Connexins constitute a family of transmembrane proteins that form channels between neighboring cells. These channels are organized in special plasma membrane domains termed gap junctions and provide for direct intercellular communication (1). Gap junction channels allow adjacent cells to exchange second messengers, ions, and cellular metabolites and are involved in the regulation of cell proliferation, differentiation, and cell death (2). There is increasing evidence that disruption of gap junctional intercellular communication (GJIC) is one important step in malignant transformation (3). The most abundantly expressed connexin in human tissues, connexin-43 (Cx43), has been identified as a tumor suppressor protein that reverses the malignancy of tumorigenic cells (4–7).

Gap junctions are highly mobile plasma membrane domains with rapid turnover rates (8, 9). Cx43 has a half-life ranging from 1.5–5 h, depending on the cell type studied (10–13). Previous work has shown that modulation of the Cx43 turnover rate might be important in the regulation of GJIC (14). Degradation of Cx43 has been shown to involve both the lysosome and the ubiquitin-proteasome system (14–16). Lysosomes are considered to be involved in the destruction of internalized gap junctions, as well as in degrading Cx43 delivered from early secretory compartments (17–20). The proteasome is required for the degradation of newly synthesized Cx43 during endoplasmic reticulum-associated degradation (21, 22). Moreover, several studies indicate that proteasomal activity is required for internalization of Cx43 at the plasma membrane (14, 16, 20, 23). However, the mechanism by which proteasomal inhibition interferes with Cx43 internalization is currently unknown.

Degradation of a protein via the ubiquitin-proteasome system occurs via two distinct and successive steps. First, the target protein is conjugated to polyubiquitin chains on lysines. Second, the ubiquitin-conjugated protein is recognized by the 26 S proteasome, a large, multicatalytic protease, and degraded (24). In contrast to polyubiquitin chains, monoubiquitin is involved in regulating proteins by proteasome-independent processes. One characterized function of monoubiquitin is as a signal for internalization and subsequent endosomal sorting of many cell surface proteins (25). Receptor tyrosine kinases were recently found to be monoubiquitinated at multiple sites in response to ligand binding (26, 27). In these cases, a single ubiquitin is sufficient for both receptor internalization and subsequent degradation. Targeting of proteins for ubiquitination is mediated by the E3 ubiquitin-protein isopeptide ligases. Often, poly- or monoubiquitination of proteins is preceded by phosphorylation that generates a docking site for the E3 ligase (25). For instance, the cell-cell adhesion protein E-cadherin is monoubiquitinated and undergoes endocytosis after activation of the tyrosine kinase Src (28).

Many growth factors, viral oncoproteins, and carcinogenic chemicals influence gap junction channels (29). The tumor-promoting phorbol ester 12-O-tetradecanoylphorbol 13-acetate (TPA) induces a rapid phosphorylation of Cx43 and inhibition of GJIC in a number of cell types (30–34). In many cell types, the TPA-induced block in GJIC is followed by loss of Cx43 gap junction plaques at the plasma membrane (34–37). TPA has
also been reported to cause a reduction in the Cx43 protein level (38). However, the mechanisms underlying the TPA-induced delocalization and loss of Cx43 protein are poorly understood.

We have previously reported that Cx43 is ubiquitinated at the plasma membrane in response to mitogen-activated protein kinase (MAPK) activation (23). In the present study we show that Cx43 is ubiquitinated after TPA treatment, in a protein kinase C (PKC) dependent manner. The TPA-induced ubiquitination of Cx43 is associated with internalization and degradation of Cx43. We provide evidence that TPA induces conjugation of multiple monoubiquitins rather than a polyubiquitin chain on Cx43. Furthermore, we show that proteasomal inhibitors counteract the TPA-induced Cx43 ubiquitination. Thus the proteasome might play an indirect role in Cx43 internalization and degradation by affecting the level of Cx43 mono-ubiquitination and subsequent trafficking of Cx43 to endosomal compartments.

EXPERIMENTAL PROCEDURES

Reagents and Antibodies—TPA, epidermal growth factor (EGF), MG132, lactacystin, N-acetyl-leucyl-leucyl-norleucine (ALLN), leupeptin, and cycloheximide were obtained from Sigma. Protein A-Sepharose was from Amersham Biosciences. The purified polyubiquitin chain was obtained from Affinity Research Products (Exeter, UK). In this chain, ubiquitin is polymerized through lysine 48. The ubiquitin moiety carries a lysine to arginine mutation at residue 29 to block ubiquitin polymerization through this lysine. The anti-Cx43 antisera was made in rabbits injected with a synthetic peptide consisting of the 20 C-terminal amino acids of Cx43 (39). The P4D1 (mouse IgG) and FK1 (mouse IgM) anti-ubiquitin antibodies were obtained from Babco (Cowan, CA) and Affinity Research Products (Exeter, UK), respectively. Both antibodies have been extensively characterized by one-dimensional Western blotting (26, 40, 41). Anti-β-catenin (mouse IgG) and anti-EGF receptor (mouse IgG) were from BD Transduction Laboratories (San Diego, CA). Alexa 488-conjugated goat anti-rabbit IgG antibodies were from Sigma. Goat anti-rabbit IgG secondary antibodies conjugated to horseradish peroxidase were from Bio-Rad. Horseradish peroxidase-conjugated donkey anti-mouse IgG and donkey anti-mouse IgM were obtained from Jackson Immunoresearch Laboratories, Inc. (West Grove, PA).

Cell Culture and Treatment—The rat liver epithelial cell line IAR20 was obtained from the International Agency for Research on Cancer (Lyon, France). The cells were originally isolated from normal inbred BD-IV rats and express endogenous Cx43 (35, 43). A431 and HeLa cells were from the American Type Culture Collection (Manassas, VA). Cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal bovine serum (Invitrogen). Cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal bovine serum (Invitrogen). Cells were washed with PBS and scraped in 500 µl SDS electrophoresis buffer and heated to 95 °C for 5 min prior to protein separation by 8% SDS-PAGE. Western blotting was performed as described above.

RESULTS

TPA Induces Ubiquitination of Cx43—In gap junctional communication-competent cells, Cx43 usually forms three major bands in SDS-PAGE reflecting different phosphorylation states of Cx43. The fastest migrating band of Cx43, termed Cx43-P0, is converted to two slower migrating species, Cx43-P1 and Cx43-P2 (Fig. 1A). TPA induced rapid hyperphosphorylation of Cx43 in IAR20 cells, seen as an increase in the Cx43-P2 band level and a loss of the Cx43-P0 and Cx43-P1 bands (Fig. 1A). The TPA-induced hyperphosphorylation of Cx43 was transient, and after 4 h of TPA treatment the level of the Cx43-P2 band was reduced and the level of the Cx43-P0 and Cx43-P1 bands recovered. In agreement with previous studies, TPA induced a rapid and transient inhibition of GJIC between IAR20 cells (Ref. 44 and Fig. 1A).

Covalent binding of ubiquitin serves as a signal for regulating the turnover of several plasma membrane proteins (25, 48). We previously showed that hyperphosphorylation of Cx43 and inhibition of GJIC in response to EGF treatment is accompanied with increased Cx43 ubiquitination (23). Here, we wanted to investigate whether the Cx43 ubiquitination level is affected by exposure to TPA. Immunoprecipitation of Cx43 and subsequent Western blotting with anti-ubiquitin antibodies revealed that the level of Cx43 ubiquitination was strongly increased in response to TPA treatment (Fig. 1B). As control, a ubiquitin smear was not detected when anti-Cx43 antibodies were replaced with preimmune serum. The TPA-induced ubiquitination of Cx43 was transient and occurred concomitantly with the Cx43 hyperphosphorylation.

Next we asked which signaling pathways are involved in the ubiquitination of Cx43 in response to TPA treatment. As shown in Fig. 2A, the change in the Cx43 phosphorylation status and GJIC in response to TPA treatment was partly counteracted by...
the PKC inhibitor GF109203X, indicating that PKC is involved in TPA-induced hyperphosphorylation of Cx43 and inhibition of GJIC in IAR20 cells. The effect of TPA on Cx43 phosphorylation status and GJIC was also partly counteracted by the MEK1 inhibitor PD98059. These observations are in accordance with previous studies in other cell types, indicating that TPA-induced hyperphosphorylation of Cx43 and GJIC inhibition involves the MAPK signaling pathway (49, 50). The TPA-induced ubiquitination of Cx43 was completely counteracted by GF109203X, indicating that this modification is mediated via PKC (Fig. 2B). Moreover, the TPA-induced ubiquitination of Cx43 was partly counteracted by PD98059, indicating a partial involvement of the MAPK pathway.

**TPA Induces Cx43 Internalization and Degradation**—Next we investigated the fate of Cx43 after its TPA-induced hyperphosphorylation and ubiquitination. It has been shown previously that the Cx43-P0 isoform of Cx43 is localized in the Golgi region, whereas Cx43-P1 and Cx43-P2 are mainly localized in the plasma membrane (46). As determined by fluorescence microscopy, most Cx43 protein in IAR20 cells is arranged as gap junctions at the plasma membrane, whereas faint Cx43 staining is also found in the Golgi area (Fig. 3A). This localization pattern of Cx43 is in accordance with the Western blot experiments showing that most Cx43 is in the Cx43-P1 or Cx43-P2 level (Fig. 1A). TPA treatment for 30 min induced disorganization of Cx43 gap junctions and internalization of Cx43 from the plasma membrane to intracellular vesicular structures (Fig. 3B). This TPA-induced loss of Cx43 gap junction plaques was strongly counteracted by GF109203X (Fig. 3C) and partly counteracted by PD98059 (Fig. 3D). It should be noted that longer incubations with GF109203X alone induced ubiquitination and internalization of Cx43 by an unknown mechanism.2 Together, these results indicate that the TPA-
induced internalization of Cx43 gap junction plaques is largely mediated via PKC and that the MAPK pathway is partly involved in this process in IAR20 cells.

TPA treatment alone for 60 min caused a nearly complete loss of Cx43 immunofluorescence staining except for a faint signal in the perinuclear area (Fig. 4). Internalized, annular gap junctions have previously been reported to be acidic (18). To investigate whether acidification is required for the TPA-induced loss of Cx43 staining, TPA was preincubated with ammonium chloride, which elevates the endosomal pH (51). Under these conditions, Cx43 staining remained in intracellular vesicles. Thus, loss of Cx43 protein in response to TPA treatment appears to require acidification of internalized Cx43 annular gap junctions. The loss of Cx43 staining observed after 60 min of TPA treatment was partly counteracted by leupeptin, an inhibitor of cathepsins B, H, and L. Several studies have shown that Cx43 internalization is counteracted by proteasomal inhibitors (16, 20, 23). The TPA-induced internalization of Cx43 in IAR20 cells was partly counteracted by the proteasomal inhibitor MG132 (Fig. 4). Similar results were obtained with the mechanistically distinct proteasomal inhibitor lactacystin. Thus, proteasomal activity appears to be required for Cx43 internalization in response to TPA treatment. Interestingly, although the TPA-induced internalization of Cx43 was partly blocked by MG132, after 60 min of cotreatment with TPA and MG132 most Cx43 was not found in plaques as in untreated cells but rather was homogeneously distributed at the plasma membrane, indicative of disorganization of gap junction plaques.

To further examine the mechanisms underlying the loss of Cx43 protein in response to TPA treatment, the Cx43 protein level was examined by Western blotting. When TPA was coincubated with the protein synthesis inhibitor cycloheximide, Cx43 protein was rapidly lost compared with treatment with cycloheximide alone (Fig. 5, A and B). In accordance with the immunofluorescence data in Fig. 4, this loss was counteracted by ammonium chloride. Under these conditions, a low molecular weight band was observed after 2 h possibly representing incompletely degraded Cx43. The TPA-induced loss of Cx43 was only slightly counteracted by preincubation with leupeptin. Under these conditions we did not observe accumulation of low molecular weight Cx43 degradation products. Together, these results indicate that TPA-induced degradation of Cx43 in IAR20 cells involves lysosomes but also that other non-lysosomal proteases might play a role in the degradation process. In accordance with the immunofluorescence data, the TPA-induced loss in the Cx43 protein level was strongly counteracted by the proteasomal inhibitor MG132. Under these conditions, Cx43 remained in the P2 status at the time points investigated.

**Fig. 4. Effects of lysosomal and proteasomal inhibitors on TPA-induced Cx43 delocalization.** IAR20 cells were treated with vehicle (MeSO), ammonium chloride (10 mM), leupeptin (200 μM), or MG132 (10 μM) for 30 min and added vehicle or TPA (100 ng/ml) for 60 min as indicated. Cells were fixed, immunostained with anti-Cx43 antibodies, and visualized using fluorescence microscopy. Bar, 20 μm.

**Fig. 5. Effects of lysosomal and proteasomal inhibitors on TPA-induced down-regulation of Cx43 protein.** A, IAR20 cells were treated with vehicle (MeSO), ammonium chloride (10 mM), leupeptin (200 μM), or MG132 (10 μM) for 30 min and added vehicle or TPA (100 ng/ml) for the time points indicated. Cell lysates were prepared and equal amounts of total cell protein were subjected to SDS-PAGE. Cx43 was detected by Western blotting with anti-Cx43 antibodies. B, the Cx43 band intensities on gels shown in A were measured using Scion Image. Values shown are the mean ± S.E. of three independent experiments. Statistical analysis was performed using one-way analysis of variance with the Tukey-Kramer multiple comparisons test. By this test, cells treated with TPA + Chx + MG132 had significantly (p < 0.05) increased Cx43 protein levels compared with cells treated with TPA + Chx at any time point investigated. Cells treated with TPA + Chx + NH4Cl had significantly (p < 0.05) increased Cx43 protein levels compared with cells treated with TPA + Chx at 60, 120, and 240 min, but not at 30 min. Cells treated with TPA + Chx + leupeptin had not significantly (p > 0.05) altered Cx43 protein levels compared with cells treated with TPA + Chx at any time point investigated.
Evidence That Cx43 Is Modified by Multiple Monoubiquitins in Response to TPA Treatment—The role of ubiquitin in the function and intracellular trafficking of Cx43 is not known. Conjugation of polyubiquitin chains at least four subunits long mediate recognition and proteasomal degradation of proteins (52, 53). In contrast, conjugation of monoubiquitin is involved in regulating localization and activity of proteins by proteasome-independent processes (25). To investigate whether Cx43 is mono- or polyubiquitinated in response to TPA treatment, we used antibodies that differentiate between different forms of ubiquitinated proteins. The anti-ubiquitin antibody P4D1 recognizes both poly- and monoubiquitinated proteins (26). In contrast, the anti-ubiquitin antibody FK1 recognizes only polyubiquitinated proteins (40). As control, both antibodies recognized a purified ubiquitin ladder as determined by Western blotting (Fig. 6A). Moreover, a typical ubiquitin smeared pattern was detected when untreated IAR20 cells were examined by SDS-PAGE and immunoblotting using the anti-ubiquitin antibodies. As expected, polyubiquitinated proteins recognized by the FK1 antibody had high molecular weight, whereas the lower molecular weight bands detected by the P4D1 antibody probably represent monoubiquitinated proteins. To further ascertain the specificity of these antibodies, EGF receptor was immunoprecipitated from EGF-treated A431 cells and subjected to Western blotting. EGF receptor was previously reported to be modified by multiple monoubiquitins in response to EGF binding (26, 27). In accordance with these previous studies, ubiquitinated EGF receptor was strongly detected by the P4D1 antibody but detected only very faintly by the FK1 antibody (Fig. 6B). In contrast, β-catenin isolated from MG132-treated HeLa cells was recognized by both the P4D1 and the FK1 antibodies (Fig. 6C) in accordance with previous studies showing that β-catenin is modified by polyubiquitin chains (26, 54). In agreement with the results presented in Fig.
ubiquitin recruitment. The potential serines and lysines on several serines in the Cx43 C-terminal tail might play a role in promoting ubiquitination within the receptor cytoplasmic tail (55).

The proteasomal inhibitors counteract TPA-induced ubiquitination of Cx43—Because only polyubiquitinated and not monoubiquitinated proteins are targeted for degradation by the proteasome, our results indicate that Cx43 is not targeted for proteasomal degradation in response to TPA treatment. Nevertheless, as shown in Figs. 4 and 5, Cx43 internalization and degradation were inhibited by proteasomal inhibitors. A possible explanation for this observation could be that proteasomal inhibitors interfere with ubiquitination of Cx43. As shown in Fig. 7A, ubiquitination of Cx43 occurred as early as 5 min after TPA treatment. Importantly, the TPA-induced ubiquitination of Cx43 was strongly counteracted by MG132. The TPA-induced ubiquitination of Cx43 was also partly counteracted by the proteasomal inhibitors lactacystin and ALLN (Fig. 7B) but not by the cathepsin inhibitor leupeptin. Together, these results suggest that the block in internalization and degradation of Cx43 caused by proteasomal inhibitors might be due to inhibition of Cx43 monoubiquitination and subsequent endosomal trafficking rather than inhibition of proteasome-mediated degradation of Cx43.

**DISCUSSION**

Internalization and degradation of gap junctions are considered to be highly regulated processes. However, our knowledge of the molecular mechanisms underlying these processes is fragmentary. Ubiquitin plays an important role in the internalization and intracellular trafficking of many plasma membrane proteins. We reported previously that EGF-induced internalization of Cx43 is associated with increased ubiquitination of Cx43 (23). In the present report, we show that the Cx43 ubiquitination level is strongly increased by the tumor promoter TPA. This process was found to occur via PKC, but the data also indicate partial involvement of the MAPK pathway. Serine phosphorylation has been shown previously to regulate the ubiquitination of lysine residues in both cytosolic and membrane proteins (25). For instance, it has been shown that serine phosphorylation of the G protein-coupled receptor in yeast accelerates receptor internalization by promoting ubiquitination within the receptor cytoplasmic tail (55). Our results indicate that ubiquitin conjugation and the subsequent Cx43 turnover are regulated by serine phosphorylation of Cx43. Moreover, the observation that ubiquitination of Cx43 is regulated by both PKC and MAPK raises the possibility that several serines in the Cx43 C-terminal tail might play a role in ubiquitin recruitment. The potential serines and lysines on Cx43 involved in TPA- and EGF-induced ubiquitination remain to be identified.

Previous studies indicate that lysosomes are involved in the degradation of internalized gap junctions (14, 16, 18–20). However, the relative importance of lysosomes in Cx43 degradation appears to vary between different cell types (14, 15). Here we have shown that Cx43 degradation in response to TPA was strongly counteracted by ammonium chloride, indicating that acidification of annular gap junctions is required in this process. These results are compatible with previous electron microscopy studies indicating that annular gap junctions are acidic (18). Importantly, ammonium chloride might block Cx43 endocytic trafficking prior to eventual fusion of annular gap junctions with lysosomes. The loss of Cx43 protein was only partly counteracted by leupeptin, an inhibitor of cathepsin B, H, and L. These results are compatible with our previous studies in which lower concentrations of leupeptin did not affect degradation of Cx43 in response to EGF treatment (23). Together, these results indicate that TPA-induced degradation of Cx43 annular gap junctions in IAR20 cells involves lysosomes, but also that non-lysosomal proteases might play a role in the degradation process. Thus, further studies are required to unravel the molecular mechanisms involved in Cx43 degradation after its internalization.

While polyubiquitination targets proteins for proteasomal degradation, monoubiquitination is involved in several proteasome-independent cellular processes (25). One important function of monoubiquitination is to regulate the activity of proteins located at the plasma membrane. In yeast, internalization of
most plasma membrane proteins requires conjugation of mono- and polyubiquitins on their cytoplasmic domains (52). In mammalian cells, it was recently shown that receptor tyrosine kinases are modified by multiple monoubiquitins after ligand-induced activation and that this modification was sufficient for their internalization (26, 27). In the present report we provide evidence that Cx43 is modified by several monoubiquitins rather than a polyubiquitin chain in response to TPA or over, such as regulating the stability of polyubiquitinated proteins involved in Cx43 internalization or intracellular trafficking. Moreover, even though our data suggest that Cx43 is modified by multiple monoubiquitins in response to TPA or EGF, they do not rule out the possibility that Cx43 may bind polyubiquitin chains under other conditions.

In conclusion, our data indicate that TPA induces conjugation of multiple monoubiquitins on Cx43, which subsequently is internalized and degraded. These processes are mediated via PKC and partly involve the MAPK pathway. We have shown that proteasomal inhibitors counteract the TPA-induced ubiquitination of Cx43. Taken together, the results suggest that the proteasome might play an indirect role in Cx43 degradation by affecting the level of Cx43 monoubiquitination. It is likely that Cx43 is subjected to rapid ubiquitination-deubiquitination reactions during its internalization and degradation process. How the equilibrium between ubiquitination and deubiquitination affects internalization and subsequent intracellular trafficking of Cx43 remains to be determined.

Acknowledgments—We thank Randi Skibak and Astri Nordahl for excellent technical assistance and Dr. Tore Sanner for critical review of the manuscript and helpful discussions.

REFERENCES

1. Goodenough, D. A., Goliger, J. A., and Paul, D. L. (1996) Annu. Rev. Biochem. 65, 475–502
2. Loewenstein, W. R. (1979) Biochim. Biophys. Acta 560, 1–65
3. Yamashaki, H., and Naus, C. C. (1996) Carcinogenesis 17, 1199–1213
4. Huang, R. F., Fan, Y., Hossain, M. Z., Peng, A., Zeng, Z. L., and Boynton, A. L. (1998) Cancer Res. 58, 5089–5096
5. Omi, Y., and Yamashaki, H. (1998) Int. J. Cancer 78, 446–453
6. Qin, H., Shao, Q., Curtis, H., Galipeau, J., Belliveau, D. J., Wang, T., Alauxi-Jamali, M. A., and Laird, D. W. (2002) J. Biol. Chem. 277, 2932–29328
7. Rose, B., Mehta, P. P., and Loewenstein, W. R. (1993) Carcinogenesis 14, 1073–1075
8. Gaietta, G., Deerinck, T. J., Adams, S. R., Bouwer, J., Tour, O., Laird, D. W., Sosinsky, G. S., Tsien, R. Y., and Ellisman, M. H. (2002) Science 296, 503–507
9. Laird, D. W. (1996) Bioenerg. Biomembr. 28, 311–318
10. Fallon, R. F., and Goodenough, D. A. (1981) J. Cell Biol. 90, 521–526
11. Laird, D. W., Puramam, K. L., and Revel, J. P. (1991) Biochem. J. 273, 67–72
12. Thomas, M. A., Zoso, N., Seeris, I., Demaurex, N., Chasson, M., and Staeh, O. (2003) J. Cell Sci. 116, 2213–2222
13. Traub, O., Look, J., Dermitzel, R., Brummer, P., Hulser, D., and Willecke, K. (1989) J. Cell Biol. 108, 1539–1551
14. Musil, L. S., Le, A. C., Van Slyke, J., and Roberts, L. M. (2000) J. Biol. Chem. 275, 25207–25215
15. Laing, J. G., and Beyer, E. C. (1995) J. Biol. Chem. 270, 26399–26403
16. Laing, J. G., Tadros, P. N., Westphale, E. M., and Beyer, E. C. (1997) Exp. Cell Res. 236, 482–492
17. Jordan, K., Chodock, R., Hand, A. R., and Laird, D. W. (2001) J. Cell Sci. 114, 763–771
18. Lown, W. J., and Hai, N. (1978) Cancer Res. 38, 25207–25210
19. Musil, L. S., and Musil, L. S. (2000) Mol. Biol. Cell 11, 1933–1946
20. Van Slyke, J. K., and Musil, L. S. (2002) J. Cell Biol. 157, 381–394
21. Leithe, E., and Rivedal, E. (2004) J. Cell Sci. 117, 1211–1220
22. Ciechanover, A. (1998) EMBO J. 17, 7151–7160
23. Hicke, L., and Dunn, R. (2000) Annu. Rev. Cell Dev. Biol. 16, 141–172
24. Haglund, K., Sigismund, S., Pole, S., Szyminczkiewicz, I., Di Fiore, P. P., and Dikic, I. (2003) Nat. Cell Biol. 5, 461–466
25. Mossessian, Z., Bregman, K., Katz, M., Zeng, Y., Vereg, G., Szillosi, J., and Yamashaki, H. (2003) J. Biol. Chem. 278, 21323–21329
26. Fujita, Y., Krause, G., Scheffner, M., Zechner, D., Leddy, H. E., Behrens, J., Sommer, T., and Birchmeier, W. (2002) Nat. Cell Biol. 4, 222–231
27. Lampe, P. D., and Lau, A. F. (2000) Arch. Biochem. Biophys. 384, 205–215
28. Berthoud, V. M., Ledbetter, M. L., Hertzberg, E. L., and Saez, A. C. (1992) Eur. J. Cell Biol. 57, 40–50
29. Brissette, J. L., Kumar, N. M., Gubala, N. B., and Dotto, G. P. (1991) Mol. Cell. Biol. 11, 5364–5371
30. Lampe, P. D. (1994) J. Cell Biol. 127, 1895–1905
31. Lampe, P. D., TenBroeck, E. M., Burt, J. M., Kurata, W. E., Johnson, R. G., and Lau, A. F. (2000) J. Cell Biol. 149, 1503–1512
32. Rivedal, E., Yamashaki, H., and Sanner, T. (1994) Carcinogenesis 15, 689–694
33. Ono, S. B., Oyama, M., al, Asumori, A., Goo, D., and Yamashaki, H. (1991) Mol. Carcinog. 4, 322–327
34. Matesic, D. F., Rupp, H. L., Bonney, W. J., Ruch, J. H., and Trosto, J. E. (1994) Mol. Carcinog. 10, 226–236
35. Yaney, S. B., Edens, J. E., Trosto, J. E., Chang, C. C., and Revel, J. P. (1982) Exp. Cell Res. 139, 329–340
36. Oh, S. Y., Grupen, C. G., and Murray, A. W. (1991) Biochem. Biophys. Acta 1094, 243–245
37. Rivedal, E., Mollerup, S., Haugen, A., and Vikhammer, G. (1996) Carcinogenesis 17, 2321–2328
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40. Fujimuro, M., Sawada, H., and Yokosawa, H. (1994) FEBS Lett. 349, 173–180
41. Fujimuro, M., Sawada, H., and Yokosawa, H. (1997) Eur. J. Biochem. 249, 427–433
42. Lee, D. H., and Goldberg, A. L. (1998) Trends Cell Biol. 8, 397–403
43. Montesano, R., Drevinon, C., Kuroki, T., Saint, V. L., Handleman, S., Sanford, K. K., DeFeo, D., and Weinstein, I. B. (1977) J. Natl. Cancer Inst. 59, 1651–1658
44. Leithe, E., Cruciani, V., Sanner, T., Mikalsen, S. O., and Rivedal, E. (2003) Carcinogenesis 24, 1239–1245
45. Opsahl, H., and Rivedal, E. (2000) Cell Adhes. Commun. 7, 367–375
46. Musil, L. S., Cunningham, B. A., Edelman, G. M., and Goodenough, D. A. (1996) J. Cell Biol. 111, 2077–2088
47. Musil, L. S., and Goodenough, D. A. (1991) J. Cell Biol. 115, 1357–1374
48. Strous, G. J., and Govers, R. (1999) J. Cell Sci. 112, 1417–1423
49. Rivedal, E., and Opsahl, H. (2001) Carcinogenesis 22, 1543–1550
50. Ruch, R. J., Trosko, J. E., and Madhukar, B. V. (2001) J. Cell. Biochem. 83, 163–169
51. Seglen, P. O. (1983) Methods Enzymol. 96, 737–764
52. Bonifacino, J. S., and Weissman, A. M. (1998) Annu. Rev. Cell Dev. Biol. 14, 19–57
53. Chau, V., Tobias, J. W., Bachmair, A., Marriott, D., Ecker, D. J., Gonda, D. K., and Varshavsky, A. (1989) Science 243, 1576–1583
54. Aberle, H., Bauer, A., Stappert, J., Kispert, A., and Kemler, R. (1997) EMBO J. 16, 3797–3804
55. Hicke, L., Zanolari, B., and Riezman, H. (1998) J. Cell Biol. 141, 349–358
56. Polo, S., Sigismund, S., Faretta, M., Guidi, M., Capua, M. R., Bossi, G., Chen, H., De Camilli, P., and Di Fiore, P. P. (2002) Nature 416, 451–455
57. Wendland, B. (2002) Nat. Rev. Mol. Cell. Biol. 3, 971–977
58. Larsen, W. J., Tung, H. N., Murray, S. A., and Swenson, C. A. (1979) J. Cell Biol. 83, 576–587
59. Mizuno, E., Kawahata, K., Kato, M., Kitamura, N., and Komada, M. (2003) Mol. Biol. Cell 14, 3675–3689
60. Rabinovitch, C., Bache, K. G., Gilksoly, D. J., Madshus, I. H., Stang, E., and Stenmark, H. (2002) Nat. Cell Biol. 4, 394–398
61. Minnaugh, E. G., Chen, H. Y., Davie, J. R., Celis, J. E., and Neckers, L. (1997) Biochemistry 36, 14418–14429