Biochemical and Biophysical Research Communications 382 (2009) 41–45

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

# Interplay between PKC and the MAP kinase pathway in Connexin43 phosphorylation and inhibition of gap junction intercellular communication

Solveig Sirnes, Ane Kjenseth, Edward Leithe, Edgar Rivedal *

*Department of Cancer Prevention, Institute for Cancer Research, Norwegian Radium Hospital, Rikshospitalet University Hospital, Montebello, Oslo, Norway*
*Centre for Cancer Biomedicine, University of Oslo, Oslo, Norway*

ARTICLE INFO

*Article history:*
Received 11 February 2009
Available online 1 March 2009

*Keywords:*
Cell–cell communication
Gap junction
Connexin43
Phosphorylation
Protein kinase C
MAP kinase

ABSTRACT

Gap junction channels are made of a family proteins called connexins. The best-studied type of connexin, Connexin43 (Cx43), is phosphorylated at several sites in its C-terminus. The tumor-promoting phorbol ester TPA strongly inhibits Cx43 gap junction channels. In this study we have investigated mechanisms involved in TPA-induced phosphorylation of Cx43 and inhibition of gap junction channels. The data show that TPA-induced inhibition of gap junction intercellular communication (GJIC) is dependent on both PKC and the MAP kinase pathway. The data suggest that PKC-induced activation of MAP kinase partly involves Src-independent trans-activation of the EGF receptor, and that TPA-induced shift in SDS–PAGE gel mobility of Cx43 is caused by MAP kinase phosphorylation, whereas phosphorylation of S368 by PKC does not alter gel migration of Cx43. We also show that TPA, in addition to phosphorylation of S368, also induces phosphorylation of S255 and S262, in a MAP kinase-dependent manner. The data add to our understanding of the molecular mechanisms involved in the interplay between signaling pathways in regulation of GJIC.



## Introduction

Gap junctions are plasma membrane domains containing channels that permit ions and small molecules to be transferred between neighboring cells [1]. The channels consist of connexin proteins, and their aberrant function has been associated with a number of pathological conditions, including cancer [2–4].

Phosphorylation of Connexin43 (Cx43), the best-studied type of connexin, has been shown to regulate both gap junction channel formation, permeability and turnover [5,6]. However, the specific role of the various phosphorylation sites in regulating gap junction channel activity is incompletely understood. This is also complicated by the fact that many inhibitors of gap junction intercellular communication (GJIC) activate more than one kinase, and more than one signaling pathway [7,8].

The phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and epidermal growth factor (EGF) are potent inhibitors of GJIC [8,9] and their action has been associated with phosphorylation of Cx43 at different sites [10,11]. TPA- and EGF-induced phosphorylation of Cx43 is associated with a decrease in SDS–PAGE gel mobility, from the so-called Cx43-P0 form to the Cx43-P1/P2 forms [12], as well as enhanced Triton X-100 solubility [13].

We and others have previously suggested that the TPA-induced inhibition of GJIC involves the MAP kinase pathway [8,14]. The aim of the present study was to obtain a better understanding of the role of Cx43 phosphorylation in inhibition of GJIC and change in mobility of Cx43 in SDS–PAGE in response to TPA. We show that TPA-induced modulation of Cx43 phosphorylation and GJIC in IAR20 cells involves a combined action of the PKC and MAP kinase signaling pathways. Evidence is provided that TPA-induced alteration in the gel mobility shift of Cx43 and effect on GJIC is caused by MAP kinase phosphorylation.

## Material and methods

*Cells.* The rat liver epithelial cell line IAR20 was obtained from The International Agency for Research on Cancer, Lyon, France [15].

*Materials.* TPA, EGF, U1026, GF109203X, AG1478, PD153035, PP2 and Lucifer Yellow were purchased from Sigma (St. Louis, MO, USA) and chlordane from Sulpelco (Bellefonte, PA, USA). All chemicals except Lucifer Yellow were dissolved in dimethyl sulfoxide (DMSO).

*Antibodies.* The anti-Cx43 antiserum was made in rabbits injected with a synthetic peptide consisting of the 20 C-terminal amino acids of Cx43 [16], rabbit anti phospho-ERK1/2 was from Cell Signaling Technology (Beverly, MA, USA). Rabbit anti phospho-(S368)-Cx43 was from Chemicon (Temecula, CA, USA). Phospho specific antibodies against P(S255)-Cx43 and P(S262)-Cx43

* Corresponding author. Fax: +47 22 93 57 67.
*E-mail address:* edgarr@rr-research.no (E. Rivedal).

0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.02.141

was obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA), and anti P-MARCKS from Sigma (St. Louis, MO, USA).

*Measurement of gap junction intercellular communication by scrape loading.* GJIC in the rat liver epithelial cell line IAR20 was determined by scrape loading of Lucifer Yellow (LY) and quantitative determination of dye spreading by image analysis as previously described [17].

*Western blotting.* Cells were scraped into SDS electrophoresis sample buffer (10 mM Tris pH 6.8, 15% (w/v) glycerol, 3% (w/v) SDS, 0.01% (w/v) bromophenol blue and 5% (v/v) 2-mercaptoethanol). The extracts were sonicated in a Branson Sonifier and heated for 5 min at 95 °C prior to electrophoresis and Western blotting. Blotting membranes were developed with 4-chloro-1-naphtol (Cx43) or chemoluminescence (phospho-specific antibodies against Cx43 and phospho-ERK1/2).

## Results

### *Pharmacological evidence that TPA-induced inhibition of gap junction channels involves EGF receptor and the MAP kinase pathway*

In agreement with previous studies, TPA induced complete block in cell communication in IAR20 cells after 30 min exposure [15,18] (Fig. 1A). As expected, the PKC inhibitor GF109203X completely blocked the TPA-induced inhibition of GJIC (Fig. 1A). Importantly, the MEK inhibitor U0126 was about as effective as the PKC inhibitor in counteracting the effect of TPA on GJIC. Only at the highest TPA concentration examined (100 ng/ml), the PKC inhibitor was slightly more effective in counteracting TPA-induced inhibition of dye transfer. These data are in accordance with our previous studies in the transformed cell line IAR6.1, and suggest that the TPA-induced inhibitory effect on GJIC is mediated through PKC-mediated activation of the MAP kinase pathway, and that the direct phosphorylation of Cx43 by PKC is of minor importance [8]. To rule out the possibility that the effect of U0126 on TPA-induced inhibition is due to unspecific inhibition of PKC, we examined the effect of U0126 on TPA-induced phosphorylation of MARCKS, a well-studied substrate for PKC [19]. As shown in Fig. 1B, TPA-induced phosphorylation of MARCKS was efficiently prevented by the PKC inhibitor GF109203X, but not by U0126. On the contrary, U0126 seemed to result in increased amount of phosphorylated MARCKS. The reason for this is unknown. Thus, the inhibitory effect of U0126 on TPA-induced block in gap junction channel activity is not a result of unspecific inhibition of PKC.

To elucidate the mechanism by which TPA induces activation of the MAP kinase pathway, we asked whether trans-activation of EGF receptor could be involved. AG1478 and PD153035 are potent and selective inhibitors of EGF receptor kinase and can block phosphorylation of EGF receptor induced by several substances. As expected, AG1478 and PD153035, as well as the MEK inhibitor U0126, completely counteracted EGF-induced inhibition of GJIC (Fig. 1C). Interestingly, AG1478 and PD153035 counteracted the TPA-induced inhibition of GJIC by approximately 20% (Fig. 1A). This suggests that TPA activates the MAP kinase pathway partly by transactivating EGF receptor.

We next examined the role of Src in TPA-induced inhibition of GJIC, since PKCδ has been shown to be able to activate EGF receptor via activation of Src in response to TPA treatment [20]. However, the selective inhibitor of Src activity, PP2, was found to have no effect on the TPA-induced inhibition of GJIC, suggesting that Src is not involved in this process (Fig. 1A).

Taken together, these results provide complementary evidence and understanding to previous studies in other cell lines, indicating that MAP kinase is of major importance in TPA-induced inhibition of GJIC [8,14]. The results also suggest that this process partly involves trans-activation of the EGF receptor.

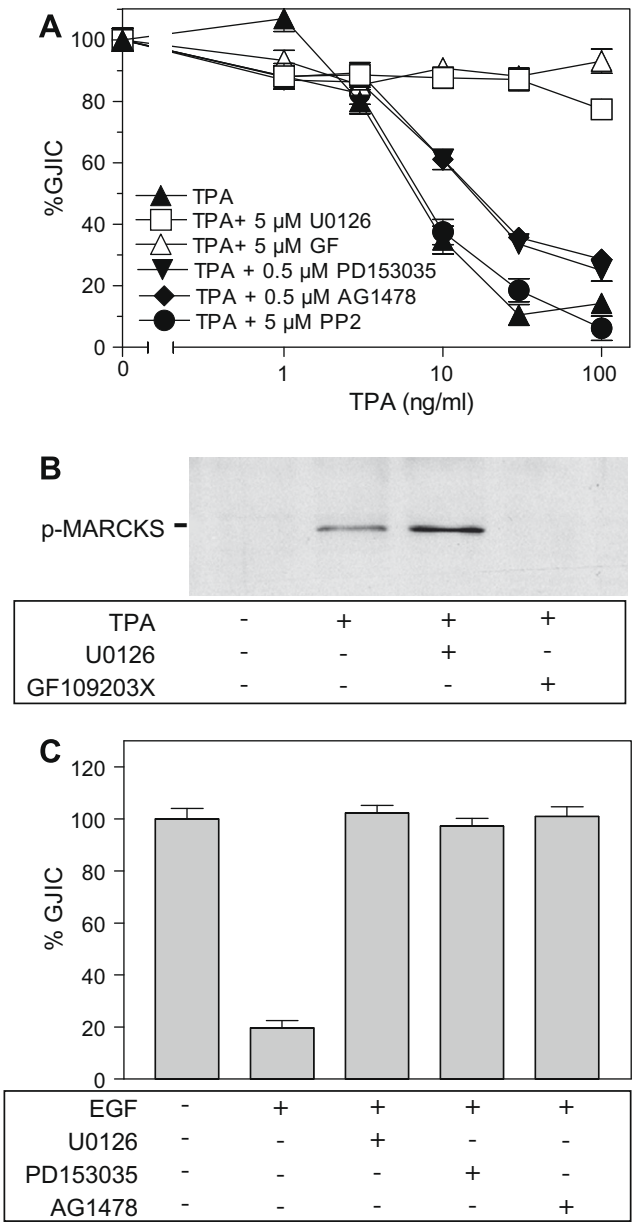


**Fig. 1.** (A) Effect of the MEK inhibitor U0126 (5 μM), PKC inhibitor GF109203X (GF) (5 μM), EGF receptor inhibitor PD153035 (0.5 μM), EGF receptor inhibitor AG1478 (0.5 μM), SRC inhibitor PP2 (5 μM), on TPA-induced inhibition of GJIC in IAR20 cells. The cells were co-exposed to different concentrations of TPA and the inhibitors for 30 min prior to determination of GJIC. (B) Effect of the MEK inhibitor U0126 (5 μM) and the PKC inhibitor GF109203X (5 μM) on TPA-induced phosphorylation of the PKC-specific cellular substrate MARCKS. The cells were co-exposed to 100 ng/ml TPA and the inhibitors for 30 min prior to preparation of extracts for Western blot. (C) Effect of the MEK inhibitor U0126 (5 μM), the EGF receptor inhibitors PD153035 (0.5 μM) and AG1478 (0.5 μM) on EGF-induced inhibition of GJIC in IAR20 cells. The cells were co-exposed to 100 ng/ml EGF and inhibitor for 30 min prior to determination of GJIC.

### *Effect of combined exposure of TPA and EGF on gap junctional communication*

To further elucidate the role of PKC and MAP kinase in TPA-induced phosphorylation of Cx43 and inhibition of gap junction channels, we next examined the effect of the combined exposure of TPA and EGF on gap junction channel activity. As expected, TPA induced complete block in cell–cell communication in IAR20 cells after 30 min exposure [15,18] (Fig. 2). The block was tran-

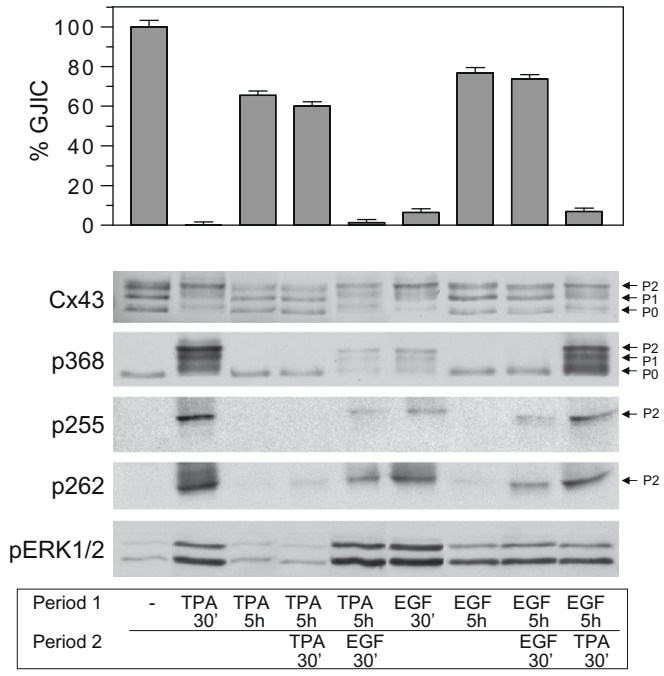


**Fig. 2.** Effect of exposing IAR20 cells for 30 min or 5 h to different combinations of TPA (100 ng/ml) and EGF (100 ng/ml) on GJIC and Western blot using five different antibodies: Cx43, antibody against the carboxy terminal part of Cx43; P368, P255 and P262, phospho-specific antibodies to detect Cx43 phosphorylated at position S368, S255 and S262; P-ERK, antibody detecting activated ERK1/2.

sient, and after 5 h of TPA exposure GJIC was restored to about 60% of that seen in unexposed IAR20 cells. At this time point, re-exposure with fresh TPA had little or no effect on the level of communication, showing that restoration of GJIC in IAR20 cells was not caused by inactivation of inhibitory compound, but by down-regulation of the mechanism of action. These results are compatible with previous studies showing that PKC is down-regulated in response to TPA treatment [21,22].

GJIC was also completely blocked 30 min after the addition of EGF (Fig. 2). Inhibition of GJIC by EGF is considered mediated through activation of a MAP kinase pathway, probably ERK1/2 [11]. TPA is, however, also activating ERK1/2 (Fig. 2) [23], and prolonged TPA exposure could therefore possibly down regulate both PKC and ERK1/2. Pre-exposure to TPA for 5 h did however not abolish the ability of EGF to down regulate GJIC or activate ERK1/2. This observation shows that although ERK1/2 is activated by TPA, it is not down regulated by TPA exposure.

EGF had similar transient effect as TPA on cell communication in IAR20 cells. From complete block of GJIC after 30 min EGF exposure, communication was restored to about 70% of normal level after 5 h continuous exposure. Communication in cells pre-exposed 5 h to EGF was however not affected by EGF re-exposure, while TPA was still able to completely down regulate GJIC (Fig. 2). Interestingly, the level of activated ERK1/2 was fully reversed after 5 h exposure to TPA, but not after 5 h EGF exposure. This could perhaps explain why the TPA-induced inhibition of GJIC after 5 h of EGF treatment was apparently not associated with increased activation of ERK1/2 (Fig. 2).

### *Effect of combined exposure to TPA and EGF on the Cx43 phosphorylation status*

We next evaluated the effect of TPA and EGF exposure on the Cx43 phosphorylation status. Cx43 was analyzed by Western blotting using four different antibodies. (1) An antibody against the last 20 amino acids of the C-terminal part of Cx43. This antibody has been used to detect total amount of Cx43 as well as some of the changes in the phosphorylation pattern of Cx43 [8]. (2) An antibody shown to be specific for Cx43 phosphorylated on serine at position 368 [10]. (3) An antibody directed against Cx43 phosphorylated at S255, and (4) an antibody specific for Cx43 phosphorylated at S262.

Cx43 often exists in several phosphorylated forms with different mobility in SDS gel electrophoresis. Unexposed IAR20 cells show three major bands (Fig. 2). The fastest migrating band, P0, has been shown to contain unphosphorylated Cx43 since phosphatase treated cell extracts have been shown to give only one single band in this position [24]. It can, however, not be excluded that the band at the P0 position also contains phosphorylated forms of Cx43.

Interestingly, the only band detected by the p368 antibody in unexposed IAR20 cells was observed at the P0 position (Fig. 2). This has also been reported for other cell types [10], and suggests that this type of Cx43 phosphorylation does not result in altered gel electrophoresis mobility. No band was detected by the S255 phosphorylation specific antibody. Only a weak band could be observed after blotting with the p262 antibody (Figs. 2 and 3). Exposure of IAR20 cells to TPA for 30 min caused strong relative increase in the P2 band for all Cx43 antibodies used. S262 was previously reported to be phosphorylated in response to $p34^{Cdc2}$ as well as in response to TPA treatment [25]. S255 is a reported MAP kinase phosphorylation site [26]. Phosphorylation of this site after exposure to TPA strengthens the hypothesis that TPA-induced inhibition of GJIC involves MAP kinase.

After 5 h exposure to TPA, the band patterns for all antibodies had returned to that of unexposed cells, except that the total amount of Cx43 was decreased (Fig. 2). Re-exposure with fresh TPA for 30 min had no effect on any of the band patterns, in concordance with its inability to inhibit GJIC under these conditions.

Thirty minutes exposure to EGF induced a change in the Cx43 band pattern similar to what was observed in response to TPA treatment. EGF did however not induce enhanced intensity of S368 phosphorylation in Cx43, although the band pattern of S368-phosphorylated Cx43 was altered compared to unexposed cells. Thus, exposure to EGF seemed to result in changed mobility in already S368-phosphorylated Cx43, indicating that phosphorylation at other sites than S368, possibly by ERK1/2, could be

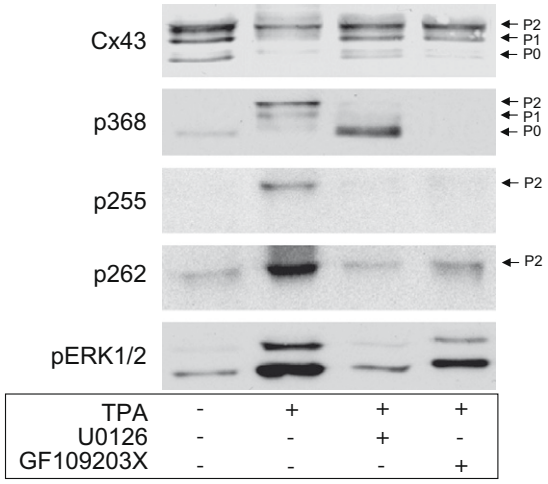


**Fig. 3.** Effect of the MEK inhibitor U0126 (5 μM) and the PKC inhibitor GF109203X (5 μM) on TPA-induced effects on Cx43 Western blots using five different antibodies: Cx43, antibody against the carboxy terminal part of Cx43; P368, P255 and P262, phospho-specific antibodies to detect Cx43 phosphorylated at position S368, S255 and S262; P-ERK, antibody detecting activated ERK1/2. The cells were co-exposed to 100 ng/ml TPA and the inhibitors for 30 min prior to preparation of extracts for Western blotting.

responsible for the change in mobility. As expected, exposure to EGF caused an increase in the P2 band for Cx43 phosphorylated on S255, but the increase was smaller than after TPA treatment. EGF also induced an increased intensity of the P2 band of Cx43 phosphorylated on S262.

Pre-exposure to TPA for 5 h did not prevent the effect of EGF on the Cx43 band pattern, neither for total Cx43 nor for the phosphorylated species of Cx43 (Fig. 2). EGF was also under these conditions able to activate ERK1/2. The data are in line with the observation that TPA pretreatment did not counteract EGF-induced effects on GJIC. Thus, TPA-induced refractoriness is likely to be caused by down-regulation of PKC and not by preventing ERK1/2 activation.

EGF exposure for 5 h resulted in Cx43 band patterns reversing to that of unexposed cells. The 5 h EGF exposure rendered the cells resistant to further ERK1/2 activation by EGF, as well as EGF-induced alterations in the Cx43 band pattern, all in agreement with the GJIC experiments.

Pre-treatment to EGF for 5 h did not, also in agreement with the GJIC experiment, prevent TPA-induced alteration in the Cx43 band pattern, neither for the antibody against the C-terminal part, nor for the phosphorylated Cx43 (Fig. 1). The shifts are, however, partly reduced compared with the TPA-induced effects in cells without EGF pre-exposure, in agreement with the observed partial role of TPA-induced trans-activation of EGF receptor in the GJIC measuring experiment.

### *TPA-induced SDS–PAGE mobility shift of Cx43 is caused by a MAP kinase mediated phosphorylation*

We have previously suggested that bands of Cx43 with decreased mobility (mainly P2) observed after TPA exposure of IAR6.1 cells could have occurred as a consequence of phosphorylation by a MAP kinase, and not by PKC [8]. The above findings do not exclude that TPA-induced phosphorylation of S368 could result in decreased mobility of Cx43 in SDS–PAGE gels. We therefore wanted to more closely investigate the role of MAP kinase in the altered migration of S368-phosphorylated Cx43 after TPA treatment. Cells were coincubated with TPA and either PKC or MEK1 inhibitor. Then the Cx43 phosphorylation status was examined with antibodies against total Cx43 or Cx43 phosphorylated at either S255, S262 or S368. As shown in Fig. 3, the MEK1 inhibitor U0126 blocked the TPA-induced mobility shift of Cx43, as well as the activation of MAP kinase ERK1/2. However, U0126 did not counteract the increased level of S368 phosphorylation induced by TPA. Interestingly, exposure to the MEK1 inhibitor caused S368-phosphorylated Cx43 to migrate at the P0 position, rather than at the P2 position. In contrast, the PKC inhibitor GF109203X, having intermediate effects on the shift in Cx43 band pattern and level of activated ERK1/2, completely counteracted S368 phosphorylation. The TPA-induced phosphorylation of both S255 and S262 was counteracted by both U0126 and GF109203X, suggesting that both PKC and MAP kinase are involved in this process. Taken together, the data are in support of the view that TPA-induced mobility shift in Cx43 is caused by MAP kinase phosphorylation, while phosphorylation by PKC on serine 368 does not change the Cx43 mobility.

## Discussion

We have previously observed that the effect of TPA on gap junctions seemed not only to be mediated by a direct effect on Cx43 by PKC [8]. In the present work we have studied this in more detail. TPA induced strong inhibition of GJIC as well as mobility shift in Cx43, observed both with an antibody against total Cx43, and when using Cx43 phospho-specific antibodies (Fig. 2). This was almost completely and equally well prevented by the MEK inhibitor and the PKC inhibitor. The MEK inhibitor was also efficiently preventing TPA-induced alteration in Cx43 electrophoresis mobility without preventing TPA-induced increase in the amount of S368-phosphorylated Cx43. The PKC inhibitor, on the other hand, completely prevented induction of S368-phosphorylated Cx43. This suggests that inhibition of GJIC by TPA is mainly mediated via MAP kinase, and not by direct phosphorylation of Cx43 by PKC. The data suggest that the TPA-induced phosphorylation of Cx43 on S368 by PKC is not causing mobility shift in SDS electrophoresis gels. This mobility shift is rather caused by MAP kinase.

EGF was found to induce mobility shift of Cx43 on SDS–PAGE, both for total and p368 Cx43 (Fig. 2). Pre-exposure to TPA desensitized the cells to itself, but not to EGF, and vice versa (Fig. 2). This is in accordance with previous findings that desensitization by TPA is caused by down regulation of PKC. Our finding that EGF inhibits GJIC also after TPA treatment is in agreement with studies on other cell types [27], suggesting that the MAP kinase pathway is not down regulated by prolonged TPA treatment. Exposure of cells to EGF has been shown to result in phosphorylation of Cx43 at S255 by a MAP kinase [11], while $p34^{Cdc2}$ as well as TPA exposure, have been found to induce phosphorylation of S262 [25]. We observed these two types of phosphorylation in the P2 position (Fig. 3) after both TPA and EGF exposure of IAR20 cells, supporting serine 255 and 262 as candidates for phosphorylation sites involved in mediating the effect of both TPA and EGF.

PKC has been shown to be able to activate the MAP kinase pathway via RAF kinase [28] as well as the EGF receptor itself via Src [20]. Here we show that two EGF receptor inhibitors block TPA-induced inhibition of GJIC by approximately 20%. On the other hand, the Src inhibitor PP2 had no effect. This suggests that a minor part of the TPA-induced effect on GJIC is mediated through a Src-independent effect on the EGF receptor, while the major effect is mediated from PKC to the MAP kinase pathway downstream of EGF receptor (Fig. 4).

The present study adds to the understanding of how TPA-induced inhibition of Cx43 gap junction channels is mediated by a cross-talk between PKC and the MAP kinase pathway. The data suggest that although PKC directly phosphorylates Cx43 at S368, this phosphorylation seems to have little effect on both GJIC and Cx43 electrophoresis mobility. Instead, the inhibitory effect of TPA on GJIC as well as the reduced SDS–PAGE gel mobility of Cx43 appear to be induced via the MAP kinase pathway.

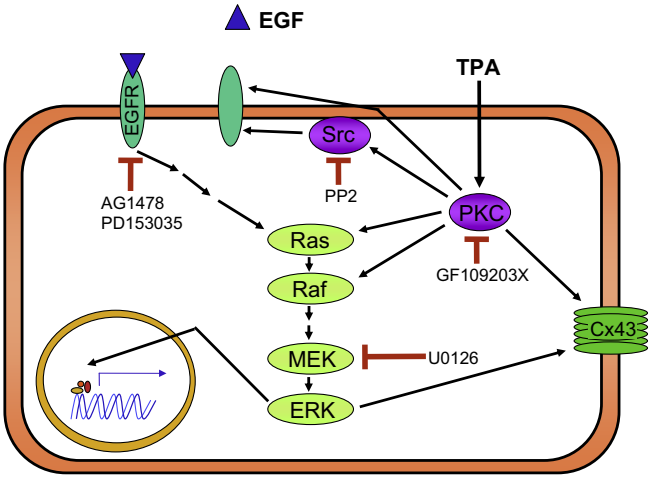


**Fig. 4.** Schematic description of the kinases and chemical inhibitors discussed in the present work.

## Acknowledgments

We are grateful to Astri Nordahl for excellent technical assistance. This study was supported by the Norwegian Cancer Society and The Research Council of Norway.

## References

[1] D.A. Goodenough, J.A. Goliger, D.L. Paul, Connexins, connexons, and intercellular communication, Annu. Rev. Biochem. 65 (1996) 475–502.
[2] E.H. Chang, G. Van Camp, R.J. Smith, The role of connexins in human disease, Ear Hear. 24 (2003) 314–323.
[3] E. Leithe, S. Sirnes, Y. Omori, E. Rivedal, Downregulation of gap junctions in cancer cells, Crit. Rev. Oncog. 12 (2006) 225–256.
[4] L. Cronier, S. Crespin, P.O. Strale, N. Defamie, M. Mesnil, Gap junctions and cancer: new functions for an old story, Antioxid. Redox. Signal. 11 (2009) 323–338.
[5] P.D. Lampe, A.F. Lau, Regulation of gap junctions by phosphorylation of connexins, Arch. Biochem. Biophys. 384 (2000) 205–215.
[6] D.W. Laird, Connexin phosphorylation as a regulatory event linked to gap junction internalization and degradation, Biochim. Biophys. Acta 1711 (2005) 172–182.
[7] M.Z. Hossain, A.B. Jagdale, P. Ao, A. Kazlauskas, A.L. Boynton, Disruption of gap junctional communication by the platelet-derived growth factor is mediated via multiple signaling pathways, J. Biol. Chem. 274 (1999) 10489–10496.
[8] E. Rivedal, H. Opsahl, Role of PKC and MAP kinase in EGF- and TPA-induced connexin43 phosphorylation and inhibition of gap junction intercellular communication in rat liver epithelial cells, Carcinogenesis 22 (2001) 1543–1550.
[9] A.F. Lau, M.Y. Kanemitsu, W.E. Kurata, S. Danesh, A.L. Boynton, Epidermal growth factor disrupts gap-junctional communication and induces phosphorylation of connexin43 on serine, Mol. Biol. Cell 3 (1992) 865–874.
[10] J.L. Solan, M.D. Fry, E.M. Tenbroek, P.D. Lampe, Connexin43 phosphorylation at S368 is acute during S and G2/M and in response to protein kinase C activation, J. Cell Sci. 116 (2003) 2203–2211.
[11] B.J. Warn-Cramer, G.T. Cottrell, J.M. Burt, A.F. Lau, Regulation of connexin-43 gap junctional intercellular communication by mitogen-activated protein kinase, J. Biol. Chem. 273 (1998) 9188–9196.
[12] J.L. Brissette, N.M. Kumar, N.B. Gilula, G.P. Dotto, The tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate and the ras oncogene modulate expression and phosphorylation of gap junction proteins, Mol. Cell. Biol. 11 (1991) 5364–5371.
[13] S. Sirnes, E. Leithe, E. Rivedal, The detergent resistance of Connexin43 is lost upon TPA or EGF treatment and is an early step in gap junction endocytosis, Biochem. Biophys. Res. Commun. 373 (2008) 597–601.
[14] R.J. Ruch, J.E. Trosko, B.V. Madhukar, Inhibition of connexin43 gap junctional intercellular communication by TPA requires ERK activation, J. Cell Biochem. 83 (2001) 163–169.
[15] M. Mesnil, R. Montesano, H. Yamasaki, Intercellular communication of transformed and non-transformed rat liver epithelial cells. Modulation by TPA, Exp. Cell Res. 165 (1986) 391–402.
[16] E. Rivedal, H. Yamasaki, T. Sanner, Inhibition of gap junctional intercellular communication in Syrian hamster embryo cells by TPA, retinoic acid and DDT, Carcinogenesis 15 (1994) 689–694.
[17] H. Opsahl, E. Rivedal, Quantitative determination of gap junction intercellular communication by scrape loading and image analysis, Cell Adhes. Commun. 7 (2000) 367–375.
[18] E. Rivedal, E. Leithe, Connexin43 synthesis, phosphorylation, and degradation in regulation of transient inhibition of gap junction intercellular communication by the phorbol ester TPA in rat liver epithelial cells, Exp. Cell Res. 302 (2005) 143–152.
[19] A. Ngezahayo, F. Lang, H.A. Kolb, Cholecystokinin-octapeptide affects the fluorescence signal of a single pancreatic acinar cell loaded with the acrylodan-labelled MARCKS peptide, a protein kinase C substrate, Pflug. Arch. 429 (1995) 805–808.
[20] S. Amos, P.M. Martin, G.A. Polar, S.J. Parsons, I.M. Hussaini, Phorbol 12-myristate 13-acetate induces epidermal growth factor receptor transactivation via protein kinase Cdelta/c-Src pathways in glioblastoma cells, J. Biol. Chem. 280 (2005) 7729–7738.
[21] H.S. Gainer, A.W. Murray, Diacylglycerol inhibits gap junctional communication in cultured epidermal cells: evidence for a role of protein kinase C, Biochem. Biophys. Res. Commun. 126 (1985) 1109–1113.
[22] E. Leithe, V. Cruciani, T. Sanner, S.O. Mikalsen, E. Rivedal, Recovery of gap junctional intercellular communication after phorbol ester treatment requires proteasomal degradation of protein kinase C, Carcinogenesis 24 (2003) 1239–1245.
[23] P.D. Adams, P.J. Parker, TPA-induced activation of MAP kinase, FEBS Lett. 290 (1991) 77–82.
[24] D.W. Laird, K.L. Puranam, J.P. Revel, Turnover and phosphorylation dynamics of connexin43 gap junction protein in cultured cardiac myocytes, Biochem. J. 273 (Pt. 1) (1991) 67–72.
[25] J.L. Solan, P.D. Lampe, Key connexin 43 phosphorylation events regulate the gap junction life cycle, J. Membr. Biol. 217 (2007) 35–41.
[26] B.J. Warn-Cramer, P.D. Lampe, W.E. Kurata, M.Y. Kanemitsu, L.W. Loo, W. Eckhart, A.F. Lau, Characterization of the mitogen-activated protein kinase phosphorylation sites on the connexin-43 gap junction protein, J. Biol. Chem. 271 (1996) 3779–3786.
[27] M.Y. Kanemitsu, A.F. Lau, Epidermal growth factor stimulates the disruption of gap junctional communication and connexin43 phosphorylation independent of 12-*O*-tetradecanoylphorbol 13-acetate-sensitive protein kinase C: the possible involvement of mitogen-activated protein kinase, Mol. Biol. Cell 4 (1993) 837–848.
[28] W. Kolch, G. Heidecker, G. Kochs, R. Hummel, H. Vahidi, H. Mischak, G. Finkenzeller, D. Marme, U.R. Rapp, Protein kinase C alpha activates RAF-1 by direct phosphorylation, Nature 364 (1993) 249–252.