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Gap junctions: multifaceted regulators of embryonic cortical development

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Abstract

The morphological development of the cerebral cortex from a primitive neuroepithelium into a complex laminar structure underlying higher cognition must rely on a network of intercellular signaling. Gap junctions are widely expressed during embryonic development and provide a means of cell-cell contact and communication. We review the roles of gap junctions in regulating the proliferation of neural progenitors as well as the migration and differentiation of young neurons in the embryonic cerebral cortex. There is substantial evidence that although gap junctions act in the classical manner coupling neural progenitors, they also act as hemichannels mediating the spread of calcium waves across progenitor cell populations and as adhesive molecules aiding neuronal migration. Gap junctions are thus emerging as multifaceted regulators of cortical development playing diverse roles in intercellular communication.

Introduction

The adult cerebral cortex is an intricate laminar structure composed of six distinct layers of neurons that together are highly adapted to process complex information. The mechanisms that control the morphological development of the cerebral cortex are of particular interest, as evolutionary advancements in cognition might be closely linked with changes in developmental patterns [1]. Neocortical brain development is orchestrated by contact, signaling and communication between cells in the developing neuroepithelium. Gap junctions, large-diameter channels that form an aqueous pore between the cytoplasm of adjacent cells, are highly expressed during embryonic development in distinct spatial—temporal patterns (for a review, see Ref. [2]). Gap junctions have been broadly implicated in the control of embryonic patterning and morphogenesis by mediating information flow between coupled populations of cells through the creation of morphogenic gradients and synchronization of electrical and/or metabolic activities (for a review, see Ref. [3]). Here we will review the role of gap junctions in the development of the rodent cerebral cortex, highlighting distinct functions in regulating cell proliferation, migration and differentiation. Gap junctions also contribute to the generation of cortical circuits in neonatal animals by mediating oscillatory patterns of electrical activity, although this topic will not be discussed (for a review, see Ref. [4]).

Gap junctions were first described in the mature brain in the late 1970s. Soon after the identification and cloning of the rat gap junction subunits connexin (Cx) 32 and Cx26 from

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the liver [5-7] and the cloning of Cx43 from the heart [8], it was shown that Cx26 and Cx43 are highly expressed in the developing embryonic cortex whereas Cx32 is upregulated after birth; this was the first evidence that there is a complex cell-specific expression pattern of Cx proteins in the developing and mature brain [9]. The formation of functional gap junctions in the developing cortex was originally demonstrated by transfer of low but not high molecular weight dyes between clusters of neuroepithelial cells in the ventricular zone (VZ); the lack of transfer seen with high molecular weight dyes excludes the possibility of cell coupling through cytoplasmic bridges [10,11]. In addition, electrophysiological recordings demonstrated that neuroepithelial cells have a low input resistance as a result of the expanded intracellular volume of coupled cells, a volume that can be effectively reduced by closing the gap junctions [10]. Electron microscopy has confirmed the presence of gap junction plaques between cells lining the ventricular surface [12]. Coupling has also been observed between neural progenitor cells derived from the embryonic ganglionic eminences using dye transfer techniques *in vitro* [13].

Today we know that there are at least 20 genes encoding connexins in rodents and humans, and that at least 5 of them are highly expressed in the rodent embryonic cerebral cortex including Cx26, Cx36, Cx37, Cx43 and Cx45 [12,14]. Each Cx has a distinct spatial and temporal pattern of expression during cortical development, which might have functional significance as each Cx family member has distinct permeability and regulation properties (for a review, see Ref. [15]). Cx26, Cx37 and Cx45 are largely evenly distributed from the VZ to the cortical plate, whereas Cx36 and Cx43 are highly expressed in the VZ and less so in the cortical plate [14,16].

The functional capabilities of gap junctions extend beyond the classical notion of intercellular coupling and include hemichannel-mediated exchange with the extracellular environment, cell-cell adhesion and intracellular signaling (Box 1). Here we examine the diverse roles of gap junctions regulating the growth, structural organization and maturation of the cerebral cortex.

Radial glial proliferation: the role of gap junction coupling and hemichannels

The identity of coupled cells in the cortical neuroepithelium remains somewhat elusive in the literature. Coupled clusters of cells were initially hypothesized to contain neuroblasts [10] and then later neural progenitors as well as at least some radial glial cells [11,17]. However, radial glial cells have more recently been identified as the neural progenitors of the embryonic cortex dividing asymmetrically to produce neurons or intermediate progenitor cells [18,19]. We suggest that coupled clusters of cells are composed largely of radial glial cells. Initial experiments did not detect all the radial fibers because they were limited by the sensitivity of microscopic techniques that were only able to detect the fiber on the brightest cell filled directly by the patch pipette. More advanced confocal microscopy allows visualization of radial glial fibers associated with secondarily filled cells in coupled clusters in the VZ (S.C. Noctor and A.R.K., unpublished). Radial glial gap junction coupling is regulated progressively during cortical development and within each cell-cycle division and might play an important role in controlling the pattern of neurogenesis [10,11,17,20] (Figure 1).

Box 1. Gap junction functions: coupling, hemichannels, adhesion and signaling

Gap junctions are channels that form an aqueous pore between the cytoplasm of two adjacent cells, allowing the exchange of electrical current and small molecules (<1 kDa). Each gap junction is made up of two hemichannels on opposing membranes that join through hydrophobic interactions. Hemichannels are composed of six connexin (Cx) subunits (connexon), each having four transmembrane domains and two extracellular loops (for a review, see Ref. [63]). Gap junction functions (Figure 1): (i) Cell coupling: the classical action of gap junctions is to allow electrical current, small molecules, metabolites or ions

to travel between cells, including but not limited to cAMP, ATP, IP3, glucose, glutamate, Ca^{2+} and K^{+} . (ii) Hemichannels: in addition to forming the opposing subunits for gap junctions, hemichannels can also exist in an unopposed form on the cell membrane, thus mediating exchange with the extracellular environment. It was originally thought that hemichannels would remain in a closed state because of high levels of extracellular Ca^{2+} ; however, recent evidence suggests that hemichannels mediate functional release of small molecules such as ATP under physiological conditions (for a review, see Ref. [64]). (iii) Adhesion: the formation of gap junctions between adjacent cells can provide an adhesive force between cells and interact with the internal cytoskeleton [16,35]. (iv) Signaling: gap junctions, especially the C terminus of the Cx subunit, have been implicated in junction-independent cell-signaling functions (for a review, see Ref. [65]).

The number of cells in a cluster of coupled cells decreases from embryonic day 15 in the rat, suggesting that coupling is more prevalent during mid-neurogenesis than in late neurogenesis [10]. However, this decrease in overall cell coupling is accompanied by an increase in gap junction hemichannel-mediated Ca^{2+} waves. It has been shown that gap junction hemichannels mediate Ca^{2+} waves in the developing VZ through the release of ATP that binds to purinergic P2Y1 receptors on neighboring radial glia, thereby activating an IP3-mediated release of Ca^{2+} from internal stores [20,21]. Interestingly, Ca^{2+} wave frequency, size and distance increase in late neurogenesis [20]. The observation that similar levels and types of Cxs are expressed in mid- and late neurogenesis [14,17] suggests that Cx proteins are regulated at a molecular level such that they underlie the formation of gap junction-coupled clusters of cells during mid-neurogenesis and the hemichannel-mediated spread of Ca^{2+} waves during late neurogenesis.

How do gap junction-mediated cell coupling and Ca^{2+} waves differ in their functional consequences and their impact on neurogenesis? In rat organotypic slices at E16, a time point when both cell coupling and Ca^{2+} waves are present, the pharmacological block of gap junction channels or P2Y1 receptors, which blocks Ca^{2+} wave propagation, both result in a decrease in the entry of cells into S phase of the cell cycle [11,20]. Cultured cortical explants or dissociated cells from E12-13 mice also show cell-cycle inhibition in the presence of pharmacological gap junction blockers [22,23], as do neurospheres derived from ganglionic eminence progenitors [13]. However, these studies do not explore whether functional coupling or hemichannel-mediated Ca^{2+} waves are involved. In the development of the chick retina, increases in intracellular Ca^{2+} are correlated with the initiation of interkinetic nuclear migration (IKNM), the stereotyped migration of the nucleus during the cell cycle [24]. Retinal Ca^{2+} waves, mediated by gap junction coupling and ATP release through hemichannels, regulate cell proliferation and IKNM [24,25]. However, it is not clear whether the effects on IKNM are independent from those on the cell cycle. In the cortex, IKNM does not proceed in the presence of cell-cycle inhibitors, suggesting a dependence on cell-cycle progression [26]. It will be interesting to determine whether, in the cerebral cortex, hemichannel-mediated Ca^{2+} waves directly regulate cell-cycle progression, IKNM or both. As IKNM occurs throughout neurogenesis and Ca^{2+} waves predominate in late neurogenesis, another signaling mechanism would be expected during early development. Overall, it remains to be seen what functional role the switch from gap junction radial glial coupling to hemichannel-mediated Ca^{2+} wave propagation plays in the progression from mid- to late neurogenesis.

Not only is gap junction and hemichannel formation regulated during the course of neurogenesis, but it is also regulated with each passage through the cell cycle. M phase cells, identified by their morphology and position near the ventricle, are excluded from cell clusters, suggesting that cells uncouple when they pass through M phase [11]. Cells appear to recouple in S phase during early neurogenesis or in late S phase or early G2 during late neurogenesis

and remain coupled until the subsequent mitosis [11]. The delay in recoupling during late neurogenesis is correlated with and might be related to the increase in Ca^{2+} wave initiation as open hemichannels, which initiate the Ca^{2+} waves, are restricted to S phase cells [20]. Thus, there is a dynamic regulation of coupling, uncoupling and hemichannel opening throughout each cell cycle.

Does regulation of Cx expression underlie the changes in coupling and hemichannel formation during the cell cycle? During both mid- and late neurogenesis, Cx26 is expressed in more dividing cells during M/G1 than during S/G2; conversely, Cx43 is expressed in more dividing cells during S/G2 than in M/G1 [17]. Thus, the uncoupling of cells in M phase cannot be simply correlated with lower overall Cx expression levels. Alternatively, it is possible that the changes in the types of Cxs expressed affect the level of coupling. Interestingly, Cx43 expression is reduced during M phase in other cell types, which might result from p34^{cdc2} protein kinase phosphorylation that triggers degradation (for a review, see Ref. [27]). The first posttranslational modifications of Cx26 have recently been reported but the functions of these modifications are unknown [28]. Nonetheless, the fluctuation in Cx levels does not appear extremely large, and more than 50% of cells express Cx26 or Cx43 during all phases of the cell cycle. It is therefore likely that the ability of Cxs to form functional gap junctions or hemichannels is also tightly regulated by signaling interactions during the cell cycle.

Loss-of-function assays can help to differentiate the specific roles of Cx26 and Cx43 in the cell cycle. For example, it seems likely that Cx43 plays a more important role than Cx26 in the mediation of Ca^{2+} waves. Cx43 is particularly permeable to ATP [29], and is expressed at high levels during S phase in late neurogenesis [17], a time point when ATP is released through hemichannels to initiate Ca^{2+} waves [20]. Furthermore, hemichannels composed of Cx43 mediate Ca^{2+} waves by releasing ATP in the chick retinal pigment epithelium [25]. It will also be important to consider the other Cx proteins expressed in the developing cortex including Cx45, Cx36 and Cx37 and whether they are regulated with and contribute to cell-cycle progression. Additionally, during their migration to the cortex, intermediate progenitor cells in the sub-ventricular zone can undergo an expansive, symmetric division [18]. It is unknown whether intermediate progenitors express Cxs and, if so, whether their divisions are also regulated by gap junctions. Furthermore, gap junctions do not act in isolation to control progenitor proliferation in the developing cortex. Soluble growth factors such as bFGF are thought to be critical in the regulation of cell division and might, in some cases, do so by controlling gap junction expression and coupling (Box 2).

Neuronal migration: the role of gap junction adhesion

After radial glia divide asymmetrically, the newborn neurons must find their way to the correct lamina of the cortical plate. Interestingly, radial glial cells serve not only as the stem cells of the developing cortex but also as a cellular highway that guides the migration of newborn neurons to their final destination. Newborn neurons migrate in very close association with a radial glial fiber [30,31]. Nadarajah *et al.* first suggested that gap junctions might mediate communication between the radial fiber and the migrating neuron [12]. They observed that gap junctions are not only expressed in the VZ but also throughout the intermediate zone and cortical plate during development, a pattern suggestive of roles other than regulating radial glial proliferation, and they were able to localize Cx43 expression to radial glial fibers as well as closely associated migrating neurons [12]. More recent work benefiting from higher-resolution confocal microscopy has localized Cx26 and Cx43 puncta at the points of contact between migrating neurons and radial glial fibers, confirming a possible role in the signaling and communication between migrating neurons and their closely associated radial fibers [16]. Using electron microscopy, gap junctions have been localized between nestin-positive (a radial glial marker) and nestin-negative cells [12]. However, a clear localization at the electron

microscopic level of a gap junction or of Cx proteins at the interface between a migrating neuron and radial fiber would lend strong support to the observations made with fluorescence microscopy.

Box 2. bFGF signaling regulates Cx43

An interesting story has emerged linking the actions of basic fibroblast growth factor (bFGF, otherwise known as FGF 2) to Cx43 [23,66]. bFGF plays a very important role as a positive regulator of cerebral cortical size and progenitor proliferation during development [67-70]. bFGF and its receptors are expressed in the proliferative VZ during development, suggesting that it might act as a paracrine signal between progenitor cells [70]. Microinjection of bFGF into the *in vivo* developing cortex increases neuronal number and cerebral cortical size, whereas bFGF knockout mice have decreased neuronal numbers and decreased cortical size [70]. In fact, bFGF along with EGF is used to maintain neural progenitor cells grown as neurospheres *in vitro*, but the effect of bFGF is only evident when cells are in contact with each other, suggesting that cell-cell contact and signaling are necessary for its actions [68]. In cortical progenitors, *in vitro* treatment with bFGF increases the expression of Cx43 but not Cx26 mRNA and protein in a concentration-dependent manner and increases dye coupling between cells as well as proliferation [23,66]. bFGF binds to receptor tyrosine kinases (RTKs) and signals through p42/p44 mitogen-activated protein (MAP) kinases (otherwise known as extracellular signal regulated kinases; ERK1/2) to increase the expression levels of Cx43 [23,66]. Furthermore, blocking gap junctions pharmacologically inhibits the proliferative effect of bFGF. Cx43 expression increases proliferation in cultures not treated with bFGF but does not increase proliferation in cultures already treated with bFGF. Together, this suggests that the upregulation of Cx43 through MAP kinase signaling is necessary and sufficient for the proliferative effects of bFGF [23,66] (Figure I). It will be interesting to determine whether Cx43 is the only gap junction subunit regulated by growth factors or whether other subunits are similarly regulated. Furthermore, it is possible that changes in bFGF release or receptor activation underlie the changes in Cx43 expression seen during development and within the cell cycle. Further *in vivo* and epistasis experiments such as injecting bFGF into the ventricles of wild-type and Cx43 knockout mice during neurogenesis and assaying coupling and proliferation will help to further unravel the connections between FGF signaling, gap junction coupling and the regulation of proliferation.

Do gap junctions play a functional role in neuronal migration? Observations from Cx43 knockout mice, Cx43 conditional knockout mice and shRNA-mediated knockdown of Cx43 and Cx26 support a functional role for gap junctions in mediating neuronal migration along radial glial fibers [16,32,33]. Fushiki *et al.* report a change in the distribution of BrdU-labeled cells in the cortex of Cx43 knockout mice such that more cells are found in the intermediate zone and fewer are found in the cortical plate [32]. Mice with a conditional deletion of Cx43 driven by Cre expression under the human GFAP promoter also have a phenotype suggestive of a failure in neuronal migration [33]. Interestingly, the mouse background has a substantial effect on the severity of the phenotype, such that those on a C57Bl/6J background do not display a phenotype whereas those on a 129SVEV background have a clear brain phenotype, of which a particularly severe inbred line has been termed Shuffler [33]. The biological significance of the background effect in these mice is yet to be fully understood, but might relate to the potential for compensation by other Cx proteins in different mouse lines. Although the smaller cortex, cerebellum and hippocampus in Shuffler mice are most likely a result of proliferative defects, other phenotypes suggest compounding migratory problems. Most strikingly, in the cerebellum, granule cell ectopies and the increased thickness of the external granule layer are suggestive of a failure of granule cells to migrate radially to the internal granule layer.

Additionally, the delamination of Purkinje cells in the cerebellum and ectopic clusters of cells in the cortex that fail to reach the cortical plate suggest a general radial migration defect as well. It is not clear from these studies whether migration defects result from a loss of Cx43 in neurons, radial fibers, or both, because disorganization of radial fibers is observed [33]. Further evidence for a migration defect comes from *in utero* injection and electroporation of shRNA constructs targeting Cx26 and Cx43 in the developing rat cortex [16]. Cells expressing shRNA targeting Cx26 or Cx43 are unable to migrate to the cortical plate and remain in the intermediate zone. No effects were observed on cell death, early differentiation or ability to exit the cell cycle. Furthermore, the requirement for Cx expression in migrating neurons was established using transplant assays showing that Cx-shRNA-expressing neurons are unable to migrate on wild-type radial glia.

Most surprisingly, experiments designed to rescue the Cx-shRNA-induced migration defect using mutated forms of the Cx protein suggest that gap junction adhesion but not the channel or the C terminus is necessary for migration (Figure 2a) [16]. One critical tool for this study was the recent discovery that point mutations in a conserved threonine in the third transmembrane domain of Cx26 and Cx43 confer a closed channel [34]. This mutation was developed as a dominant-negative inhibitor of gap junctions, but the fact that it is sufficient to rescue neuronal migration suggests that it might be an extremely useful construct for exploring other non-channel-mediated functions of Cx proteins. Gap junctions have been previously reported to mediate adhesion in rat C6 glioma cells [35]; however, a functional role for adhesion is a departure from the classical notion of gap junctions. Cortical cells, however, can use gap junctions to promote cell-cell adhesion, and time-lapse experiments of shRNA-expressing neurons and of Cx puncta in migrating neurons together suggest that gap junction adhesions play a role in the stabilization of the leading processes and the trans-location of the nucleus (Figure 2b) [16].

Future work needs to explore the way in which gap junction adhesions interact with the internal cytoskeleton. Gap junction adhesions in migrating neurons co-localize with actin puncta and the centrosome [16], and Cx43 has been shown to bind several actin-interacting proteins including vinculin and drebrin [36,37]. Furthermore, in avian tenocytes, a mechanosensitive cell type, Cx43 colocalization with actin increases with mechanical strain and can be reduced by inhibition of myosin II, suggesting a dynamic functional interaction between the actin cytoskeleton and Cx43 [38]. The mechanism by which gap junctions co-localize with the centrosome, a structure that plays a very important role in coordinating migration (for a review, see Ref. [39]), is yet to be understood. Interestingly, the C terminus of Cx43 directly binds microtubules [40], but this interaction does not appear to be necessary for migration as removal of the C terminus of Cx43 or of the very short C terminus of Cx26 (for which there is no evidence of microtubule binding) does not disrupt the ability to mediate migration. Thus, further investigation concerning the mode of Cx interaction with the actin cytoskeleton and the centrosome as well as other adhesion molecules such as integrins will provide insights into the mechanistic role of gap junction adhesions during migration.

Neuronal differentiation: the role of gap junctions, hemichannels and signaling

The expression of Cxs is dynamic during the time period of neuronal differentiation. During early postnatal ages in the developing cortex, the overall expression of Cx43 increases, that of Cx26 initially increases and then decreases by the third postnatal week and that of Cx32 increases [9,12]. These fluctuations are accompanied by changes in the cell types that express each Cx. Cx43 and Cx26 are expressed in neurons and radial glia during development [12, 16], but their expression is largely restricted to astrocytes in the adult, whereas Cx26 and Cx45

are the most abundant Cxs in neurons and Cx32 is found in oligodendrocytes. Are the changes in Cx expression levels important for neuronal maturation?

Cx43 is highly expressed in newborn neurons derived from cultured immortalized hippocampal progenitor cells; however, upon differentiation, Cx43 levels are reduced whereas Cx40 and then Cx33 increase [41]. The decrease in Cx43 might be specific to neuronal and not glial differentiation, as RT4 rat peripheral neurotumor cells decrease levels of Cx43 when differentiated into neurons but not when differentiated into glia [42]. In cultured human NT2/D1 and rat P19 cells, both of which are pluripotent carcinoma cell lines in which cells are coupled and express Cx43, differentiation along the neural lineage with retinoic acid reduces levels of Cx43 expression [43,44]. Interestingly, treatment of NT2/D1 or P19 cells with pharmacological gap junction blockers, but not inactive analogs, profoundly reduces the number of mature neurons generated, as assayed by microtubule-associated protein 2 (MAP2) expression, and increases the proportion of cells expressing immature markers such as Nestin and Vimentin [45,46]. Furthermore, in the developing cortex, shRNA targeting Cx43 but not Cx26 reduces the proportion of cells that express mature neuronal markers postnatally such as NeuN and MAP2 (L.A.B.E. and A.R.K., unpublished) with no change in the proportion of immature neurons [16]. Together, this work suggests that functional gap junction channels mediate signaling that promotes neural differentiation.

Recent studies suggest that Cx32, Cx43 and Cx31 might play a role in neurite outgrowth [47, 48]. Neural crest-derived PC12 cells do not express appreciable levels of endogenous connexins, but when Cx32 or Cx43 are over-expressed during NGF-induced neural differentiation, neurite length is increased about twofold [47]. Interestingly, this increase in neurite growth is a result of hemichannel-mediated ATP release and its binding to purinergic receptors, as the effect can be mimicked by ATP treatment and blocked with purinergic antagonists or with gap junction channel blockers [47]. Furthermore, NGF treatment stimulates the opening of hemichannels [47]. Contrary to this finding, expression of Cx31 in SH-SY5Y human epithelial-derived neuroblastoma cells increases neurite length independent of connexin trafficking to the cell membrane [48]. Thus, signaling or transcriptional changes that result from Cx31 expression produce increased neurite outgrowth [48]. Further studies of neuronal differentiation *in vivo*, rather than in cell culture, will be critical to understanding the role of Cxs in neuronal differentiation. Furthermore, although it appears that the gap junction channel plays a central role, it is possible that the adhesive properties of gap junctions and their interaction with the cytoskeleton might contribute to cytoskeletal remodeling, especially during neurite outgrowth.

Functional redundancy and compensation among Cx family members

All of the functional studies reviewed suggesting that gap junctions regulate radial glial cell-cycle dynamics and neuronal differentiation have used pharmacological manipulations, whereas the studies that suggest a role in migration have used shRNA or genetic manipulations. Pharmacology allows determination of channel function when it is involved in gap junction intercellular exchange or hemichannel opening. As expected if migration is independent of channel function, none of the pharmacological studies note a migration defect. Alternatively, knocking down or genetically eliminating a Cx protein interferes with all aspects of its function including channel, adhesion and signaling capabilities. Although we have discussed migratory defects in relation to the loss of Cx proteins, is there also evidence for proliferation defects when the levels of Cx proteins are manipulated? In the conditional Cx43 knockout Shuffler strain, the smaller hippocampus, cortex and cerebellum are suggestive of a proliferation defect [33]. Furthermore, shRNA-induced knockdown of Cx26 or Cx43 has effects on radial glial proliferation (L.A.B.E. and A.R.K., unpublished). However, the genetic knockout of Cx43 does not have a phenotype suggestive of proliferative defects, and the migratory defects are relatively mild.

This brings up a common thread in gap junction research, that of compensation. In fact, when the Cx43 genetic knockout was first described, no apparent neurological defects were reported [49]. This might be a result of genetic compensation by other Cx family members and is reminiscent of the disconnect in other areas of gap junction research, such as embryonic axis formation, between the observation of strong phenotypes with pharmacological or knockdown studies and mild or absent phenotypes with genetic manipulations [3]. To test this rigorously, approaches to studying other highly redundant protein families could be employed [50]. Double and triple knockouts of Cx26, Cx43 and possibly Cx45, Cx36 or Cx37 could be created and studied. The embryonic lethality of some Cx proteins could complicate such an approach, but it might be possible to combine traditional and conditional genetic knockouts as well as targeted shRNA knockdown to eliminate multiple Cx family members.

To complicate matters, pannexins, homologs of invertebrate gap junction innexin proteins, might also contribute to gap junction function in the developing brain (for a review, see Ref. [51]). Pannexins are four-transmembrane domain proteins that can form gap junctions and hemichannels in *Xenopus* oocytes [52]. Pannexin 1 is expressed at early embryonic time points in the cortex peaking around mouse embryonic day 18, whereas pannexin 2 expression increases postnatally [53,54]. As pannexin channels are blocked by pharmacological agents used to block connexin channels such as carbenoxolone, pannexin-mediated gap junction function might have previously been attributed to connexins [55]. However, there is at present no evidence for the formation of functional gap junctions by pannexins in the brain [56]. Alternatively, pannexins might act as hemichannels mediating ATP release and Ca^{2+} waves as seen in other cell types [57,58]. It is thus still unclear what role pannexins might play in cortical development.

Conclusions

Here we review data suggesting that gap junction channels, hemichannels and adhesions act in distinct ways to mediate radial glial division, neuronal migration and neuronal differentiation. The scope of gap junction-mediated signaling continues to expand when considering the largely unknown downstream signaling pathways, which are arguably the most fascinating and least understood aspects of gap junction biology. For example, identifying the functionally relevant molecules exchanged between cells through gap junction channels is, at present, very difficult. Although it is typically thought that small metabolites and ions are the main signaling molecules, the possibility that short RNAs pass through gap junctions suggests alternative possibilities [59]. In understanding the role of gap junctions in radial glial division, it will be critical to identify the molecules exchanged between coupled cells and how they regulate the cell cycle. Likewise, the current data suggest that hemichannels on radial glial cells mediate ATP release that activates purinergic receptors and initiates Ca^{2+} waves in the cortical VZ [20], but it is not known how purinergic Ca^{2+} waves in turn regulate proliferation. A recent study of *Xenopus* development demonstrates that purinergic signaling induces the transcription factor Pax6, thereby initiating eye development [60]. Pax6 also plays a critical role in radial glial divisions in the cortex (for a review, see Ref. [61]), and it is possible that Pax6 levels might be affected by purinergic activation. Furthermore, the gap junction adhesions that mediate radial migration are likely involved in a dynamic crosstalk with cytoskeletal elements and other signaling molecules to mediate neuronal migration. Finally, recent reports also suggest that gap junction expression might coordinate the expression of networks of genes, the so-called connexin transcriptome, which might have diverse effects that are largely unexplored [62]. Thus, when designing future studies to discern the role of gap junctions in cortical development, not only will it be necessary to consider the possible roles of gap junctions as channels, hemichannels, adhesions or signaling molecules but also how these functions are integrated into the cells' other signaling pathways and communication machinery.

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Figure I.
Functional properties of connexins, hemichannels and gap junctions.

Figure 1.

The role of gap junction coupling and hemichannels in radial glial cell proliferation. Radial glial coupling and hemichannel activity are regulated during the course of neurogenesis and within each cell cycle. Radial glial cell gap junction coupling is greatest during mid-neurogenesis and decreases in late neurogenesis [10]. During the cell cycle, cells uncouple from clusters during M phase and recouple during S phase in mid-neurogenesis or late S or G2 phase in late neurogenesis [11]. During late neurogenesis, hemichannels on S phase radial glia initiate Ca^{2+} waves by releasing ATP which binds to P2Y1 receptors on adjacent cells inducing an IP_3 -mediated release of Ca^{2+} from intracellular stores [20]. Additionally, during each cell cycle, the levels of Cx26 and Cx43 fluctuate such that Cx26 and Cx43 levels are at their peak during M phase and S phase, respectively [17]. Pharmacologically blocking coupling or Ca^{2+} waves inhibits entry into S phase of the cell cycle [10,20].

Figure I.
bFGF-induced proliferation is dependent on Cx43 upregulation.

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Figure 2.

The role of gap junction adhesion in neuronal migration. (a) Several studies suggest that gap junctions mediate the radial migration of neurons to the cortical plate [16,32,33]. One approach to studying this phenomenon is through the use of in utero injection and electroporation of shRNAs targeting Cxs (Cx-shRNA) into the VZ of the developing cortex [16]. Control electroporated neurons are able to migrate to the cortical plate (CP), whereas those expressing Cx26-shRNA or Cx43-shRNA are unable to migrate to the CP and remain in the intermediate zone (IZ). The shRNA-induced migration defect can be rescued by co-expression of Cx43 or Cx26 with conservative mutations (CM) that confer resistance to shRNA knockdown.

Interestingly, selective rescue experiments using a variety of Cx mutants that selectively impair the channel, adhesion or the C terminus suggest that adhesion, but not the channel or C terminus, is necessary for migration [16]. SVZ = sub-ventricular zone. (b) Time-lapse imaging of Cx-shRNA-expressing neurons as well as the dynamics of Cx puncta localization in migrating neurons suggests that gap junction adhesions play two major roles in neuronal migration. (i) Gap junction adhesions, especially Cx43 but also Cx26, localize to the dominant but not the transient branch of bifurcated migrating neurons, thereby stabilizing the dominant leading process (yellow puncta) [16]. (ii) Gap junction adhesions, especially Cx26 but also Cx43, localize to the cell body of migrating neurons, specifically co-localizing with the centrosome, and move into the dilatation in the leading process before the translocation of the nucleus, possibly stabilizing the centrosome during nuclear translocation (orange punctum) [16]. Actin puncta also co-localize with gap junction adhesions and might provide a link to the internal cytoskeleton [16]. (Adapted from [16].)