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Connexins in migration during development and cancer

Maria Kotini, Roberto Mayor*

Department of Cell and Developmental Biology, University College London, Gower Street, London WC1E 6BT, UK

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ABSTRACT

Connexins, the gap junction proteins, through their multitude of actions are implicated in a variety of cell processes during animal development and cancer. They allow direct or paracrine/autocrine cell communication through their channel and hemi-channel functions. They enable adhesion and interact with a plethora of signalling molecules. Here, we review the common themes in developmental and pathological processes and we focus in their involvement in cell migration in four different systems: neurons, astrocytes, neural crest and cancer.

Gap junctions overview

Cell co-operation is concomitant with the organisation found in multicellular systems. Throughout animal life, from embryonic development to tissue homeostasis and in pathophysiological conditions, as in cancer metastasis, cells rely on the exchange of information between collaborating individuals in order to coordinate their action. One way in which cells achieve this coordination is through direct cell –cell communication.

In metazoans, direct cell communication is achieved by gap junctions, cell membrane junctional structures that display a 20–30 angstroms “gap” between adjacent cells, as first observed in electronic micrographs from mouse heart and liver (Revel and Karnovsky, 1967). Gap junctions are characterised as channels, which allow the intercellular exchange of small molecules and ions between neighbouring cells. Electron micrographs revealed the presence of gap junctions in numerous metazoan cell types, such as in astrocytes, neurons and ependymal cells in the brain of various vertebrates (Brightman and Reese, 1969), in smooth muscle cells (Uehara and Burnstock, 1970) and in embryonic amphibian cells (Decker and Friend, 1974).

In chordates the proteins that are involved in the formation of gap junctions are called connexins (Goodenough, 1974; Nicholson et al., 1981). The participation of connexins in animal development is evident by (a) their presence in various developmental stages and organisms, and (b) their requirement in early development, since loss-of connexin expression leads to dramatic and often lethal developmental phenotypes (Kruger et al., 2000; Kumai et al., 2000; Reaume et al., 1995). Thus, cell-cell communication is established early during embryogenesis. In mouse embryos, six different connexin genes have been transcribed by the eight-cell stage (Davies et al., 1996). In Xenopus, five different connexins are reported in early development, from fertilised egg till early tailbud stage (Landesman et al., 2003). Furthermore, specific connexins are linked with distinct morphogenetic processes. For instance, connexin43 (Cx43) gap junction protein plays an essential role in morphogenesis of the embryonic chick face (McGonnell et al., 2001), Cx43.4 is required for left-right axis formation in zebrafish Kupffer's vesicle (Hatler et al., 2009) and Cx45 is crucial for mouse heart development (Alcoléa et al., 1999). In cancer, the role of connexins is shown to be both enhancing (Ezumi et al., 2008; Ito et al., 2000; Plante et al., 2010) and inhibitory (Eghbali et al., 1991; Hellmann et al., 1999; Hirschi et al., 1996; Zhu et al., 1991) depending on the stage of disease and the tissue involved. For a review on the role of connexins in cancer see (Naus and Laird, 2010).

Structure and protein domains

In a cell, a hexamer of connexins is required to form a cylindrical shaped structure, which is called connexon, and corresponds to half of a complete channel, also known as “hemichannel” (Fig. 1A). The hexagonal structure of connexons was revealed by freeze fracture electron microscopy (Peracchia, 1973a, 1973b). Alignment and docking of two connexon counterparts belonging to adjacent cells is required to form a complete gap junction channel (Fig. 1A). The binding between connexons occurs

* Corresponding author.
E-mail address: r.mayor@ucl.ac.uk (R. Mayor).

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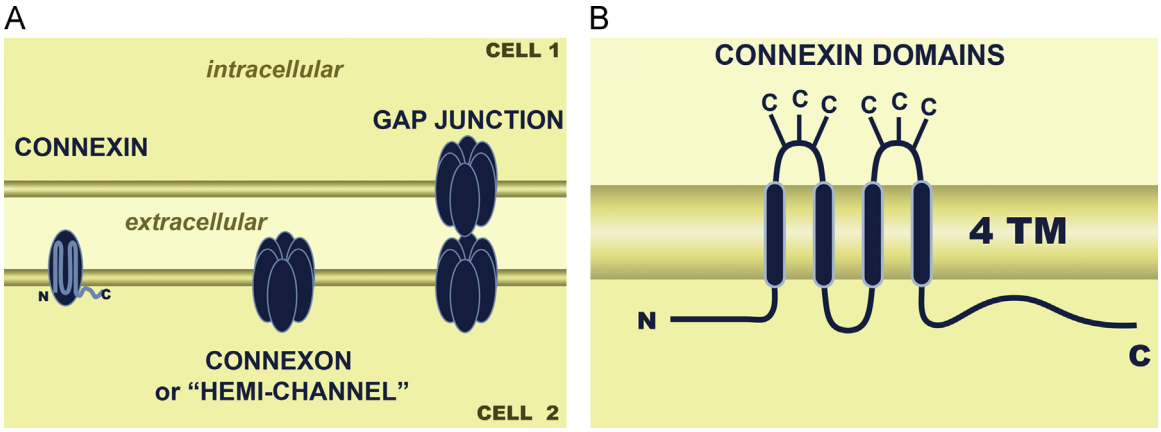

Fig. 1. Connexins are the structural basis of Gap Junction. (A) Connexins are depicted as blue ellipses (left); six connexins are required to form a connexon, also known as hemi-channel (middle). Two connexons align and form the complete gap junction channel (right). (**B**) Connexin Structural Domains. Connexin family of proteins share distinct structural motifs: cytoplasmic N-terminus, four trans-membrane domains, two extra-cellular loops with three cysteine residues on each one, one intra-cellular loop, cytoplasmic c-terminus.

through non-covalent bonds of the cysteine residues of the extracellular loops (Yeager and Nicholson, 1996). Gap junctions often assemble in a more complex multi-gap junctional structure along the adjoining cell membranes, called the gap junction plaque (Musil and Goodenough, 1991).

Depending on the connexin profile of the connexons, the gap junction channels are defined as homomeric or heteromeric and homotypic or heterotypic. Homomeric/homotypic are channels that are composed of the same connexin type, while heteromeric channels are composed of two different connexins and heterotypic channels are formed by two different connexons. Not all connexins can interact with one another to generate heterotypic or heteromeric channels, since the compatibility between different connexin types is a selective process (Elfgang et al., 1995; White et al., 1995).

All connexins share a conserved tertiary structure that can be divided into the following motifs: four trans-membrane domains, two extracellular loops with 3 Cysteine residues on each one, a cytosolic loop and a cytosolic N-terminus and C-terminus (Fig. 1B) (Unger et al., 1999a, 1999b).

Connexin activities

Channel functions

Gap junction channel

Initially, connexin activity was solely attributed to the channel function of gap junctions. The presence of connexins in a tissue was synonymous to have gap junction intercellular communication (GJIC). In this model, gap junctions serve as direct links between neighbouring cells, enabling tissues to function as a syncytium.

Indeed, in early development of Xenopus embryonic stages, most blastomeres are communicating through gap junctions (Landesman et al., 2000). During development, after several divisions, this communication is restricted to more localised areas and this localised communication has been suggested to control the compartmentalisation of the embryo (Bruzzone et al., 1996). Moreover, gap junction mediated communication by Cx32 and Cx43 is required for maintenance of embryonic cell adhesion and concomitant positional information during Xenopus development (Paul et al., 1995). In zebrafish development, the spatiotemporal expression of Cx43.4, a connexin with low conductance properties, coincides with the initiation of morphogenetic processes, further implicating connexin channel activity with pattern formation (Hatler et al., 2009).

Further evidence for connexin channel activity is demonstrated in studies related to heart development. In the developing heart, gap junctions are essential for conduction of electrical impulses of the heart and for synchronous beating (Simon et al., 1998). The spatiotemporal expression of heart connexins (Cx30.2, Cx40, Cx43 and Cx45) is tightly regulated in all vertebrates and connexin inhibition or misexpression results in perturbation leading to heart arrhythmias (Kirchhoff et al., 1998; Van der Velden et al., 1998). In a recent study, Cx40, the predominant connexin in the atrial myocardium has been shown critical for the conduction properties of the atrium (Benes et al., 2014).

The channel activity of gap junctions is normally studied by the passage of fluorescent dyes that are membrane impermeable. The role of gap junctions as channels has been revealed by their inhibition with various blocking agents (Davidson et al., 1986; Harks et al., 2001; Srinivas and Spray, 2003; Xia and Nawy, 2003). However, these blocking reagents cannot discriminate among the different connexins and their specificity is unclear. In a recent study, a more refined inhibition of the channel function became possible through a single point mutation at a threonine amino acid of the third trans-membrane helix of a-type and b-type connexins (Beahm et al., 2006). This point mutation can lead to a closed gap junction channel, without affecting other connexin related activities (as discussed below). Use of targeted channel inhibition of specific connexins could reveal their distinct roles in development and cancer.

Hemi-channel

Connexins can function in a paracrine and autocrine manner through their hemi-channel activity. This is the activity of the open unpaired connexon with exchange of large membrane impermeable molecules between the cytoplasm and the extracellular environment. In physiological conditions hemi-channel activity is limited (Buvinic et al., 2009; Cherian et al., 2005; Solan and Lampe, 2009), while uncontrolled hyperactivity could lead to cell death (Contreras et al., 2002). In response to stress factors or specific physiological signals (Buvinic et al., 2009; Cherian et al., 2005) opening of hemi-channels can lead to autocrine-paracrine cell signalling. Thus, tight regulation of hemi-channel opening is essential. It is proposed that ATP release through hemi-channels leads to activation of purinergic receptors (P2Y, P2X7) and consequently to the control of Ca^{2+} influx (Scemes et al., 2003; Weissman et al., 2004). The role of ATP

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