

Review

Connexin phosphorylation as a regulatory event linked to gap junction channel assembly

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Abstract

Gap junctions, composed of proteins from the connexin family, allow for intercellular communication between cells and are important in development and maintenance of cell homeostasis. Phosphorylation has been implicated in the regulation of gap junctional communication at several stages of the cell cycle and the connexin “lifecycle”, such as trafficking, assembly/disassembly, degradation, as well as in the gating of “hemi” channels or intact gap junction channels. This review focuses on how phosphorylation can regulate the early stages of the connexin life cycle through assembly of functional gap junctional channels. The availability of sequences from the human genome databases has indicated that the number of connexins in the gene family is approximately 20, but we know mostly about how connexin43 (Cx43) is regulated. Recent technologies and investigations of interacting proteins have shown that activation of several kinases including protein kinase A, protein kinase C (PKC), p34^{cdc2}/cyclin B kinase, casein kinase 1 (CK1), mitogen-activated protein kinase (MAPK) and pp60^{src} kinase can lead to phosphorylation of the majority of the 21 serine and two of the tyrosine residues in the C-terminal region of Cx43. While many studies have correlated changes in kinase activity with changes in gap junctional communication, further research is needed to directly link specific phosphorylation events with changes in connexin oligomerization and gap junction assembly.

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Keywords: Connexin; Gap junction; Phosphorylation; Cell signaling; PKC; PKA; Casein kinase

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Abbreviations: Cx, connexin; PKA, cAMP-dependent protein kinase; MAPK, mitogen-activated protein kinase; PKC, protein kinase C; CK1, casein kinase 1; PMA, phorbol 12-myristate 13-acetate; FSH, follicle stimulating hormone; NP, non-phosphorylated; SDS-PAGE, sodium dodecylsulfate-polyacrylamide gel electrophoresis

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1. Introduction

Gap junctions are tightly packed clusters of intercellular channels that directly connect the cytoplasm of adjacent cells. They coordinate cell-to-cell communication within tissues and allow for the transfer of molecules less than 1000 Da between cells such as ions, amino acids, nucleotides and second messengers (e.g., Ca^{2+} , cAMP, cGMP, IP_3) and other metabolites [1–3]. In vertebrates, gap junctions are composed of proteins from the connexin family, which is comprised of approximately 20 members in humans [2–4]. Connexins are commonly designated with numerical suffixes referring to the molecular weight of the deduced sequence in kilodaltons (e.g., connexin43 or Cx43) [3]. Connexins are differentially expressed in tissues with some being significantly expressed in only a few tissues and some, like Cx43, being more widespread. Gap junctions play significant regulatory roles in embryonic development, electrical coupling, apoptosis, differentiation, tissue homeostasis and metabolic transport in non-vascularized tissue [2,4].

Deficient or improper gap junction function has recently been associated with a variety of diseases, including some forms of neuropathy, hereditary deafness, cataracts, skin disease, heart disease, and cancer [3]. These disparate phenotypes not only show the diversity of the expression pattern of connexins, but they also illustrate that gap

junctions play different roles in different tissues. Mouse “knock in” experiments, in which one connexin isotype is replaced with another, have shown that individual connexins play distinct roles in specific cellular processes. For example, when Cx43 was replaced by Cx32 or Cx40, the mice survived past birth but the developmental defects were only moderated by replacement with Cx32 and several additional defects became apparent in both groups of mice [5]. In other mouse studies, ablation of the lens fiber cell connexins Cx46 or Cx50 led to cataract formation while lens growth was only impaired in the Cx50^{−/−} mice. Interestingly, targeted replacement of Cx50 with Cx46 corrected defects in differentiation and prevented cataract formation but did not restore growth control [6].

Connexin proteins possess four hydrophobic membrane-spanning domains (see Fig. 1). Two conserved, extracellular domains are involved in paired hemichannel docking with counterparts from the adjacent membrane leaving three cytoplasmic domains corresponding to the amino-terminal region, a loop between transmembrane domains 2 and 3, and the carboxy-terminal tail region [3]. During intercellular channel formation, six connexin proteins oligomerize into a hexameric hemi-channel, or connexon, which traffics to the plasma membrane. One hemi-channel docks with a second in an opposing cell to form an intact channel. Once assembled, groups of these intercellular channels (termed

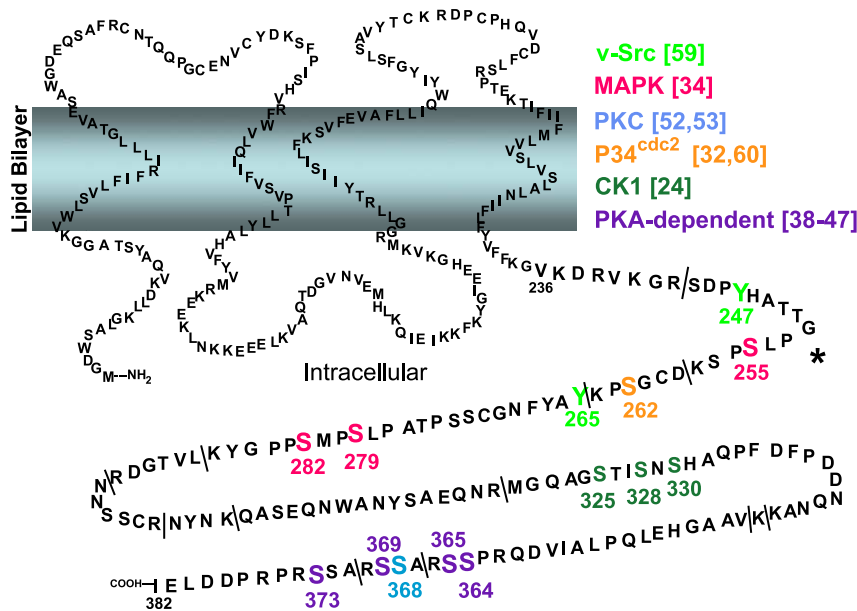


Fig. 1. Phosphorylation sites in Cx43. Phosphorylation sites targeted by known kinases are indicated by different colors (Src—lime green; MAPK—red; PKC—blue; p34^{cdc2}—orange; CK1—dark green, PKA-dependent—purple). The primary references for these assignments are indicated in brackets. The lines dividing the sequence represent tryptic cleavage sites and the fragment labeled with an * is significantly more phosphorylated in S than G₀ phase cells. Adapted from Ref. [12].

gap junctional plaques) allow coordinated function in post-mitotic cells (Fig. 2). Formation and degradation of gap junctions is a very dynamic process with reports of half-lives of less than 2–5 h in cultured cells and tissues [7–11]. Therefore, the regulation of gap junction assembly and turnover is likely to be critical in the control of intercellular communication. These channels can also be gated in response to various stimuli, including changes in voltage, pH, and connexin phosphorylation [1–3,12].

Many of the connexins not only contain “consensus phosphorylation sequences” for protein kinases, but have also been demonstrated to be phosphorylated by kinases in vitro and in some cases in cell culture or tissues. In addition, many connexins (Cx31, Cx32, Cx37, Cx40, Cx43, Cx45, Cx46, Cx50, and Cx56) have been shown to be phosphoproteins by either a shift in their electrophoretic mobility or direct incorporation of ^{32}P [12,13], and we expect this list to grow as more recently discovered family members are investigated.

This review starts by discussing what is known about the life cycle of connexin proteins and how phosphorylation could regulate connexin assembly into gap junctions; then it discusses how these early stages are affected as cells pass through the cell cycle, during which very dynamic changes in gap junction assembly and disassembly occur.

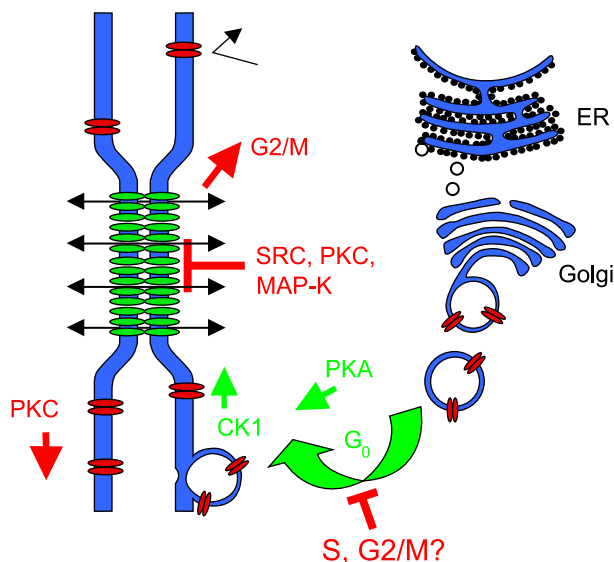


Fig. 2. Protein kinases act at different steps in the life cycle of Cx43. Cx43 produced in the endoplasmic reticulum (ER) assembles into a hexameric connexon as it is transported through the Golgi and *trans*-Golgi network (not to scale relative to the plasma membrane). After export and fusion with the plasma membrane, randomly dispersed Cx43 connexons or hemichannels will accumulate at pre-existing or newly formed gap junctions. Cell communication (indicated by double headed arrows) occurs upon gap junction formation. Presumably hemichannels are not normally open (indicated by the bent-back arrow). The possible subcellular sites of action of different protein kinases are shown and kinases or phases of the cell cycle that lead to increased communication are indicated in green and conditions leading to a reduction are in red.

2. The life cycle of Cx43 in homeostatic cells

Live cell imaging studies have clearly shown that gap junctions are dynamic plasma membrane structures with rapid turnover rates [14–16]. Although there is some controversy as to whether all connexins follow the conventional pathway of synthesis in the endoplasmic reticulum, transport through the Golgi and export to the plasma membrane, essentially all data indicate that Cx43 follows this pathway (see Fig. 2). However, whether all connexins oligomerize into a connexon in the endoplasmic reticulum, *cis*-Golgi compartments or the *trans*-Golgi Network is not as clear. Cross-linking studies using various inhibitors have indicated that Cx43 oligomerizes in the *trans*-Golgi network [17], but other studies have indicated that Cx26/Cx32 oligomer assembly occurred in the ER/Golgi intermediate compartment [18]. These seemingly conflicting results may be explained by a study, using connexins engineered with different endoplasmic reticulum retention signals, showing that the site of oligomerization might be connexin-dependent [19]. Connexons can then be transported to the plasma membrane where they are available for gap junction assembly. There is considerable evidence that connexons exist in the non-junctional plasma membrane outside of gap junctions (reviewed in Ref. [4]). In fact, a growing body of work suggests that these connexons or hemichannels may play important biological roles in the regulation of calcium, ATP and NAD^+ levels in cells [20,21]. Clearly, functional hemichannels would have to be tightly regulated since unrestricted connections between the cytoplasm and extra-cellular spaces would result in cell death. Both mitogen-activated protein kinase (MAPK)- and protein kinase C (PKC)-mediated phosphorylation have been implicated in keeping Cx43 hemichannels closed [4]. Opening of hemichannels might be a regulated step in apoptotic pathways [22]. Finally, gap junctions must be assembled from a plasma membrane pool of connexons (Fig. 2) which, at least for Cx43, appears to be a process that is correlated with Cx43 phosphorylation [23,24].

Evidence for the acute regulation of gap junction assembly/disassembly can be found in the myometrium where gap junctions are necessary for the synchronization of electrical and metabolic activities of uterine smooth muscle contractions during delivery [25]. A 5–10-fold increase in Cx43 mRNA and protein levels was detected immediately prior to and during labor. Cx43 protein accumulates within cytoplasmic membranes until parturition, when it is rapidly transported to the plasma membrane and assembled into gap junctions, followed by a rapid loss of the protein after delivery [26].

3. Cx43 phosphorylation

Several reports have shown that Cx43 has a half-life in the range of 1–3 h in cultured cells or in tissues [7–11]. A

fast turnover rate could imply a high level of posttranslational regulation. Indeed, the pioneering work of Musil and Goodenough, the Lau laboratory and several other investigators have shown that Cx43 is differentially phosphorylated throughout its life cycle in homeostatic cells [8,9,11,23,27–30]. Cx43 demonstrates multiple electrophoretic isoforms when analyzed by polyacrylamide gel electrophoresis (SDS-PAGE), including a faster migrating form that includes non-phosphorylated (P0 or NP) Cx43, and at least two slower migrating forms, commonly termed P1 and P2. Both P1 and P2 co-migrate with P0 following alkaline phosphatase treatment, suggesting that phosphorylation is the primary covalent modification detected in SDS-PAGE analysis [9,30]. However, we and others have found that phosphorylated species can migrate with the P0 band in SDS-PAGE [31]. Phosphoamino acid analysis indicates the majority of the phosphorylation events occur on serines [30,32–34], although tyrosine phosphorylation has been observed in the presence of activated pp60^{src} [9,35]. The C-terminal region of Cx43 appears to be the primary region that becomes phosphorylated, but Cx56 can be phosphorylated within the cytoplasmic loop region, in addition to its C-terminal domain [36]. Cx43 does not contain serine residues in its intracellular loop region (Fig. 1), and no reports of phosphorylation of the N-terminal region of connexins have been presented.

The initial phosphorylation of Cx43 can occur within 15 min of synthesis in cultured fibroblast cells [9]. Treatment of cells with the trafficking inhibitors monensin or Brefeldin A results in the loss of the P1, P2, and higher phosphorylated forms of Cx43 and the appearance of a phosphatase-sensitive band migrating just above the NP form [33,37]. A fraction of monomeric Cx43 is phosphorylated and migrated at the P1 position [17]. Whether these observations represent sequential phosphorylation events is unknown, but clearly, phosphorylation of Cx43 can occur before it reaches the plasma membrane. The functional relevance of these early phosphorylation events has yet to be demonstrated but they could be involved in the proper trafficking of Cx43 from the ER, oligomerization of Cx43 into a connexon, or the exit of Cx43 connexons from the *trans*-Golgi network. No direct evidence has been presented linking Cx43 phosphorylation with the regulation of oligomerization into a connexon. In fact, no effect on oligomerization was observed upon truncation of Cx43 after residue 251 (removing amino acids 252–382 and 20 of 21 C-terminal region serines), but the truncated protein was transported to the plasma membrane less efficiently than the full-length protein [38]. Because some non-phosphorylated Cx43 can be found at the plasma membrane [23,30], these early phosphorylation events could be transient or simply unnecessary for transport to the plasma membrane. Consistent with the latter idea, phosphorylation of only one or two subunits of the oligomerized connexon may be sufficient for efficient Golgi trafficking or export to the plasma membrane. Although Cx43 in nonjunctional plasma

membrane regions is not necessarily phosphorylated, Cx43 located in gap junctions has been reported to be primarily phosphorylated to the P2 form and resistant to solubilization by Triton X-100 in several cell types (e.g., Ref. [23]). Cx43 in nonjunctional plasma membranes or cytoplasmic membranes is soluble in 1% Triton X-100 [23]. Whether the phosphorylation event(s) leading to P2 formation are necessary for the formation of or stabilization of gap junctions is unknown, but the phosphorylation event(s) is unlikely to be without consequence.

Based on several studies, Cx43 undergoes multiple phosphorylation events, as at least five phosphorylated serines have been detected on Cx43 isolated from unstimulated cells and several more in growth factor or kinase activator treated cells [12]. These mostly uncharacterized phosphorylation events have been correlated with changes in assembly, acquisition of Triton X-100 insolubility, and degradation of Cx43 gap junction channels, and hence, could play critical roles in regulating gap junctional communication. Several protein kinases, including PKC, PKA and casein kinase 1 (CK1), are known to be important in the phosphorylation and regulation of gap junction assembly (Figs. 1 and 2) and these are described in detail below.

3.1. Phosphorylation of Cx43 in response to increased cAMP levels

Agents that elevate intracellular cAMP have been shown to increase the amount of Cx43 that is phosphorylated and Cx43-mediated intercellular communication. Depending on the cell type, many different agents can increase cAMP levels and, thus, many different stimuli can increase gap junction formation. Such enhancement has been shown to result from elevated synthesis [39] and/or increased trafficking of connexons to the plasma membrane resulting in increases in the size and number of gap junctions and the assembly of new gap junctions [40–43]. Cyclic AMP-enhanced gap junctional assembly has been shown to be mediated by PKA [41], and microinjected PKA catalytic subunit can have rapid effects on gap junctional communication [44,45]. Although these effects may result in changes in gap junction channel gating, PKA effects on channel assembly can also be very rapid [46]. S364 is a major phosphorylation site in cellular Cx43 and phosphorylation of this site appears to be critical for cAMP-enhanced but not basal gap junctional assembly [46]. However, Cx43 is a relatively poor substrate for PKA compared to PKC or MAPK [46–48]. Whether PKA activates another kinase that actually phosphorylates Cx43 at S364 or PKA inefficiently directly phosphorylates this site, implying some physiologic advantage to Cx43 being a poor substrate [47], is unknown. There is significant biological interest associated with S364. S364 resides in the first of a tandem RXSSR repeat found from R362–R374. A serine to proline conversion at S364 (S364P) was

first identified as a mutation in a subset of patients with visceral atrial heterotaxia, and transfected cells expressing the S364P mutant Cx43 displayed altered gap junctional properties in response to PKC or PKA activity [44]. In spite of the fact that Cx43 mutations have not been identified in other populations of visceral atrial heterotaxia patients [49], the S364P mutation significantly affects left–right patterning in the early *Xenopus* embryo when misexpressed dorsally resulting in a significant increase in heterotaxia [50]. The S364P mutation also alters the pH sensitivity of Cx43 gating in the *Xenopus* oocyte expression system [51].

3.2. Protein kinase C

PKC has received considerable attention because PKC activators (e.g., phorbol 12-myristate 13-acetate or PMA) both increase Cx43 phosphorylation and decrease gap junction communication in a number of different cell types [7,27,28,52,53]. PMA treatment has been shown to reduce the permeability of Cx43 channels via phosphorylation of S368 [54]. In addition to its effects on channel gating, PMA has also been shown to dramatically decrease gap junction assembly. Time course, pulse chase and cell surface biotinylation experiments indicated that Cx43 present in the plasma membrane is the target and that PMA acts by destabilizing newly forming gap junctions while not affecting larger ones [7]. However, PMA effects on assembly have not as yet been linked to phosphorylation at a specific site in Cx43. The kinetics of PMA action can be complex since it can affect channel gating, gap junction assembly, and connexin half-life. In addition, PKC becomes down-regulated over time in the presence of PMA, thereby reversing many of these effects. Thus, reports that show differences in the effects of PMA or PKC activation in disparate or even the same cell types need to be compared cautiously.

Recent reports have begun to dissect the regulation of Cx43 by PKC on a molecular basis by defining which specific residues can be phosphorylated by PKC, which residues show increased phosphorylation in response to PMA treatment and which specific PKC isozymes might be involved in this process. PKC has been shown to phosphorylate Cx43 at S368 [47,54,55] and S372 [47,54,55] in vitro. Phosphorylation of S262 and S368 has been shown to be increased in response to PMA. The former has been linked to increased cellular proliferation through an unknown mechanism [56] while the latter has been shown to underlie the PMA-induced reduction in intercellular communication and alteration of single channel behavior [54]. However, these or other potential PKC sites might be involved in regulating assembly or degradation of gap junctions and channels. The introduction of several inhibitors and activators of PKC that are specific for certain isoforms has led to investigations of the roles that these PKC isoforms may play in the down-regulation of communication. For example, overexpression

of PKC γ or PMA treatment of lens epithelial cells caused a reduction in cell surface Cx43 and allowed co-immunoprecipitation of PKC γ and Cx43 [57]. Inhibition of gap junctional communication was found to be dependent on PKC α , β , and δ to different extents in various fibroblast systems [58]. PKC α and ϵ were found to associate with Cx43 in cardiomyocytes [59]. Fibroblast growth factor-2, which decreases cardiomyocyte gap junctional permeability and increases Cx43 phosphorylation, increased colocalization of PKC ϵ with Cx43 [60]. Thus, it appears that several members of the conventional and novel PKC families likely influence gap junctional communication. One confounding factor is that different cells express distinct isoforms and adaptor proteins and respond differently to these agents, thus generalizing about the importance of specific isoforms in regulating gap junctional communication may be somewhat premature. An understanding of the actual sites of phosphorylation and the consequences of these events will probably be necessary before we fully understand the importance of different PKC isozymes in regulating gap junctional communication.

3.3. CK1 and other kinases

CK1, particularly the δ isoform, has been shown to interact with and phosphorylate Cx43 on serine(s) 325, 328 or 330 in vitro [24]. Cx43 may also be a direct substrate for CK1 in vivo as these residues are major phosphorylation sites of cellular Cx43 [24]. Immunofluorescence and cell surface biotinylation experiments in the presence of a CK1 inhibitor showed increases in Cx43 plasma membrane localization, but reduced gap junction formation, indicating that this pathway is important in gap junction assembly.

Certainly, other kinases phosphorylate Cx43. Several residues in the tandem serine repeat region can be phosphorylated (i.e., serines 365, 368, 369 and 373) in response to follicle-stimulating hormone (FSH), probably at least partially through a PKA-mediated mechanism [48]. There is experimental evidence that Cx43 can be phosphorylated on at least 12 of the 21 serines [12] and two of the tyrosines [61] (Fig. 1) in the cytoplasmic tail region (amino acids 250–382). Certainly, it is possible that multiple kinases may phosphorylate the same residue. For example, serine 255 can be phosphorylated by both MAPK and cdc2 [34,62]. Thus, considerable evidence indicates that Cx43 is a highly phosphorylated and a highly regulated protein. The challenge is to link these specific phosphoregulatory events with the different steps in the Cx43 life cycle.

4. Changes in Cx43 phosphorylation during the cell cycle

The amount of Cx43 phosphorylation has been reported to increase 2.5 \times during S and 4.5 \times during M relative to the G0/G1 level [62]. Gap junctional communication also

changes during the cell cycle. Gap junctional communication was reported to be moderate during G1/S, increased through S and decreased in G2/M [63,64]. In addition, uncoupling of gap junctional communication has been reported to inhibit cell progression into S phase [65,66]. 3T3 cells expressing a Cx43 truncation mutant (aa 1–256), which lacks 19 of the 21 serines found in the C-terminus, showed slower progression through G2/M [67]. During mitosis, gap junctional communication levels decrease dramatically [63,68], and gap junctional communication is gradually restored as cells proceed through G1 [63,68].

4.1. The localization of Cx43 changes during the cell cycle

Immunofluorescence studies indicate that Cx43 is almost exclusively in the plasma membrane during G0 (Fig. 3). Immunoblot analysis indicates that about half of the protein resides in the Triton X-100 insoluble fraction (Fig. 3) and migrates as the P2 isoform. However, Cx43 is found both at the plasma membrane and in numerous cytoplasmic vesicles and intracellular compartments during S phase. In these cells, the ratio of Triton X-100 insoluble to whole cellular Cx43 decreases and more Cx43 migrates in the P0 and P1 positions. During mitosis, Cx43 is found almost entirely in the cytoplasm, seen as large, discrete puncta clustered in the interior of the cell (Fig. 3) [32,68], and Cx43 is phosphorylated to a distinct phosphoisoform (Pm or P3) in a manner

that is dependent on p34cdc2 kinase [32,62]. Interestingly, the amount of Triton X-100 insoluble material in mitotic cells is about the same as in the S phase cells and contains the P3 form, despite reduced plasma membrane staining (Fig. 3).

Gap junction assembly also is cell cycle-regulated with G0 cells being about 50% more efficient at assembly than S phase cells [31]. This difference may account for the accumulation of intracellular Cx43 in S and G2/M cells. Experiments utilizing an antibody specific for Cx43 that has been phosphorylated at S368 have shown that this decrease in assembly corresponds with increased phosphorylation at S368 [31]. As discussed above, this site has previously been reported to be phosphorylated in response to PMA, which also causes a dramatic decrease in gap junction assembly [7].

Other PKC-mediated effects during cell cycle progression have been shown. In a study of clone 9 cells, a PKC-mediated decrease in gap junctional communication and a shift in phosphoforms were observed as cells progressed from G0 to S phase [69]. Mitogenic stimulation of cardiomyocytes has also been associated with decreased gap junctional communication and PKC-mediated phosphorylation on S262 [56]. In this system overexpression of wild-type Cx43 or a S262A mutant led to a decrease in the number of cells entering S phase. However, a S262D mutant, which mimics the phosphorylated charge, did not

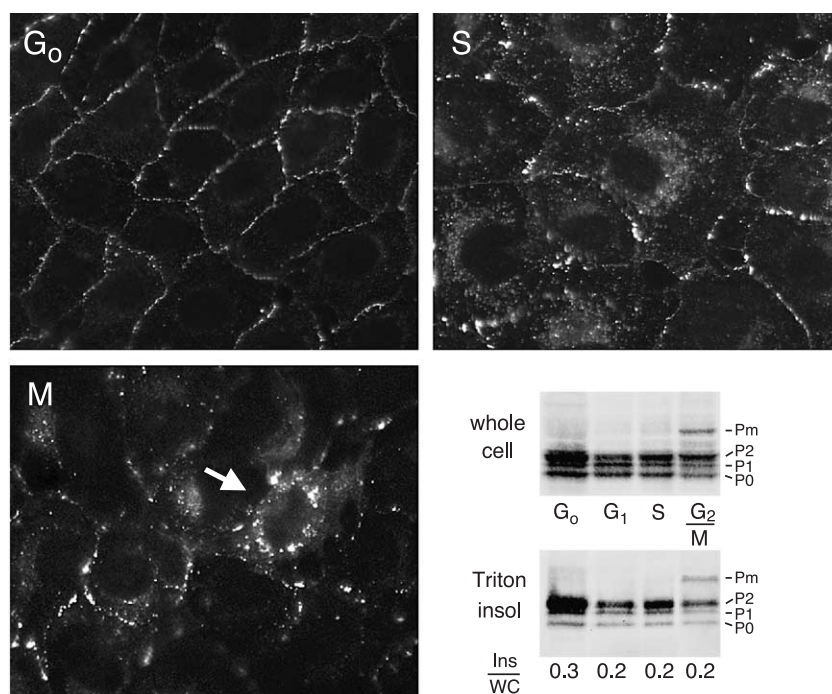


Fig. 3. Cx43 localization and phosphorylation status alter during the cell cycle. Immunofluorescence staining for Cx43 in NRK cells synchronized in G₀, S or G₂/M (M) phase of the cell cycle is shown. An arrow points to a mitotic cell in the M panel. Cells were grown on coverslips, fixed in 50% MeOH/50% acetone and probed with a monoclonal anti-Cx43 antibody. The lower right panel shows an immunoblot analysis of synchronized NRK cell lysates. Whole cell lysates (WC) and 1% Triton X-100 insoluble fractions (INS) were run on SDS-PAGE, blotted to nitrocellulose and probed with an antibody to the N-terminus of Cx43. Quantitative analysis of immunoblot was performed using the LI-COR fluorescence system. The bottom line shows the ratio of insoluble Cx43 over whole cell (INS/WC) obtained using this system.

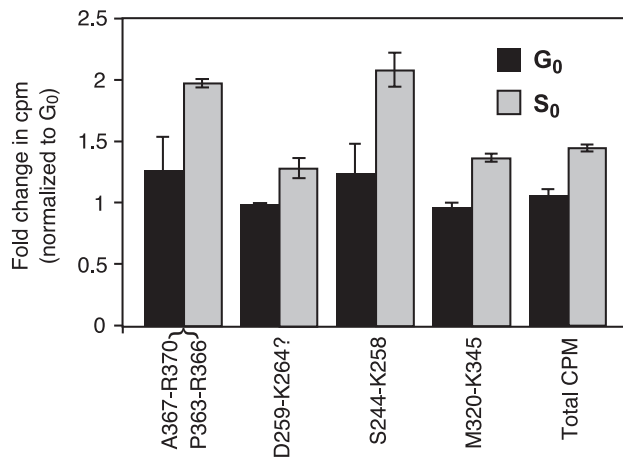


Fig. 4. Two peptides derived from metabolically labeled Cx43 are more phosphorylated in S phase than G₀ phase. Synchronized NRK cells were metabolically labeled with ³²PO₄ for 4 h, lysed in RIPA buffer containing 0.6% SDS, and Cx43 was immunoprecipitated using a Cx43 antibody. Cx43 was trypsinized, fragments were separated via reverse phase HPLC, fractions were collected and analyzed by Cerenkov counting. Experiment was performed in duplicate. Peaks of radioactivity were quantified and normalized to one of the G₀ values to evaluate changes on a peak by peak basis. Histogram shows the fold change in radioactivity of fragments. Error bars represent the range of duplicate samples.

affect the number of cells entering S phase. This indicates that phosphorylation on this site may be important in allowing cells to enter S phase.

4.2. Site-specific phosphorylation of Cx43 during the cell cycle

While Cx43 phosphorylation, in general, increases as cells progress through S and G₂ phases of the cell cycle, examination of specific cell cycle-regulated phosphorylation events has been primarily focused on mitotic cells. Phosphotryptic peptide/2-D gel electrophoresis analyses of Cx43 isolated from metabolically labeled cells have shown that there are several distinct sites of phosphorylation during

mitosis which are dependent on cdc2 kinase activity [32,62]. In vitro kinase reactions using purified cyclin B/cdc2 to phosphorylate recombinant Cx43 [32] or peptides containing S255 and S262 [62] have shown that these sites can be direct substrates of cdc2. However, these represent only a subset of sites phosphorylated during mitosis. Increased phosphorylation on S368 has also been shown using a phosphospecific antibody [31]. Thus, cdc2 appears to have a direct role in phosphorylating Cx43 during mitosis as well as an indirect role, likely by activating as yet unidentified kinases.

Tryptic analysis of Cx43 isolated from metabolically labeled G₀ and S phase cells shows site-specific regulation of phosphorylation. The total Cx43 pool from S phase cells incorporated 35% more phosphate than that from G₀ phase cells (Fig. 4, Total CPM). When individual tryptic fragments were separated by HPLC and radioactivity was normalized to the G₀ population, we found two tryptic fragments (Figs. 1 and 4) that were preferentially labeled, revealing 57% (A367–R370 and/or P363–R366) and 69% (S244–K258) increases in incorporated radioactivity (Fig. 4). Confirmation of the actual sites and identification of the kinases responsible will be important in the elucidation of their function.

4.3. Functional relevance

Interestingly, in spite of the preponderance of gap junction plaques in G₀ phase cells, they are less efficient at dye transfer than S phase cells [31]. When synchronized cells were co-injected with dyes of differing size (Alexa 488 and Alexa 594, FW 570 and 760, respectively), we found (Fig. 5) that more than three times as many cells received dye in the S phase population than in the G₀ population. In both S and G₀ phase cells, Alexa 488, the smaller of the dyes, showed more extensive dye transfer (Fig. 5). Increased gap junctional communication during S phase has also been observed when compared to G₁ phase cells [66].

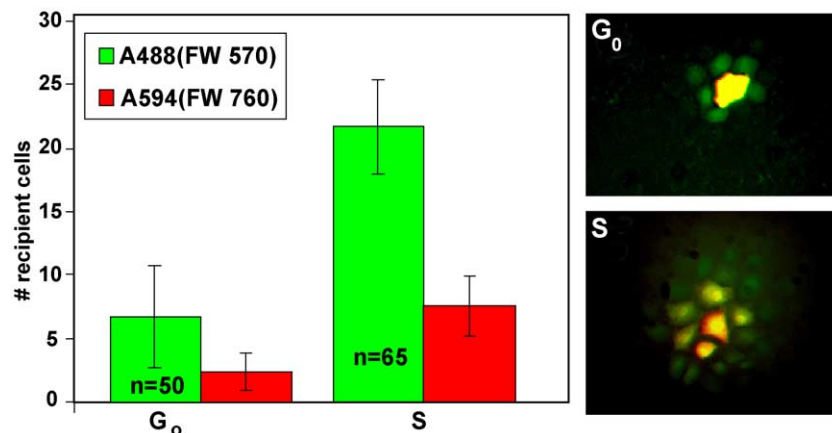


Fig. 5. S phase cells are more efficient at dye transfer than G₀ phase cells. Synchronized cell populations were co-microinjected with 10 mM Alexa 488 and Alexa 594 hydrazide; images were taken 10 min after injection and the number of cells receiving dye was assessed. *N* shows total number of cells injected in three separate experiments. Error bars depict standard error. The difference between S and G₀ cells is highly significant ($P < 0.001$).

In mitotic cells, gap junctional communication is markedly reduced [63,68], consistent with the protein being intracellular. Presumably, shutdown of gap junctional communication during mitosis is important in isolating mitotic cells and division-related signals from their non-dividing neighbors. It is also possible that intracellular Cx43 itself plays an important functional role as there is increasing evidence that Cx43 mediates growth control independently of intercellular communication [56,70].

5. Concluding remarks

Phosphorylation has been implicated in the regulation of gap junctional communication at several stages of the cell cycle and the connexin “life cycle”. Evidence indicates the trafficking, assembly/disassembly, and degradation, as well as the gating of “hemi” channels or intact gap junction channels are affected by several kinase pathways that can mediate connexin phosphorylation. Regulation occurs during the early stages of the connexin life cycle through assembly of functional gap junctional channels. Gap junction formation and Cx43 phosphorylation also appear to be correlated and highly regulated during the cell cycle. Changes in phosphorylation likely affect Cx43 subcellular localization and presumably binding partners leading to differences in not just Cx43 regulation but other aspects of cellular regulation including control of growth and proliferation. A more thorough understanding of these functions will require linkage of specific phosphorylation events to changes in binding partners and connexin function.

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