Stat3 Activity Is Required for Centrosome Duplication in Chinese Hamster Ovary Cells*

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The centrosome is the major microtubule organizing center in mammalian cells. During interphase, the single centrosome is duplicated and the progeny centrosomes then serve as the spindle poles during mitosis. Little is known about the signals that drive centrosome doubling. In these studies, various inhibitors and molecular approaches were used to demonstrate a role for the Stat pathway in regulating the events of centrosome doubling. Both piceatannol and a dominant negative behaving Stat3 adenovirus were able to disrupt centrosome duplication in hydroxyurea-arrested Chinese hamster ovary cells, demonstrating that Stat3 is a key signaling molecule in the events of centrosome duplication. Investigation into the role of Stat3 signaling during centrosome production demonstrated that Stat3 does not directly regulate the transcription of the centrosome genes encoding γ-tubulin and PCM-1. Instead, Stat3 apparently regulated γ-tubulin levels through post-transcriptional mechanisms whereas PCM-1 levels actually increased when Stat3 was inhibited, suggesting more complex mechanisms for regulating PCM-1 production. These studies demonstrate that Stat3 plays a vital role in centrosome duplication events, although the downstream targets of Stat3 activation leading to centrosome production remain to be established. The proposed signaling pathway utilizes Stat3 as a fundamental signaling molecule that directs the production of the various centrosome proteins indirectly.

The centrosome, composed of a pair of centrioles plus pericentriolar material, is responsible for nucleating and organizing microtubules in animal cells. As the cell cycle proceeds, the centrosome is duplicated only once to allow formation of a bipolar spindle during mitosis. Improper doubling of the centrosome leads to either failed mitosis or to the formation of an aberrant multipolar spindle, which results in improper chromosome segregation. The net effect of these abnormal mitotic events is the formation of either polyploid or aneuploid cells, both of which can have deleterious effects on the cell and may have devastating consequences for a multicellular organism by contributing to the formation of cancers. The mechanisms that link centrosome doubling to other cell cycle progression events are beginning to be defined. Previous studies using Chinese hamster ovary (CHO) cells demonstrated that activation of the EGF receptor (EGF-R) is critical for driving centrosome doubling (1–3). Ultimately, activation of the EGF-R sets in motion a series of events leading to phosphorylation of pRb and activation of E2F (4), followed by synthesis of cyclins E and A, both of which are important for centrosome doubling through their interaction with Cdk2 (4–7). In addition, the activities of the tumor suppressor p53 and various kinases have also been shown to be critical for accurate centrosome doubling (8–12). However, the signal transduction pathways linking EGF-R activation to the cell cycle regulators that control centrosome doubling have never been established. Studies have shown that ligand binding to EGF-R triggers a series of molecular events involving the cytoplasmic domain of the receptor (13). Ligand activation induces receptor dimerization followed by tyrosine autophosphorylation, and the activated receptor then transduces the signal in the cytoplasmic compartment. Multiple EGF-R signaling relay intermediates have been identified, including phosphatidylinositol 3-kinase (14–16), Ras/MAP kinase (17, 18), phospholipase Cγ (19–21), and Stats (13, 22–25). Ultimately, activation of these signaling intermediates results in a cellular response, which often includes regulated cell growth and proliferation.

The present study investigated the mechanism of signal transduction used for controlling centrosome doubling in CHO cells. We report that Stat3 activity is necessary for centrosome doubling, whereas inhibition of either phosphatidylinositol 3-kinase, Ras/MAP kinase, or phospholipase Cγ had no effect on production of centrosomes. Moreover, it is demonstrated that Stat3 does not target centrosome genes directly. This suggests more complex mechanisms, which include transcriptional and translational processes, for regulating centrosome production. These studies are the first to demonstrate a role for signal transduction pathways in regulating centrosome doubling during the cell cycle.

EXPERIMENTAL PROCEDURES

Cell Culture—CHO cells were maintained as detailed previously (3). To induce overproduction of centrosomes, CHO cells were arrested for 40 h with 2 mM hydroxyurea (HU). To visualize centrosomes, HU-arrested CHO cells were either fixed and processed directly (see below) or were treated with 5 mM caffeine to induce mitosis (3) because centrosomes were easier to count in mitotic cells. For some experiments, U0126 (1–25 μM), LY294002 (25–100 μM), SB202190 (1–25 μM), wortmannin (25–200 μM), U73122 (5–25 μM), or piceatannol (10–25 μM) was added to culture media at the time HU was added. Microscopy—Cells were fixed and processed for immunofluorescence

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‡ The abbreviations used are: CHO, Chinese hamster ovary; Stat, signal transducer and activator of transcription; EGF, epidermal growth factor; EGF-R, epidermal growth factor receptor; HU, hydroxyurea; RT, reverse transcriptase; GFP, green fluorescent protein; MAP, mitogen-activated protein; DAPI, 4',6-diamidino-2-phenylindole dihydrochloride.
microscopy using one of two methods. For some studies, cells on coverslips were fixed directly by immersion in $-20^\circ$C MeOH and then processed for immunofluorescence using previously reported procedures (3). For other experiments, cells were driven into mitosis by addition of 5 mM caffeine, rounded cells were collected by mitotic shake-off, and then the cells were centrifuged onto polylysine-coated coverslips. The coverslips were then fixed by immersion in $-20^\circ$C MeOH and processed for immunofluorescence microscopy. Antibodies used for immunofluorescence microscopy included a previously characterized anti-PCM-1 antiserum (3), a previously described autoimmune anticentrosome antiserum (3), and commercially available antibodies against $\alpha$-tubulin (Sigma-Aldrich), $\gamma$-tubulin (Covance, Princeton, NJ), and pericentrin (Covance).

Electron microscopy was performed essentially as described previously (3, 27). Briefly, CHO cells were treated with either HU alone or HU plus piceatannol, fixed, and then processed.

Immunoblot Analysis—Immunoblot analysis was performed using previously reported methods (3, 27). Briefly, extracts prepared from either randomly cycling, HU-arrested, or HU- and piceatannol-treated CHO cells were resolved by SDS-PAGE, the proteins were transferred to nitrocellulose, and then the blots were probed. The blots were developed using chemiluminescence procedures (27). Primary antibodies used were a previously characterized rabbit antibody against PCM-1 (3), anti-$\gamma$-tubulin (Covance), and antibodies against Stats 1, 3, and 5 (Santa Cruz Biotechnology, Inc). Blots were stripped and reprobed with anti-$\alpha$-tubulin to verify equal loading per well.

Stat3 phosphorylation status also was determined by immunoblot analysis using anti-phospho-Stat3 antibody that specifically recognized phosphotyrosine forms of Stat3 (Santa Cruz). For these studies, control CHO cells, HU-treated CHO cells, and CHO cells treated with both HU and piceatannol were collected and lysed in a buffer composed of 10 mM Tris-HCl, pH 8.0, 5 mM EDTA, 50 mM NaCl, 50 mM NaF, 1 mM Na$_3$VO$_4$, 1 mM okadaic acid, 1% Triton X-100, and 1 µg/ml each of chymostatin, leupeptin, antipain, and pepstatin. After 20 min on ice, the preparations were centrifuged at 13,000 x $g$ for 30 min at 4 $^\circ$C. The supernatants were retained, and protein concentrations were determined using the Bradford reagent (Bio-Rad). 150 µg of each extract was incubated twice for 60 min with 100 µl of Protein A-agarose beads, and then 10 µl of anti-Stat3 was added overnight at 4 $^\circ$C. The supernatants were retained, and protein concentrations were determined using the Bradford reagent (Bio-Rad). 150 µg of each extract was incubated twice for 60 min with 100 µl of Protein A-agarose beads, and then 10 µl of anti-Stat3 was added overnight at 4 $^\circ$C. The following day, 50 µl of Protein A-agarose was added, and the incubation was continued at 4 $^\circ$C for 90 min. The agarose beads were then pelleted and rinsed three times with phosphate-buffered saline followed by three rinses in phosphate-buffered saline + 0.5 M NaCl. The beads were then suspended in SDS-PAGE sample buffer and assayed for phospho-Stat3 by immunoblot analysis using anti-phospho-Stat3 antibody.
Semiquantitative RT-PCR—Total RNA was isolated from randomly cycling control CHO cells, HU-arrested CHO cells, and HU- and piceatannol-treated CHO cells using RNA Stat60 (Tel-Test, Inc., Friendswood, TX) according to manufacturer recommendations. RNA samples were then amplified with the SuperScript One-Step RT-PCR kit (Invitrogen) using the protocol recommended by the manufacturer. Samples were collected after varying numbers of PCR cycles, and the samples were analyzed by agarose gel electrophoresis. For quantitation, 

\[ ^{32}PdCTP \text{ was added to reaction mixtures. Primers used for amplification were as follows: } \]

- PTT TTT CTA GAC GG.
- CCA AAG GGT CAT CAT CTC TGC.
- ATT TGG CAG

\[ \text{forward, } \]

- GAG AAG GAT BGG TTC TGG ATG; PCM-1, forward, ATG

\[ \text{reverse, } \]

- CGY ACA GGA GGM GTT CC; reverse, GAN GAY TGN GGA GAW ATA RC; CEP135, forward, ATG ACA ACA GCT GCC GCA AGG; reverse,

\[ \text{GCT GTG TAC TCG ATT TGC ATC; and GAPDH (control), forward, } \]

- CCA AAG GGT CAT CAT CTC TGC; reverse, ATT TGG CAG GTT TTT CTA GAC GC. 

Transfection—Although Stat3β has been shown to have functions distinct from Stat3α in cells (25), overexpression allows it to serve as a dominant negative factor by specific inhibition of Stat3 function. A dominant negative acting form of Stat3β in an adenoviral vector (Stat3-EVA) was obtained from Dr. Eric Hauri (Moffitt Cancer Center, University of South Florida). The dominant negative behaving form of Stat3β (which is controlled by a cytomegalovirus promoter) and the adenoviral construct have been described previously (26). CHO cells were infected in complete medium at a multiplicity of infection of 50–100, which resulted in >99% infection as judged by GFP expression (not shown). After 24 h, the media were replaced and HU was added. After 40 h, centrosome doubling was assayed by immunofluorescence microscopy using methods outlined earlier. Controls were infected with adenovirus containing a vector encoding GFP alone.

RESULTS

Initial experiments were designed to ascertain which of the major cellular signaling mechanisms are involved in centrosome doubling. Broad spectrum inhibitors that disrupted the signal transduction of these various pathways were utilized. The inhibitors used were either wortmannin or LY294002. These inhibit phosphatidylinositol 3-kinase, or U0126, U73122, or SB202190, which are known to inhibit extracellular regulated kinases, phospholipase Cγ, and MAP kinase pathways, respectively. Cells were also treated with piceatannol, an inhibitor of the Jak/Stat pathway. Using a centrosome replication assay that was previously developed by this laboratory, centrosome overproduction was induced by the addition of HU to the media. Cells were then either fixed directly after the appropriate incubation or treated with caffeine, which drives cells into mitosis and induces centrosome separation, allowing easier centrosome counting. To determine the effects of these various inhibitors, cells were incubated with an individual inhibitor while arrested with HU and then analyzed for centrosome synthesis by immunofluorescence microscopy using anticientrosome autoantiserum. As shown in Fig. 1A, HU-treated CHO cells synthesized multiple centrosomes with the average number of centrosomes being 4–5 per cell (Fig. 1). Also note that the HU-treated cells contained condensed and fragmented chromatin as reported previously (3). Cells treated with either LY294002 (similar results obtained using wortmannin (data not shown)), U0126, U73122, or SB202190 contained centrosome numbers close to those in cells treated with HU alone. Most of these cells also underwent nuclear envelope breakdown with fragmented chromatin. In contrast, cells treated with piceatannol and HU failed to synthesize centrosomes, and displayed only a single centrosome (Fig. 1). Moreover, few of the cells treated with piceatannol progressed to mitosis following caffeine addition, indicated by the presence of an intact nucleus. These findings suggest that the Stat pathway is a likely candidate for controlling centrosome doubling in HU-arrested CHO cells.

Additional studies were performed to investigate a potential role for Stats in centrosome doubling in CHO cells. Initially, CHO cell extracts were analyzed by immunoblot analysis using antibodies against Stats 1, 3, and 5. Both Stat3 and Stat5 were shown to be present in CHO cells, but Stat1 was not identified (Fig. 2). To verify that piceatannol was inhibiting Stat activity, Stat3 was immunoprecipitated from treated cells, and the phospho-Stat3 level was measured by immunoblot analysis. As shown in Fig. 2, Stat3 phosphorylation was inhibited by >80% (n = 3) in piceatannol-treated cells. To determine whether the activity of Stats is important for centrosome doubling, a dominant negative behaving adenoviral construct of Stat3β, called Stat3βΔ, was utilized to disrupt the normal function of the Stat3 signaling pathway. Cells were infected with either a GFP control adenovirus or a GFP dominant negative Stat3, HU was added, and then infected cells were analyzed by immunofluorescence using anticientrosome autoantiserum. Cells that were treated with either HU alone or HU following infection with GFP control adenovirus overproduced centrosomes (Fig. 2). However, centrosome synthesis was blocked in cells treated with HU following infection with the dominant negative form of Stat3.

To demonstrate clearly that centrosome doubling was arrested by inhibition of Stat signaling, electron microscopy was utilized. As shown in Fig. 3, cells treated with HU alone produced multiple centrosomes, in agreement with previous studies (3, 27). In contrast, cells treated with HU and piceatannol contained only one centrosome, as indicated by the presence of only a single or double centriole cylinder. Collectively, these experiments established a role for Stat3 in centrosome doubling events, suggesting that Stat3 is pivotal for controlling this event in CHO cells.

To understand further the molecular mechanisms controlled by Stat3 signaling during centrosome doubling, semiquantitative RT-PCR and Western blot analysis were used to measure the levels of centrosome mRNA and protein, respectively, in treated cells. Initially, RT-PCR determined that levels of γ-tubulin mRNA (Fig. 4) and CEP135 mRNA (not shown) were approximately equal in control, HU-treated, and HU-
piceatannol-treated CHO cells. Similar results were obtained when dominant negative Stat3 infected cells were analyzed (not shown). In contrast, levels of PCM-1 mRNA were increased in cells treated with HU and piceatannol (PIC + HU). Levels of γ-tubulin protein were analyzed by Western blotting using anti-γ-tubulin antibody. c, labeling of control cells (A) and cells treated with HU and piceatannol (C) using anti-γ-tubulin antibody. The corresponding DAPI images are shown in B and D. γ-Tubulin could not be detected in cells treated with piceatannol. Bar, 10 μm.

Fig. 4. Effects of Stat inhibition on γ-tubulin. a, semiquantitative RT-PCR analysis of the γ-tubulin mRNA levels. Cells were either randomly cycling controls (C), arrested with HU alone (HU), or treated with HU and piceatannol (PIC + HU). RNA was isolated and analyzed by RT-PCR. PCR products were collected at 15, 30, and 40 cycles of amplification and then were analyzed by agarose gel electrophoresis. Left lane, the 1-kb ladder. b, CHO cell extracts were made from control (C), HU-treated (HU), or HU- and piceatannol-treated (PIC + HU) cells. Levels of γ-tubulin protein were analyzed by Western blotting using anti-γ-tubulin antibody. c, labeling of control cells (A) and cells treated with HU and piceatannol (C) using anti-γ-tubulin antibody. The corresponding DAPI images are shown in B and D. γ-Tubulin could not be detected in cells treated with piceatannol. Bar, 10 μm.

Fig. 5. Effects of Stat inhibition on PCM-1. a, semiquantitative RT-PCR was performed utilizing RNA isolated from control (C), HU-treated (HU), or HU- and piceatannol-treated (PIC) cells. Samples were collected after PCR cycles 10, 20, 30, and 40 and then analyzed by agarose gel electrophoresis. b, Western blot analysis of PCM-1 protein levels in control (C), HU-treated (HU), and HU- and piceatannol-treated (PIC) cells. c, anti-PCM-1 labeling of either control CHO cells (A) or cells treated with both HU and piceatannol (C and E). The corresponding DAPI images (B, D, and F) are shown. The perinuclear distribution of the overexpressed PCM-1 in cells treated with HU and piceatannol can be observed. Bar, 10 μm.
tisereum. As shown in Fig. 5, PCM-1 protein levels were increased in HU-treated cells relative to levels in untreated controls, which was expected because centrosome numbers were increased in HU-treated cells. However, in contrast to what was observed for γ-tubulin, levels of PCM-1 protein were higher in cells treated with HU and piceatannol relative to levels in either untreated controls or HU-arrested CHO cells. Immunofluorescence microscopy of HU- and piceatannol-treated cells showed that the overexpressed PCM-1 protein was localized in the perinuclear region, although it did not appear to be organized into the centrosome complex (Fig. 5).

Because γ-tubulin is critical for proper microtubule nucleation and organization, immunofluorescence microscopy using antibodies against α-tubulin was performed on control, HU-treated, and HU- and piceatannol-treated CHO cells. As shown in Fig. 6, both control and HU-treated CHO cells contained elaborate and organized microtubule arrays. In contrast, cells treated with the combination of HU and piceatannol contained very few, poorly organized microtubules, and the present microtubules were poorly organized (Fig. 6).

**DISCUSSION**

Centrosome doubling is a vital process that ensures that a functional bipolar spindle is formed during mitosis. The signaling pathways that are involved in controlling centrosome duplication need to be elucidated. In this study, experiments were performed to identify which signal transduction pathways are involved in centrosome duplication during the cell cycle, and it was demonstrated that the Stat pathway plays a role in controlling centrosome doubling events. Broad spectrum inhibitors studies, plus more specific inhibition using a dominant negative acting Stat3β construct, revealed a role solely for the Stat pathway and failed to identify a role for any of the other main cellular signaling pathways in centrosome doubling. These results, verified using both immunofluorescence and electron microscopy, compellingly demonstrate a role for Stat3 in regulation of centrosome doubling in CHO cells.

In this laboratory, previous studies using HU arrest of CHO cells demonstrated a role for EGF in triggering the events of centrosome duplication (3). Activation of the EGF receptor has been shown to trigger multiple signal transduction pathways inside cells including phosphatidylinositol 3-kinase (14–16), MAP kinase (17, 18), phospholipase Cγ (19–21), and Stat5 (13, 22–26). The proposed studies investigate which of these pathways was involved in signal relay from the EGF receptor to the actual events controlling centrosome doubling. The data shown in Figs. 1–3 clearly demonstrate a role for Stat3 in activating centrosome doubling in CHO cells. Whether Stat3 acts exclusively to control centrosome duplication or functions as a heterodimer with Stat5 remains to be established. However, the observation that Stat5 knock-out mice are viable (28) argues against a role for Stat5 in a fundamental process such as centrosome doubling. Moreover, the observation that Stat3 regulates centrosome doubling events may be important for various reasons. For example, Stat3 has been implicated as an oncogene, and constitutive activation of Stat3 has been detected in various cancer cell types (26, 29–32). Centrosome overproduction has also been detected in various cancers, and overproduction of centrosomes leading to the formation of multipolar spindles may contribute to the aneuploidy associated with various cancers (33). It will be interesting to investigate tumors with constitutively active Stat3 for centrosome overproduction. In addition, these results may help to explain why Stat3 knock-out mice die early during embryogenesis (28). Although there are several possible causes for embryonic lethality when Stat3 is knocked out, clearly the inability to duplicate centrosomes would lead to a mitotic arrest phenotype.

The potential mode of action for Stat3 to regulate synthesis of various centrosome components was also investigated. The results demonstrate that different centrosome genes and proteins are regulated differentially by Stat3. Specifically, γ-tubulin levels appear to be regulated post-transcriptionally by Stat3 whereas levels of PCM-1 mRNA and protein both increase during Stat3 inhibition. These data may indicate one of two possibilities. First, each centrosome component may exhibit a unique mechanism of gene regulation by Stat3. Alternatively, the different behavior of these two proteins may indicate differential regulation of separate classes of centrosome proteins. For example, γ-tubulin and various other proteins are involved in regulating microtubule nucleation directly (34), whereas other proteins fulfill structural roles within the centrosome (35). PCM-1, in contrast, performs an unknown function related to cell cycle progression (36). It is possible that different classes of centrosome genes will exhibit separate mechanisms of regulation related either to an essential function of the centrosome (i.e. microtubule nucleation) or to a proliferation-specific function of the centrosome (i.e. duplication). Additional studies investigating numerous centrosome components are needed to test these possibilities.

The regulation of γ-tubulin and PCM-1 levels by Stat3 does not appear to be direct, and more complex mechanisms of regulation must be proposed. If Stat3 regulated these two genes directly, the levels of PCM-1 and γ-tubulin mRNAs would drop when Stat3 activity was blocked. However, the levels of PCM-1 mRNA and protein increased whereas the level of γ-tubulin mRNA was unchanged in either HU or piceatannol-treated cells relative to controls. Instead, γ-tubulin protein levels dropped when Stat3 activity was blocked. The results related to PCM-1 can be explained by one of two mechanisms. In one scheme, Stat3 controls the synthesis of a factor that regulates the synthesis of PCM-1 mRNA. When Stat3 is inhibited, this putative factor is lost and PCM-1 mRNA and protein are overproduced. Alternatively, Stat3 may control the synthe-
sis of a factor that regulates PCM-1 mRNA degradation during the cell cycle. Failure to synthesize this putative molecule would lead to build-up of PCM-1 mRNA and protein. Because little is known about the regulation of PCM-1 synthesis, it is not possible to test these two hypotheses at present.

The results concerning γ-tubulin are more difficult to explain. As shown in Fig. 4, γ-tubulin mRNA levels remain relatively unchanged during either HU treatment (when centrosomes are overproduced) or when Stat3 is inhibited (and centrosome production is blocked). Along with previous results, which demonstrate that γ-tubulin levels do not fluctuate during the cell cycle (37), the data indicate that γ-tubulin behaves more like a housekeeping gene, in that its mRNA production does not depend on growth factor activation or cell cycling. However, it is difficult to explain the precipitous drop in γ-tubulin protein levels when Stat3 activity is blocked. Perhaps Stat3 regulates the synthesis of a component that controls either the production or degradation of γ-tubulin. Like PCM-1, little is known about the regulation of γ-tubulin synthesis during the cell cycle, so these possibilities cannot be tested. Regardless, the data concerning PCM-1 and γ-tubulin synthesis in cells where Stat3 is inhibited indicate that production of centrosome subunits is a complex process with varied mechanisms of regulation.

Cells in which Stat3 activity was inhibited contained few microtubules and perinuclear aggregates of PCM-1. The simplest interpretation for the decrease in microtubules is that in the absence of γ-tubulin (which is necessary for microtubule nucleation (38)) microtubules cannot be nucleated or organized properly. Concerning the perinuclear aggregates of PCM-1 (Fig. 5), it was recently demonstrated that PCM-1 contains two domains that control self-association (39). Moreover, when PCM-1 fragments containing these self-association domains were overexpressed, the PCM-1 fragments were able to assemble into aggregates similar to those observed in CHO cells following piceatannol treatment. In preliminary studies to test directly whether untreated CHO cells would assemble perinuclear aggregates of PCM-1, plasmids were transfected encoding full-length PCM-1 into CHO cells (data not shown). However, aggregation of PCM-1 in these cells was not achieved because obtained levels of PCM-1 overexpression were not equivalent to levels detected in piceatannol-treated cells in the PCM-1 transfection experiments.

In summary, these studies demonstrate a role for Stat3 in the events leading to accurate centrosome doubling in CHO cells. Previous studies utilizing Hu-arrested CHO cells for studying the regulation of centrosome doubling have contributed significantly to our understanding of how centrosome duplication is coordinated with other cell cycle progression events. Activation of the EGF receptor in CHO cells (3) ultimately sets in motion a series of events leading to inhibition of Rb and activation of E2F (4) followed by synthesis of cyclins E (6) and A (4), activation of Cdk2 (4, 7), and assembly of centrosomes. The reported studies demonstrate that Stat3 acts in the signal transduction pathway in CHO cells leading from EGF receptor activation to the nucleus during centrosome doubling. However, these studies also demonstrate that Stat3 does not control the centrosome genes directly (at least γ-tubulin and PCM-1) and suggest that downstream targets between Stat3 and regulation of centrosome gene expression remain to be identified. Identifying these downstream targets will be critical to elucidating the mechanisms that control the regulation of the synthesis of centrosome subunits and assembly of those subunits into a functional centrosome during the cell cycle. Finally, the reported data demonstrate that expression of centrosome genes is controlled by multiple mechanisms (including translational and pre-translational processes) in mammalian cells, highlighting the complexity of the coordination of centrosome duplication and cell cycle progression.

REFERENCES
1. Sherline, P., and Mascardo, R. (1982) J. Cell Biol. 95, 316–322
2. Mascardo, R., and Sherline, P. (1984) Diabetes 33, 1099–1105
3. Balcin, R., Bao, L., Zimmer, W. E., Brown, K., and Zinkowski, R. P. (1995) J. Cell Biol. 130, 105–115
4. Meraldi, P., Kukas, J., and Fry, A. M., Bartek, J., and Nigg, E. A. (1999) Nat. Cell Biol. 1, 88–93
5. Hinchcliffe, E. H., Chuan, L., Thompson, E. A., Maller, J. L., and Sluder, G. (1999) Science 283, 851–854
6. Lacey, K., Jackson, P., and Stearns, T. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 2817–2822
7. Matsumoto, Y., Hayasaki, K., and Nishida, E. (1999) Curr. Biol. 9, 429–432
8. Fukasawa, K., Chiu, T., Kuriyama, R., Rulong, S., and Vande Woude, G. F. (1996) Science 271, 1744–1747
9. Zhou, H., Huang, J., Zhang, Q., Wu, L. W., Gray, J. W., Sahin, A., Brinkley, B. R., and Sen, S. (1998) Nat. Genet. 20, 189–193
10. Lane, H. A., and Nigg, E. A. (1996) J. Cell Biol. 135, 1701–1713
11. Mayor, T., Meraldi, P., Stierhof, Y. D., Nigg, E. A., and Fry, A. M. (1999) FEBS Lett. 452, 92–95
12. Liu, X., and Erikson, R. L. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 8672–8676
13. Ulrich, A., and Schlessinger, J. (1990) Cell 61, 203–212
14. Hann, C. Y., and Nathans, D. (1994) Curr. Biol. 4, 555–562
15. Kim, H. H., Sterke, S. L., and Koland, J. G. (1994) J. Biol. Chem. 269, 24747–24755
16. Soltos, S. P., Carraway, K. L., Prigent, S. A., Glick, W. G., and Cantley, L. C. (1994) Mol. Cell. Biol. 14, 3550–3558
17. Campos-Gonzalez, R., and Glenney, J. R. (1992) J. Biol. Chem. 267, 14535–14538
18. Selva, E., Raden, D. L., and Davis, R. J. (1993) J. Biol. Chem. 268, 2250–2254
19. Zhu, G., Decker, S. J., and Saltiel, A. R. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 9559–9563
20. Gergel, J. R., Mcnamara, D. J., Dobrusin, E. M., Zhu, G., Saltiel, A. R., and Miller, W. T. (1994) Biochemistry 33, 14671–14678
21. Chen, P., Xie, H., Sekar, M. C., Gupta, R., and Wells, A. (1994) J. Biol. Chem. 269, 127, 847–857
22. Park, O. K., Schaefer, T. S., and Nigg, E. A. (1996) FEBS Lett. 389, 237–242
23. Quelle, F. W., Thierfelder, W., Wittthun, B. A., Tang, B. C., Cohen, S., and Ihle, J. N. (1995) J. Biol. Chem. 270, 20775–20780
24. Grewal, S. I., and Dutta, A. (1999) Science 286, 2129–2132
25. Maritan, D., Gualeri, M., and Nigg, E. A. (2000) J. Cell Biol. 150, 175–179
26. Song, L., Turkson, J., Karras, J. G., Jove, R., and Haura, E. B. (2003) Stem Cells 21, 107–116
27. DeArmond, D., Brattain, M. G., Jessup, J. M., Kreisberg, J., Malik, S., Zhao, S., and Freeman, J. W. (2003) Oncogene 22, 7781–7785
28. Scholz, A., Heinzl, S., Steen, K. M., Peters, M., Wolfel, M., Haufl, P., Schirmer, M., Wiedemann, B., and Rosewicz, S. (2003) Gastroenterology 125, 891–905
29. Cao, V., Mignavacca, M., Bocca, V., Barnabas, G., Usevich, M., Gebbia, N., and Russ, A. (2003) J. Cell. Physiol. 197, 157–168
30. Brinkley, B. R., and Goepfert, T. M. (1998) Cell Motil. Cytoskeleton 41, 281–288
31. Weise, C., and Zheng, Y. (2000) Nat. Cell Biol. 2, 358–364
32. Dietzberg, J. B., Zimanyi, W., Stoffers, A., and Weis, J. (1999) J. Cell Biol. 145, 163–174
33. Akira, S., Sato, K., and Akira, K. (1999) J. Cell Biol. 145, 183–192
34. Balcin, R., Simerly, C., Takahashi, D., and Schatten, G. (2002) Cell Motil. Cytoskeleton 52, 183–192
35. Dumontet, C., Duran, G. E., Steger, K. A., Murphy, G. L., Sussman, H. H., and Sible, B. L. (1996) Cell Motil. Cytoskeleton 35, 49–58
36. Shu, H. B., and Joshi, H. C. (1995) J. Cell Biol. 130, 1137–1147
37. Kubo, A., and Tsukita, S. (2003) J. Cell. Sci. 116, 919–928
