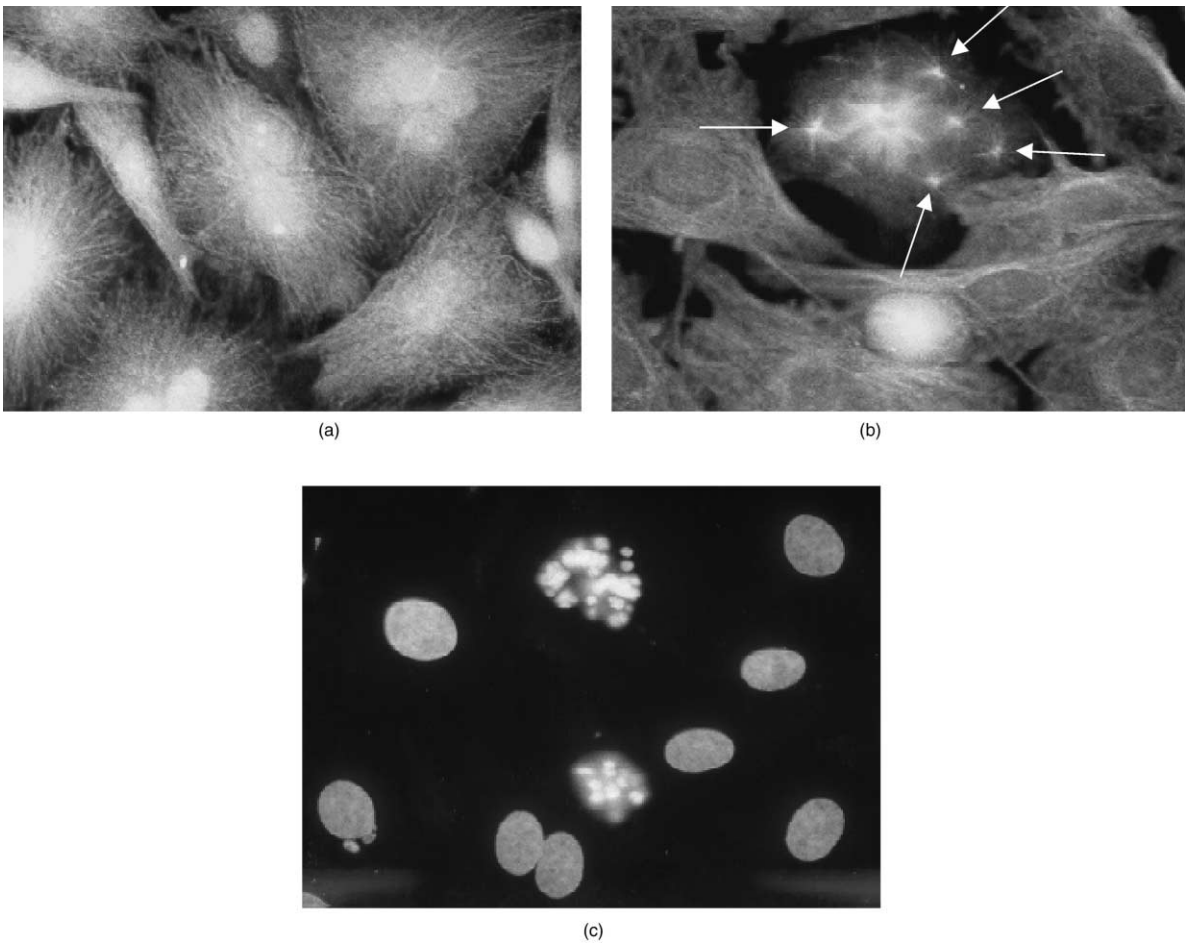

Fig. 6. Microtubule organising centre (MTOC), in V12Rac cells. V12Rac cells were cultured for 4 days in the presence of 2 mM IPTG (a), the last 24 h including 5 μM paclitaxel (b and c), and stained for β-tubulin (a and b) or with DAPI (c) as described in Materials and methods. Arrows in b indicate the MTOCs.

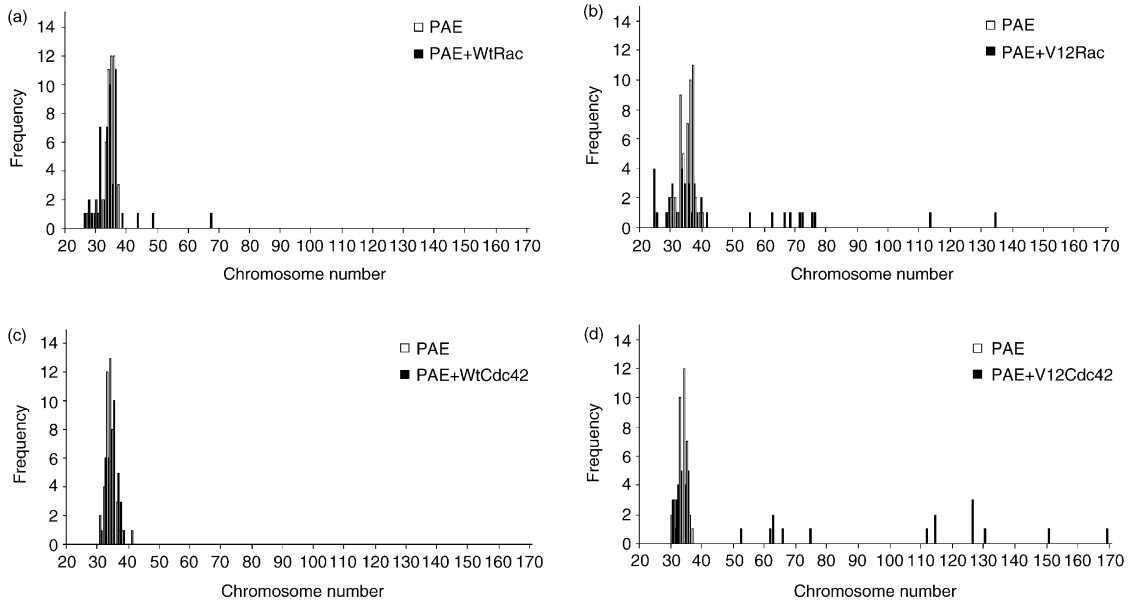

Fig. 7. Karyogram of WtRac, WtCdc42 porcine aortic endothelial (PAE) cells and of their V12 variants after 4 days of culturing in the presence of 2 mM IPTG. The number of chromosomes of 100 mitotic nuclei were counted for each cell variant as described in Materials and methods.

V12Cdc42 stabilises the bridge-like cytoplasmic structure formed between the unseparated daughter cells (Figs. 4 and 5). Surprisingly, however, this inhibition of cytokinesis does not lead to a complete inhibition of DNA synthesis, but finally results in endoreplication by which multinucleated cells become generated (Figs. 3 and 4), a process known as 'mitotic slippage'. Uncoupling of nuclear replication and cytokinesis leads, furthermore, to genomic instability (Fig. 7). Activation of Rac has recently been reported as a crucial mediator of integrin-specific control of cell cycle in endothelial cells [15]. Our findings suggest that sustained activation of V12Rac, and also of V12Cdc42, results in genomic instability which usually leads to cell death, but from which occasionally (more) tumorigenic variants may emerge.

Acknowledgements

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References

1. Van Aelst L, D'Souza-Schorey C. Rho GTPases and signaling networks. *Genes Dev* 1997, **11**, 2295–2322.
2. Hall A. Rho GTPases and the actin cytoskeleton. *Science* 1998, **279**, 509–514.
3. Sanders LC, Matsumura F, Bokoch GM, *et al*. Inhibition of myosin light chain kinase by p21-activated inase. *Science* 1999, **283**, 2083–2085.
4. Olson MF, Ashworth A, Hall A. An essential role for Rho, Rac, and Cdc42 GTPases in cell cycle progression through G1. *Science* 1995, **269**, 1270–1272.
5. Gjoerup O, Lukas J, Bartek J, *et al*. Rac and Cdc42 are potent stimulators of E2F-dependent transcription capable of promoting retinoblastoma susceptibility gene product hyperphosphorylation. *J Biol Chem* 1998, **273**, 18812–18818.
6. Welch H, Equinoa A, Stephens LR, *et al*. Protein kinase B and rac are activated in parallel within a phosphatidylinositide 3OH-kinase-controlled signaling pathway. *J Biol Chem* 1998, **273**, 11248–11256.
7. Van Leeuwen FN, van Delft S, Kaine HE, *et al*. RAC regulates phosphorylation of the myosin-II heavy chain, actinomyosin disassembly and cell spreading. *Nature Cell Biology* 1999, **1**, 242–248.
8. Sanger JM, Reingold AM, Sanger JW. Cell surface changes during mitosis and cytokinesis of epithelial cells. *Cell Tissue Res* 1984, **237**, 409–417.
9. Allen WE, Jones GE, Pollard JW, *et al*. Rho, Rac and Cdc42 regulate actin organization and cell adhesion in macrophages. *J Cell Sci* 1997, **110**, 707–720.
10. Moore KA, Sethi R, Doanes AM, *et al*. Rac1 is required for cell proliferation and G2/M progression. *Biochem J* 1997, **326**, 17–20.
11. Dutarte H, Davoust J, Gorvel JP, *et al*. Cytokinesis arrest and redistribution of actin-cytoskeleton regulatory components in cells expressing the Rho GTPase CDC42Hs. *J Cell Sci* 1996, **109**, 367–377.
12. Kosako H, Goto H, Yanagida M, *et al*. Specific accumulation of Rho-associated kinase at the cleavage furrow during cytokinesis: cleavage furrow-specific phosphorylation of intermediate filaments. *Oncogene* 1999, **18**, 2783–2788.
13. Van Leeuwen F, Kain HE, Kammen R, *et al*. The guanine nucleotide exchange factor Tiam1 affects neuronal morphology; opposing roles for the small GTPases Rac and Rho. *J Cell Biol* 1997, **139**, 797–807.
14. Yasui Y, Amano M, Nagata K, *et al*. Roles of Rho-associated kinase in cytokiments. *J Cell Biol* 1998, **30**, 1249–1258.
15. Mettouchi A, Klein S, Guo W, *et al*. Integrin-specific ativation of Rac controls progression through the G1 phase of the cell cycle. *Mol Cell* 2001, **8**, 115–127.