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Review

Channel-independent influence of connexin 43 on cell migration ☆

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ABSTRACT

In this review we focus on the role of connexins, especially of Cx43, as modulators of migration — a fundamental process in embryogenesis and in physiologic functions of the adult organism. This impact of connexins is partly mediated by their function as intercellular channels but an increasing number of studies support the view that at least part of the effects are truly independent of the channel function. The channel-independent function comprises extrinsic guidance of migrating cells due to connexin mediated cell adhesion as well as intracellular processes. Cx43 has been shown to exert effects on migration by interfering with receptor signalling, cytoskeletal remodelling and tubulin dynamics. These effects are mainly dependent on the presence of the carboxyl tail of Cx43. The molecular basis of this channel-independent connexin function is still not yet fully understood but early results open an exciting view towards new functions of connexins in the cell. This article is part of a Special Issue entitled: The Communicating junctions, composition, structure and characteristics.

Contents

1. Introduction

Cell migration is a fundamental process that has not only a pivotal role in early life when embryogenesis occurs. It is also essential for many physiological functions of the adult organism e.g. for immune surveillance [1], angiogenesis [2] and wound healing [3,4]. Moreover, cell migration plays an important role in pathophysiological processes such as tumour growth and metastasis [5,6] as well as vascular remodelling in atherosclerosis [7]. A number of proteins have been shown to be involved in the control of cell migration or its modulation. These include connexins (Cx), which are classically considered to act as gap junction channel forming proteins only. However, there is growing evidence, that connexins exert functions in the cell which are independent of their channel forming capacity. Here we aim to review current knowledge about the role of connexins in the control of cell migration with special reference to channel-independent mechanisms.

1.1. Connexins: proteins with a multitude of functions

Connexins consist of a family of more than 20 proteins which basically cluster in homomeric or heteromeric groups of six to form a hemichannel (also called connexon) in the cell membrane.

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Hemichannels allow for exchange of molecules between the cytosol and the extracellular space [8]. Two hemichannels from adjacent cells can form together a complete gap junction channel that directly connects their respective cytosols. Gap junction channels are clustered in larger groups in the membrane forming so called plaques [9]. All connexins are tetraspanning membrane proteins, the N-terminus and the C-terminus of the proteins being located in the cytosol. We will refer to the C-terminus as carboxyl tail throughout this manuscript. Connexins form two extracellular loops by which they contact with their counterparts from adjacent cells. There is also one intracellular loop which is thought to be involved in channel permeability regulation [10]. Of particular interest is the carboxyl tail, whose length varies among the different connexins. The carboxyl tail of Cx43 comprises the amino acids (aa) 232–382 (http://www.uniprot.org/uniprot/P23242). It is subject to posttranslational modifications such as phosphorylations by several kinases. Of particular importance for this review is the fact that the carboxyl tail is also able to bind other proteins including transcription factors and cytoskeletal elements. Thus, connexins can potentially act as scaffolding proteins e.g. as part of a signalosome or can interact with the cytoskeleton. Particularly this property of connexins may represent a structural basis for their "channel-independent" functions which by definition do not require exchange of molecules between adjacent cells (gap junction channels) or between the cytosol and the extracellular space (hemichannels).

1.2. Connexins are involved in cell migration

In recent years an increasing number of publications have shown that connexins are involved in the control of cell migration *in vitro* and *in vivo*.

In vitro, a role of connexins in migration has been demonstrated in brain derived cells (glioma cells [11], neuro2a cells [12], astrocytes [13]), fibroblasts (NIH3T3 cells [14,15]), endothelial cells and endothelial progenitor cells [4,16,17], epitheloid cells (HeLa cells, [16]) and cardiac cells (H9c2 cells, [18]) suggesting that connexins exert their role in cell migration control rather ubiquitously. *In vivo*, high expression of Cx43 was found in migrating neural crest cells being involved in brain and heart development [19,20] and in migrating endothelial cells during wound repair [21,22] suggesting a role of this protein during migration. Indeed, several *in vivo* studies, particularly on developmental processes in the brain [19,23,24,25], the heart [20,26] as well as in coronary vessels [27] have confirmed that connexins exert a functional role in development by controlling cell migration.

However, with regard to the topic of this short review, it remains often unclear in these studies, whether the reported influence of connexins on cell migration is dependent ("channel-dependent") or independent ("channel-independent") of the presence of functional gap junction channels or hemichannels, respectively. In many publications, the effects of Cx mutations or knockouts on cell migration have been studied without assessing for the involvement of gap junctional transfer (or transferability) of molecules (e.g. by transfer of fluorescent dyes) or electric signals. Therefore, in these studies it was not directly shown that these cells expressed functional gap junction channels and it was not tested whether channel functions were necessary for migration. For instance, Jia and coworkers convincingly showed that angiotensin II (AngII) induced expression of Cx43 and gap junctional intercellular communication (GJIC) in vascular smooth muscle cells. They also showed, that siRNA against Cx43 inhibited the AngII induced migration. However, they neither used gap junction blockers nor studied the role of genetically designed connexins without channel forming properties [28]. In C6 glioma cells it was also found that high expression of Cx43 enhanced the cell motility. Conversely, depletion of Cx43 with siRNA resulted in decreased motility and tissue invasivity of these cells but it remained unclear whether GJIC was important for this effect of Cx43 [11]. This connexin has also been shown to be involved in the response of cultured astrocytes to injury. Downregulation of Cx43 by miRNA resulted in a slower wound closure due to decreased proliferation and migration of these cells. It is likely that this was a channel-independent effect as exposure to the gap junction blocker carbenoxolone did not affect the rate of wound healing [13] but unfortunately, most pharmacological gap junction blockers are unspecific to some extent.

To solve some of these experimental problems, modifications of the connexin proteins have been performed. Especially genetic modifications of the carboxyl tail have been studied. However, transfection with such connexin mutants does not only alter putative connexin signalling but also permeability of the gap junction channels. A study in glia cells revealed that Cx43 truncated at the C-terminal end (Δ244–382) reduced motility compared to full length Cx43 suggesting that the carboxyl tail played a role for glia cell migration [11]. Likewise, stable transfection of 3T3 A31 fibroblasts with a truncated Cx43 (expressing aa 1–256, i.e. a mutant that nearly complete lacks the cytosolic carboxyl tail) resulted in a decreased cell motility compared to full length Cx43 as assayed both in a wound assay and in a Boyden chamber assay [15]. However, these effects of carboxyl-tail truncation on cell migration could still be related to an altered channel function: we [16] as others before [11,29–32] have shown that expression of Cx43 without most of the carboxyl tail (stops at aa 239, 245, 258 or 303) goes along with reduced gap junction permeability. Therefore a reduced migration in cells with truncated Cx43 could still be due to this reduced channel function.

It should be mentioned however, that the stimulatory effects of Cx43 on migration may be partly cell type specific or depend on other not yet identified factors. Indeed, some studies even showed directly or indirectly an inhibitory effect of Cx43 in migration (e.g. in cultured astroglial cells, skin lesions or in breast carcinoma cells [33–36]). Even in the same tissue contrasting effects have been described. For example, in neuronal tissue several groups reported an impaired cell migration after acute downregulation of Cx43 with shRNA [24,25], whereas another group [37] reported that the chronic knockout of Cx43 showed a normal pattern of cortical lamination which is not indicative for any impairment in migration. Contradictory effects of connexins on cell migration may therefore depend on differences between cell types, on compensatory effects of other Cx proteins or on specific properties of various migration stimulatory pathways as discussed by [16].

In this short overview we will, however, focus on migration supporting and enhancing effects of connexins. Most of this work is focused on Cx43 due to its ubiquitous distribution. Results on other connexins are therefore rather rudimentary. Therefore, this review concentrates predominantly on Cx43 mediated effects.

2. Channel-dependent connexin effects on migration

Channel-dependent effects of connexins require by definition either intact gap junctions or functional hemichannels. Gap junctional communication may be especially important in cells migrating collectively as it is observed during embryogenesis or cancer development [5]. In a wound assay, an established model of cell migration, cultured adrenocortical cells were shown to exert intact gap junctional communication between cells whilst they were migrating collectively [5]. It is conceivable that in such a cell cluster, calcium waves propagating among these cells via gap junctions could play a role in coordination of the migration [38] as calcium waves within smooth muscle cells play an important role for polarization and migration [39]. In a wound assay using immortalised, endothelial type cells, Cx43 expression was positively associated with migration and wound closure. Conversely, expression of a dominant negative Cx43 mutant reduced cell coupling and migration. Interestingly, migration of single cells was not found to be reduced under these conditions [4] suggesting that the coupling of cells was important for the collective migration

in streams or sheets. Heterocellular coupling with host cells may also be necessary to foster migration in parenchymal tissue: intact gap junctional coupling (via Cx43) of glioma cells and astrocytes was shown to be a prerequisite for glioma cell invasion and migration in brain parenchymal tissue [40].

In addition to gap junctional coupling, hemichannels are known to play a role in the control of cell migration under certain conditions. It has been demonstrated, that cells can release ATP via hemichannels. ATP release via hemichannels seems not to be specific for a certain connexin: ATP release has been shown to occur in glioma, glioblastoma and HeLa cells being transfected with either one of the connexins Cx43, Cx32, and Cx26 [41] as well as being impaired in macrophages exhibiting a Cx37 polymorphism [42]. This "channel-dependent" release of ATP via hemichannels from certain cells may play a role in inducing or coordinating calcium signalling required for the migration process of neighbouring cells via P_2Y_1 receptors [41]. ATP induced calcium signalling has been shown to modulate neuronal proliferation and, probably, migration of neuronal cells [43].

3. Channel-independent connexin effects

An increasing number of experimental observations support the view that part of the observed effects of connexins on cell migration are truly channel-independent. This means that the effects are not due to the exchange of signals such as ions or other small molecules across intercellular gap junction channels or hemichannels. There is indeed accumulating evidence that connexins may play a role in the control signalling cascades regulating cell migration and/or physically interfere with proteins that are known to be involved in cell motility. Moreover, connexins have a function in cell adhesion and guidance of migrating cells within the tissue.

3.1. Connexins as adhesion mediators in external cell guidance

An adhesive function of connexins has originally been demonstrated *in vitro*. Lin and colleagues [44] showed that cultured glioma cells expressing Cx43, Cx40 or Cx32 hemichannels aggregated with astrocytes whereas connexin free control cells did less so. 50–70% of Cx expressing cells showed an aggregation whereas in mock transfected cultures only about 10–15% of the cells aggregated spontaneously. Antibodies raised against their extracellular loops reduced aggregation of Cx43 expressing cells. Though adhesion is mediated via connexons or hemichannels it is a channel-independent function as defined before, since cells expressing connexin chimaeras without channel forming capacity showed also an enhanced aggregation. These results are in line with our observations that Cx43 expressing HeLa cells freely floating in a suspension rapidly aggregated in a Cx43 dependent manner (Kameritsch and Pohl, unpublished). In an elegant systematic study [45] showed not only adhesive aggregation of normally incompatible cell types as soon as both of them expressed Cx43 hemichannels. Adhesion between two cell types could also be substantially increased, when Cx43 expression was upregulated in only one of the partners. Moreover, studies with cells expressing either Cx32 or Cx43 suggest that the docking rules for connexon dependent adhesion may be similar as with gap junction formation [45].

Further evidence for a role of connexins in supporting cell adhesion comes from elegant *in vivo* experiments. In the developing brain, precursors of pyramidal cells have to migrate from the ventricular zone towards the cortical plate. During this process they need guidance by radial glia cells to their final destination. Immunohistochemistry revealed that contacts between these two types of cells in areas are enriched with Cx43. Acute downregulation of Cx43 reduced the radial migration (at maintained motility) *in vivo* with the result of impaired cortical layer formation. This suggests a pivotal role of Cx43 in this directional process [25,46]. Interestingly, using Cx43 acute loss of function models by RNA interference, Elias et al. [24] showed that this control of radial migration did not require an intact channel function of Cx43. However, a direct interaction between extracellular loops of Cx43 located in neural and glial cells was necessary since the conservative exchange of an amino acid in the external loops preventing the adhesive interaction abolished the radial migration of neuronal cells. The authors concluded that Cx43 regulated migration in a channel-independent manner probably by controlling mutual adhesion of the two cells types [47]. The effect of Cx43 on radial migration of neuronal cells was mimicked to some extent by Cx26. This effect must be considered independent of Cx43 since Cx43 and Cx26 neither form heteromeric hemichannels [48] nor do the two homotypic hemichannels couple to form a functional gap junction channel [49].

In further studies, these authors described that Cx43 also plays a specific role for the switch of the direction of migration in the brain. On their way from the ventral telencephalon towards the appropriate lamina of cortical plate certain inhibitory interneurons need to change from a tangential direction towards a radial one to reach their destination. It was shown that loss of Cx43 did not affect the original tangential migration but impaired the following migration in a radial direction [47]. Circumstantial, *in vivo* evidence for Cx mediated cell adhesion comes also from the observation that microglia in brain stab wounds showed rapid aggregation which was associated with a high Cx43 immunoreactivity [50].

It remains unclear however, whether the adhesive effect of the connexins is sufficient to control migration or whether other Cx-dependent processes are additionally necessary. A potential role of additional factors is suggested by conflicting results concerning a role of Cx43 in directional migration in the brain, whereas developmental changes in the cortex have also been observed in another conditional Cx43 knockout mouse specific for glia cells [51]. In two chronic knockout models of Cx43 little effect of Cx43 on cortical lamination or radial migration in the cortex, respectively, was found [37,46]. In the conditional knockout model described, the severity of phenotype changes was indeed decisively dependent on the genetic background. Another explanation for the discrepancies could be that the lack of a certain connexin could be functionally compensated by another one. *In vitro* studies with astrocytes isolated from these animals have indeed revealed persistent expression of other connexins in Cx43 KO mice [37].

Overall, these studies suggest that adhesive interaction via connexins represents an additional or alternative pathway for extracellular guidance which is generally thought to occur via the interaction of cellular integrins with extracellular matrix (ECM) proteins [52]. It remains unclear, however, whether adhesion to other cells via connexins alone is sufficient for controlling the directional cell movement or whether it requires downstream intracellular signalling elicited by adherence of connexins, possibly via interaction with other connexin associated proteins.

Unfortunately, to the authors' best knowledge, there are no studies directly measuring the adhesion forces exerted by connexins and comparing them e.g. to integrin-mediated adhesion forces. Earlier studies with leukocytes revealed, however, that adhesion forces via connexins were relatively weak as compared to specialised adhesion molecules [53] raising questions about an exclusive mechanic role of gap junctions e.g. as firm extracellular anchoring points during migration. Rather, these connexin derived adhesion points may represent a mechanic link to intrinsic migration control mechanisms. Connexins are known to interact with membrane complexes controlling the localization of cytoskeletal rearrangement and alignment processes [26,36,54]. Interestingly, Cx43 seems indeed to be important for the stability of leading processes of the neuronal cells determining the migratory pathway along the glial fibres [24]. It will be exciting to study the mechanisms that control the localisation of Cx43 in the cellular extensions of migrating neurons in a way that radial migration along the glia becomes possible.

3.2. Connexins in intrinsic migration control

In vitro, several groups have observed an enhancing effect of connexin expression on single cell migration which is unlikely to be mediated by connexin adhesive guidance due to the lack of corresponding, connexin expressing cells [11,14,15,18]. Therefore, a migration enhancing effect seems to be dependent on intracellular actions of connexins.

3.2.1.. Potential molecular mechanisms of intrinsic, channel-independent connexin effects on migration

The molecular basis for such channel-independent functions of connexins is poorly understood. Channel-independent functions of connexins are thought to be mainly due to the direct or indirect interaction of connexins with other proteins. As a result, the membrane expression of proteins that are important for cell adhesion/migration could be stabilized by such interactions. For example, the membrane expression of N-Cadherin or of ZO-1 is significantly higher in the presence of Cx43 [14].

Connexins may particularly be important for forming signalling complexes together with other proteins. Indeed, the known gap junction proteome comprises now more than 40 molecules [55]. In principle, all cytoplasmic substructures of the connexins can be posttranslationally modified [56,57] and may, therefore, interact with proteins. However, it is particularly the variable carboxyl tail [58] which contains the largest part of potential binding sites for other proteins [55,59–61]. Indeed yeast two-hybrid approaches using the isolated carboxyl tail of Cx43 allowed to identify a number of interaction partners [61]. This is in accordance with experimental findings that assign the major or even only part of connexin dependent migration control to the carboxyl tail [16,24,62]. Indeed, we and others have shown in HeLa [16] and glioma cells [62] that the migration control exerted by Cx43 was fully dependent on the presence of its C-terminus alone. This corresponds with the *in vivo* observations of Cina et al. [25] and Elias et al. [47] showing that migration dependency on Cx43 was blunted when the cells expressed a truncated Cx43 lacking its carboxyl tail. Crespin and coworkers also showed that the carboxyl tail of Cx43 (aa 243–382) alone is sufficient to promote motility of human glioma cells [62]. However, a Cx43 lacking the C-terminus had also positive effects on migration, implying the possibility of at least two distinct signalling mechanisms existed to modulate cell motility [62]. However, this second, carboxyl tail independent mechanism was found only in a subset of the glioma cell lines studied [62]. Interestingly, the two different mechanisms were apparently reflected by a different filopodia and lamellipodia density. It was higher in cells expressing the carboxyl tail than in cells expressing the truncated connexin [62].

In order to understand which protein interactions at the carboxyl tail may be functionally relevant, basic mechanisms controlling cell motility and directionality shall be shortly considered.

3.2.2.. Factors controlling cell motility and migration

Given its relevance for a wide spectrum of fundamental biological processes, the basic mechanisms of cell motility and of directed cell migration have been studied intensely. Despite existing differences between cells, the so called cell motility cycle basically includes protrusion of the leading edge, changes of adhesion to the underlying substrate as mediated by integrins, cytoskeletal remodelling and,

Table 1
Selection of Cx43 associated proteins and their effects on the control of cell migration.

Cx43 — associated proteins	Effects on Cx43/gap junctions	Ref.	Main effect in the context of cell migration	Ref.
Cytoskeletal proteins				
Microtubules (α- and β-tubulin)	Targeted delivery of newly synthesized connexin hemichannels along microtubules to existing gap junctions in the plasma membrane.	[97] [71] [82] [98]	Involved in control of cell polarity and migration.	[99] [100]
Actin	Stabilization of gap junction expression at the plasma membrane.	[101] [102]	Essential for formation of protrusive structures like lamillipodia and filopodia.	[103] [104] [105]
Drebrin	Drebrin may stabilize Cx43 gap junctions at the membrane.	[106] [90]	Involved in organisation of the cytoskeleton and transduction of contractile forces necessary to allow cellular motility.	[92]
Cortactin	Interaction may stabilize association of Cx43 with actin bundles and exert signalling effects in myogenesis.	[88] [80]	Connects the actin cytoskeleton to several proteins that control actin based cellular processes involved in migration.	[107]
Interactions with tight junctional proteins				
Zonula occludens-1 (ZO-1)	Controlling redistribution of Cx43 within the plasma membrane.	[108] [55]	-ZO-1 directly binds to a kinase MRCKβ, which is modulating the cell motility.	[72]
Interactions with adherens junction proteins				
Cadherins	Cx43 and N-cadherin control each others expression at the cell-surface. @Cx43 movement to the plasma membrane is dependent on E-cadherin-mediated adherens junction formation.	[14] [109]	N-cadherin modifies migration behaviour from "single cell" migration to "wavefront" migration. Different isoforms control neuronal migration in the CNS and are involved in transmigration of melanoma cells.	[110] [111] [112]
Catenins	Catenins control assembly and trafficking of connexins to the membrane.@ Cx43 associates with β-catenin in the cell membrane.	[94] [113]	May modulate gene expression in a Cx dependent manner. Activated β-catenin (key molecule in the Wnt pathway) interacts with transcription factors to control genes involved in migration.	[114] [115]
-p120 (catenin-related protein)	p120 co-localizes with Cx43 and N-cadherin.	[70]	Increase in p120 expression promotes cell migration.	[116]
Other connexin 43-interacting partners				
Aquaporins	AQP-4 and Cx43 are co-regulated.	[59] [67]	AQP-4 is involved in the control of glioblastoma cell migration.	[117] [118]
COX-2	Cx43 binds/co-localizes with COX-2 in intestinal adenoma cells.	[119]	Endothelial cell COX-2 promotes integrin αVβ3-mediated endothelial cell adhesion, spreading and migration.	[120]

finally, partial detachment of the cells [52]. The underlying polarized signalling events include a plethora of proteins: small G-proteins, including RhoA, Rac and Cdc42 play a key role in forward recruitment. Membrane protrusions are formed by the assembly of actin filaments and a reorientation of the Golgi apparatus and of the microtubules of the cell is observed [52,63]. Tail retraction and disassembly of focal adhesions is again mainly a small G-protein mediated process involving myosin phosphatase and the regulatory myosin light chain [52,63]. These processes are coupled with dynamic changes of the actin cytoskeleton and modulations of its interaction with integrins and, indirectly, with the cellular localization of the integrins and their avidity for the ECM. Integrins have short cytoplasmic domains that lack catalytic activities. Therefore, their signal transduction critically relies on the recruitment of scaffolding and signalling proteins such as kinases to the focal adhesion [64,65]. Directional migration requires this basic motility machinery to be coupled to further steering mechanisms. Processes underlying such steering mechanisms occur in a highly localised manner within the migrating cells suggesting an important role of scaffolding proteins controlling adherence and hence, localization of multiprotein complexes.

Cx43 interactions controlling cell motility

Connexins interact with junctional proteins, cytoskeletal proteins and enzymes, many of them known to play a role in cell motility [55,66]. In addition, direct or indirect interactions with ion channels and transporters have been reported [67] and these in principle can also play a role in controlling cell motility. Due to the ubiquitous distribution of Cx43, most studies have been performed focussing on this connexin. In some cases, scarce information about other connexins is also available. Table 1 shows an overview of a selection of proteins interacting with Cx43 and the potential role of these proteins in the control of cell motility. However, most of these interactions still await experimental evidence that their binding by Cx43 and the protein's effect on migration are functionally coupled. In the following a selection of these interactions will be briefly described, where the interaction of Cx43 and the respective protein has been studied with regard to their role for migration. Nevertheless, some of the proposed mechanisms remain speculative to some extent.

Junctional proteins and small G-proteins. Connexins are typically found in close proximity to adherens and tight junction proteins [55]. These junctions are thought to allow effective traction forces in migrating cells [68]. The interaction is also considered to stabilize gap junctional plaques. Accordingly, Cx43 and N-cadherin control each other's expression in the membrane of NIH3T3 cells [14]. The interaction of Cx43 with members of the cadherin family of adhesion sites may not only be important for the mechanics of cell migration but also generates signals. Interaction of Cx43 and cadherins has been shown to activate Rho GTPases which are promoting cell motility and invasion [26,69,70] (Fig. 1b). This activation is an important step in the control of cell migration [68]. In migrating single cells Rac1 was found to be pivotal in forming actin-rich structures which in conjunction with E-cadherin are considered responsible for the generation of traction forces of germ cells *in vivo* [68].

Several groups [14,54,59,66,71] have also described an interaction of the zonula occludens-1 protein (ZO-1) with multiple connexins. Experimental data suggest that ZO-1 has also a potential role in cell migration [72–74]. However, the importance of this interaction for migration is not clear. It has been shown that e.g. a tagging of the carboxyl tail of Cx43 abolishes the interaction of the connexin with ZO-1 [71]. Since we still have observed Cx43 mediated effects on migration also with GFP-tagged Cx43, though these were somewhat reduced, the interaction of ZO-1 and Cx43 is unlikely to play the only role in this context [16]. Rather, recent experiments suggest that the interaction regulates gap junction channel formation from hemichannels [75].

Receptor activation. In progenitor cells derived from mouse embryonic striatum, Scemes et al. showed that the purinergic P_2Y_1 receptor has an important function in the control of calcium modulated cell migration [76]. Deletion of Cx43 reduced both, the function of the receptor and cell migration. This effect was channel-independent since carbenoxolone did not mimic the effect of Cx43 deletion in wild type (WT) cells. More recently, in Cx43 deficient astrocytes it was shown that expression of the carboxyl tail alone or of a short peptide sequence of the tail (aa 260–280, representing part of a src binding site) could rescue the enhancing effect of Cx43 on the P_2Y_1 receptor mediated calcium activation, giving another example for Cx43 mediated, carboxyl tail dependent control of cell migration (Fig. 1c) [77].

Cytoskeleton. Since cytoskeletal proteins play an important role in the control of cell motility, interactions of connexins and cytoskeletal proteins are of particular interest. The carboxyl tail of Cx43 is indeed interacting with several cytoskeletal proteins (F-actin, α/ß-tubulin, α-actinin, IQGAP, actin-binding proteins like ezrin, Drebrin or cortactin) [26,59,66,78–80].

Microtubules have an important role for the control of cell polarization and migration as well. They are highly dynamic in migrating as opposed to resting cells [81]. The proper alignment of the microtubule organizing centre is a prerequisite for directional movement during migration (Fig. 1b). In mouse epicardial cells which are precursors of coronary artery system, Cx43 deficiency causes an impaired polarization, reflected by a non directional alignment of the microtubule organizing centre. As a consequence, a loss of directionality of migration of epicardial cells can be observed, resulting in an impaired development of coronary arteries in these mice. The binding of tubulin to the known binding site in the carboxyl tail of Cx43 seems to be essential for polarity and migration. A Cx43 mutant with a defect in the carboxyl tail (Δ234-243, i.e. lack of the tubulin binding site) fully mimicked the phenotypic pattern of Cx43 deficiency [27]. Such a role of Cx43 would probably require a polarized distribution of Cx43 in the membrane as well. A potential mechanism for a local accumulation of connexins could be the recently described highly dynamic targeted transport of Cx43 (or Cx26) containing vesicles to existing gap junction plaques via microtubules. The microtubules were shown to connect indirectly to cadherin [82]. The connection to cadherin involved the microtubule plus end tracking protein EB1 as well as the EB1-interacting protein p150 and β-catenin [82] (Fig. 1b). Accordingly, the alternative knockdown of either Cx43 or N-cadherin has been shown to inhibit motility of NIH3T3 cells [14].

Actin fibre growth and alignment are also basic processes in forming filopodia, which play an important role in cell migration [83,84,85]. It has been shown that filopodia are more frequent in cells expressing Cx43 [62]. Intriguingly, proteins important for filopodia formation, such as VASP and cofilin [84] have been described to be associated with Cx43 [36,86]. Moreover, Cx43 expression has been reported at the tip of filopodia-like structures in astrocytes [87] (Fig. 1a). In migrating neuronal cells *in vivo*, the role of the two expressed connexins Cx43 and Cx26 has been tested by downregulation with shRNA. Both connexins were shown to be important for migration control by stabilizing leading cell protrusions. However, according to the phenotypes observed, their role was somewhat different. Cx43 was apparently controlling branch stabilization of actin bundles in the cell protrusions whereas Cx26 seemed to be more involved in the soma, controlling the succeeding translocation of the nucleus [24].

The effects of connexins are likely to be mediated by interaction with several actin binding proteins. Such an interaction has been shown for example between cortactin and Cx43 by coimmunoprecipitation in mouse tubulus seminiferous cells [88] and in mouse myoblasts [80]. However, it was not shown whether this was a direct or indirect interaction and whether the carboxyl tail was in fact the binding site. Cortactin promotes cell motility by enhancing

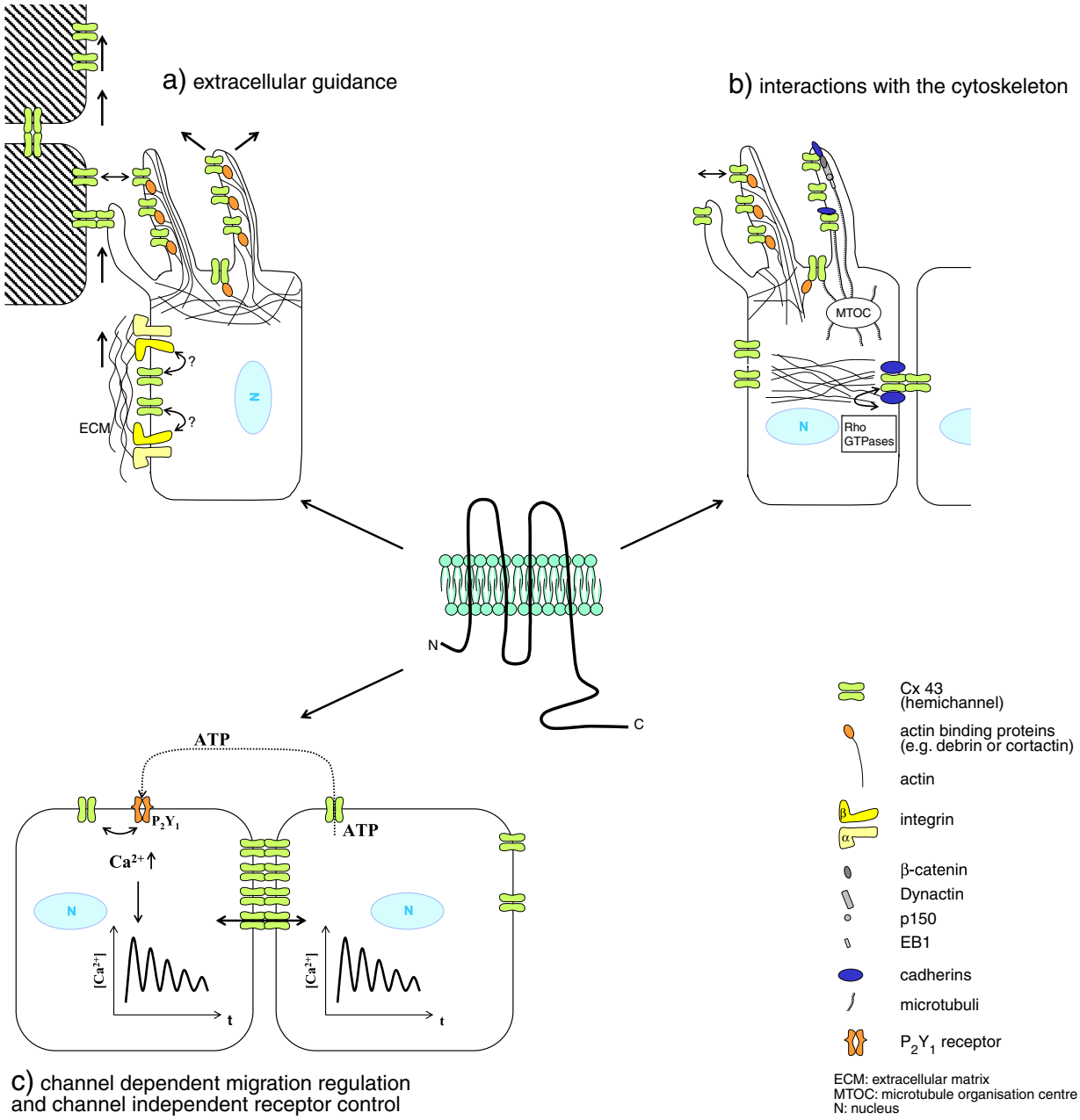

Fig. 1. Schematic illustration of interactions between Cx43 and proteins that are (a) linked to the extracellular matrix, (b) part of the cytoskeleton or (c) receptors mediating Ca^{2+} signalling which is transmitted to neighbouring cells via gap junctional communication.

lamellipodia formation. A knockdown of this protein reduces the rate of formation of new adhesion sites in lamellipodia [89]. The interaction of cortactin with Cx43 seems to be well controlled. In C2c12 murine myoblasts, sphingosine-1-phosphate (S1P) stimulated the interaction of both proteins. Since this interaction was blocked by p38 inhibition, the authors proposed that a p38 induced phosphorylation of Cx43 leads to an enhanced binding of cortactin. It was concluded that this interaction induced a cytoskeleton mediated differentiation of myoblasts which was channel-independent [80]. Potential effects on migration were not studied. However, own preliminary studies in HeLa cells stimulated with serum did not confirm that binding of cortactin to Cx43 was blocked by inhibition of p38 (Pogoda and Pohl, unpublished).

A direct interaction of the carboxyl tail of Cx43 with another actin binding protein, drebrin, has also shown in mouse brain homogenates, astrocytes and various cell models [90]. However, its role in Cx43 mediated control of migration is still unclear though an association between migration and an isoform of drebrin (drebrin E2) has been demonstrated in melanoma cells and fibroblasts where the protein contributes to retraction of cell body and tail during migration [91]. Moreover migrating neuroblasts in the adult brain showed expression of drebrin E in the cell body which disappeared with cessation of migration. This suggests that drebrin may act as a molecular switch for neuronal migration [92]. Current data suggest that drebrin plays a predominant role in the stabilisation of Cx43 in the membrane [90]. Therefore, further studies are required to elucidate the exact pathways by which Cx43 controls actin dynamics in cell migration.

Of particular interest is the potential interaction between integrins and connexins since both can play a role for the cytoskeletal reorganisation within the cell. Some reports show that there is indeed such a crosstalk. In cardiac neural crest cells, a loss of Cx43 expression was associated with significant changes of $\beta_1$integrin and vinculin distribution and a severe reduction of focal contacts [26]. In the same

cells a severe loss of directionality and a loss of migratory speed were observed, effects, which were dependent on the density of fibronectin in the extracellular matrix [26]. This suggests a crosstalk between Cx43 and $\beta_1$integrin (Fig. 1 a). However, it is unclear at present whether this was a channel-independent phenomenon and which signalling cascade was involved. Of note, in cardiomyocytes, there also existed a dependency of Cx43 expression on the matrix composition which was mediated by the $\beta_1$integrin [93].

With regard to cytoskeletal arrangements, a caveat has to be made with regard to a spatial control by Cx43 since expression of the carboxyl tail alone also enhanced the migration [16]. Of note, its subcellular distribution was diffusely cytosolic, lacking any apparent preferential localisation within the cells [16,62]. Its localisation therefore differed considerably from that of the full-length connexin. Though it cannot be excluded that still sufficient quantities of the carboxyl tail were available at the membrane as well, the preserved ability of the isolated carboxyl tail to enhance migration could be therefore due to mechanisms other than polarizing. It is also hardly conceivable that the effects of the isolated carboxyl tail are consistent with a role in development of cellular traction forces which requires membrane anchoring [68]. The ubiquitous subcellular distribution and putative concomitant decoy effects of the cytosolically distributed carboxyl tail may also explain the observation that a strong overexpression of the carboxyl tail alone had even inhibitory effects on migration in some experiments [47].

Enzymes. Connexins, in particular Cx43, are also known to interact – permanently or temporarily – with several protein kinases and phosphatases, including ERK1/2, PKC, PP1, PP2a, RPTP [55,59]. Particularly Src has been shown to bind directly to the carboxyl tail at different sites [61]. The functional role of these interactions is considered to lie predominantly in the phosphorylation of the carboxyl tail and subsequent alterations of gap junction permeability and connexin recycling. However, it is tempting to speculate that the carboxyl tail may not only serve as a substrate for these enzymes. Rather a binding of these enzymes may result in (de)phosphorylation of other proteins whilst the latter interact with the carboxyl tail. Our observation that HeLa cells expressing Cx43 showed an increased activation of p38 after stimulation with serum, points towards such a possibility [16]. However, further studies addressing this question are clearly necessary.

4. Open questions

It seems clear that connexins and in particular, Cx43 can affect cell migration in several channel-independent ways exerting profound effects in organ development as well as in the adult organism. However, there are still many open questions which require more investigations in order to understand the physiologic relevance and basic mechanisms of connexin mediated migration control. Clearly we do not yet know whether the influence of Cx43 on migration represents a class effect. Due to the fact that most studies on channel-independent effects have been performed with Cx43 there is a lack of information whether the channel-independent effects are specific for Cx43 or shared with other connexins. Of note, at least in the studies on neural crest cells [24] there were striking similarities regarding the effects of Cx43 and Cx26.

It is also not known whether the connexins need to be clustered to connexons to exert channel-independent functions or whether they can act as single proteins possibly outside cell membranes. In addition, it remains unclear whether the channel-independent interaction of connexons leading to a guidance of migrating cells has also an outside in signalling component, e.g. by conformational changes of connexins upon binding of two connexons. Another open question is the understanding of possible interactions of connexins with integrins. Whilst there are some reports, which could be interpreted as crosstalk between both classes of proteins (see above), there is a considerable lack of data in this particular field.

A general caveat has to be made with regard to interpretation of the results of genetically modified cells used in most cases reviewed here. Reduction of Cx43 may increase the transactivation potential of β-catenin thereby modulating the expression of genes [94]. Connexins are also known to affect gene expression e.g. through connexin-responsive elements (CxRE). For example, it has been shown, that connexins and/or gap junctional communication can induce recruitment of sp1 and sp3 transcription factors to the CxRE [95]. It remains to be determined to which extent so far identified channel-independent effects of connexins on migration as studied in Cx43 knockout models or with Cx43 mutants are alternatively dependent on bystander effects of an altered gene expression. Microarray analyses of gene expression in brains (at day E19) showed altered expression of up to 5% of the spotted genes in Cx43 null brains [96].

Finally, we do not know whether the described Cx43 effects on migration are only detectable when specific migration stimuli are used or whether they represent a more general phenomenon during cell migration, which is independent of specific signalling pathways.

5. Summary

In this review we focus on the role of connexins, especially of Cx43, as modulators of migration — a fundamental process in embryogenesis and in physiologic functions of the adult organism. This impact of connexins is partly mediated by their function as intercellular channels but an increasing number of studies support the view that at least part of the effects are truly independent of the channel function. The channel-independent function comprises extrinsic guidance of migrating cells due to connexin mediated cell adhesion as well as intracellular processes. Cx43 has been shown to exert effects on migration by interfering with receptor signalling, cytoskeletal remodelling and tubulin dynamics. These effects are mainly dependent on the presence of the carboxyl tail of Cx43. The molecular basis of this channel-independent connexin function is still not yet fully understood but early results open an exciting view towards new functions of connexins in the cell.

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