Biochimica et Biophysica Acta 1864 (2017) 728–736

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Review

Recruitment of RNA molecules by connexin RNA-binding motifs: Implication in RNA and DNA transport through microvesicles and exosomes

Marta Varela-Eirin [a], Adrian Varela-Vazquez [a], Marina Rodríguez-Candela Mateos [a], Anton Vila-Sanjurjo [b], Eduardo Fonseca [a], José L. Mascareñas [c,d,e], M. Eugenio Vázquez [c,d,e], Maria D. Mayan [a,*]

[a] CellCOM research group, Instituto de Investigación Biomédica de A Coruña (INIBIC), CH-Universitario A Coruña (XXIAC), Servizo Galego de Saúde (SERGAS), University of A Coruña, Xubias de Arriba, 84 15006 A Coruña, Spain
[b] Grupo GIBE, Departamento de Bioloxía Celular e Molecular, Facultade de Ciencias, Universidade de A Coruña (UDC), Campus Zapateira, s/n 15.071, A Coruña, Spain
[c] Centro Singular de Investigación en Química Biolóxica e Materiais Moleculares (CIQUS), Universidade de Santiago de Compostela, 15782, Santiago de Compostela, Spain
[d] Departamento de Química Orgánica, Universidade de Santiago de Compostela, 15782, Santiago de Compostela, Spain
[e] Departamento de Química Inorgánica, Universidade de Santiago de Compostela, 15782, Santiago de Compostela, Spain

ARTICLE INFO

Article history:
Received 9 November 2016
Received in revised form 26 January 2017
Accepted 2 February 2017
Available online 4 February 2017

Keywords:
Connexin43
Connexin26
Cellular communication
Microvesicles
Exosomes
RNA-binding domains

ABSTRACT

Connexins (Cxs) are integral membrane proteins that form high-conductance plasma membrane channels, allowing communication from cell to cell (via gap junctions) and from cells to the extracellular environment (via hemichannels). Initially described for their role in joining excitable cells (nerve and muscle), gap junctions (GJs) are found between virtually all cells in solid tissues and are essential for functional coordination by enabling the direct transfer of small signalling molecules, metabolites, ions, and electrical signals from cell to cell. Several studies have revealed diverse channel-independent functions of Cxs, which include the control of cell growth and tumourigenicity. Connexin43 (Cx43) is the most widespread Cx in the human body. The myriad roles of Cx43 and its implication in the development of disorders such as cancer, inflammation, osteoarthritis and Alzheimer's disease have given rise to many novel questions. Several RNA- and DNA-binding motifs were predicted in the Cx43 and Cx26 sequences using different computational methods. This review provides insights into new, groundbreaking functions of Cxs, highlighting important areas for future work such as transfer of genetic information through extracellular vesicles. We discuss the implication of potential RNA- and DNA-binding domains in the Cx43 and Cx26 sequences in the cellular communication and control of signalling pathways.

1. Introduction

Connexins (Cxs) and pannexins in vertebrates and innexins in invertebrates are a family of proteins involved in cell to cell and cell to the extracellular space communication [1,2]. In humans, 21 isoforms of the Cx multigene family have been identified [3]. Cxs show cell-type-specific but overlapping patterns of expression, as well as shared topology and functions, such that some Cxs can functionally replace others [4]. However, genetically modified animal models have demonstrated that distinct properties and functions of Cxs [5] make them largely non-interchangeable [4,6].

Cx hemichannels (connexons) consist of a hexameric unit of six Cxs. Hemichannels allow the direct exchange of molecules and metabolites with the extracellular matrix. Gap junction (GJ) channels are formed by the apposition of connexons from adjacent cells and allow direct exchange between contacting cells. Cxs are known to play a role in cellular functions such as cell guidance, cellular adhesion and cell growth, in both gap junction-dependent and gap junction-independent manners. Cx43 is the most completely characterized Cx isoform in terms of its channel gating properties, identified phosphorylation sites, protein interactions and channel assembly and turnover. Cx43 consists of 382 amino acids (Fig. 1A) and has versatile functional properties reflected by its distribution in multiple tissues and cell types [7,8]. Cx43 is a conditional tumour suppressor gene and participates in the synchronous contraction of muscle cells, bone remodelling, embryonic development

Abbreviations: Cxs, connexins; Cx26, connexin26; Cx43, connexin43; CL, cytoplasmic loop; CTD, C-terminal domain; EGFRvIII, epidermal growth factor receptor variant III; E1-E2, extracellular loops; GJs, gap junctions; GJIC, gap junction intercellular communication; MVs, microvesicles; mtDNA, mitochondrial DNA; NMR, nuclear magnetic resonance spectroscopy; NTD, N-terminus; M1-M4, transmembrane domains; RPBs, RNA-binding proteins.

* Corresponding author at: CellCOM Research Group, INIBIC, CH-Universitario A Coruña (XXIAC, SERGAS), University of A Coruña, Xubias de Arriba 84. 15006, A Coruña, Spain.
E-mail address: Ma.Dolores.Mayan.Santos@sergas.es (M.D. Mayan).

http://dx.doi.org/10.1016/j.bbamcr.2017.02.001

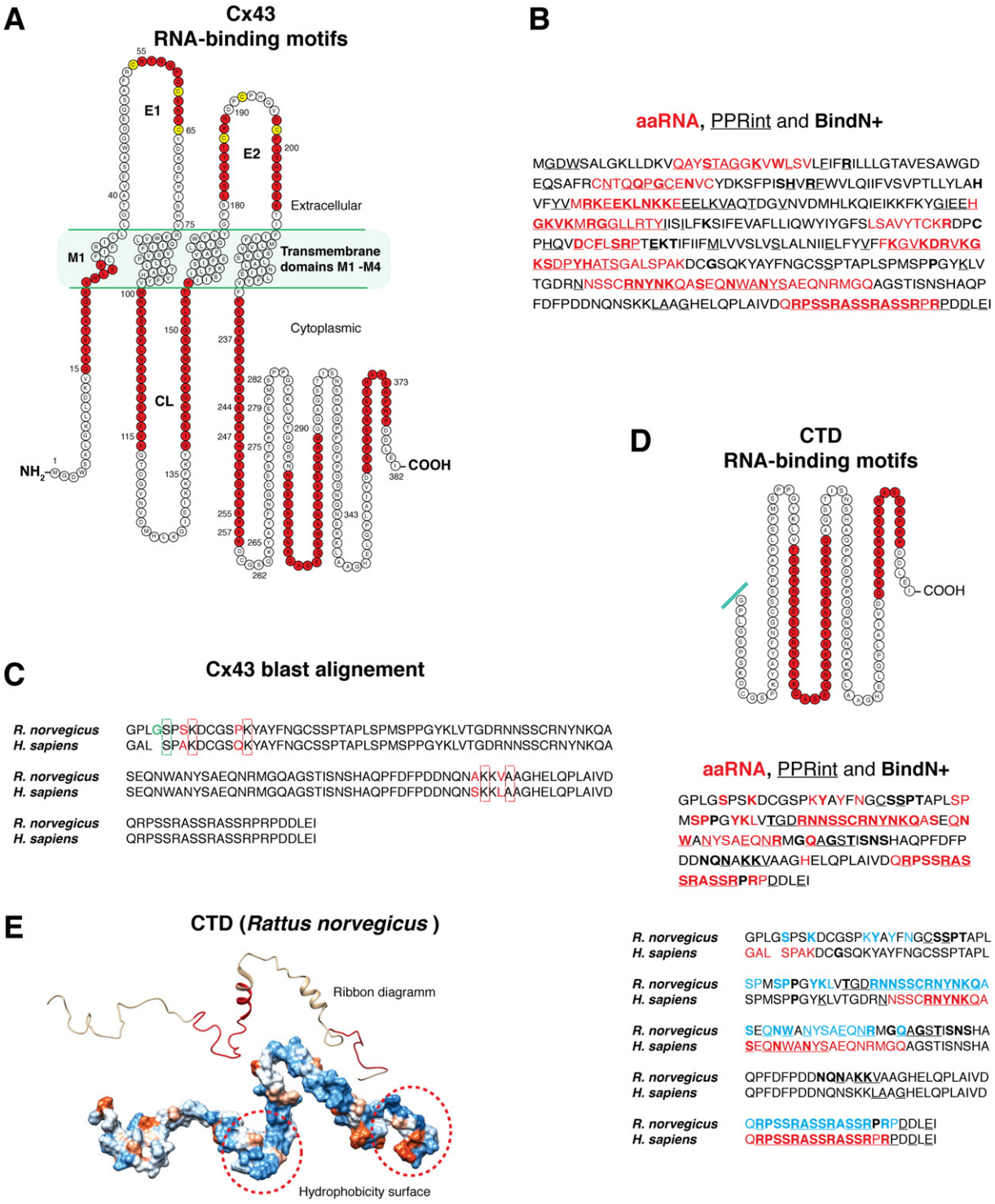

Fig. 1. Schematic representation of Connexin43 and the predicted RNA-binding domains. (A) Topological diagram of the Cx43 structure, with an N-terminal end, four transmembrane domains (M1–M4), two extracellular loops (E1, E2), an intracellular loop (CL) and the C-terminal domain (CTD). The membrane region is shown in light green. Yellow circles represent extracellular cysteine residues. Amino acid sequences in which the three computational methods coincide to predict RNA binding propensity are shown in red. (B) Amino acid sequence of Cx43 with the predicted representation of three computational methods. aaRNA in red (highest score), PPRint (SVM threshold: −0.2) underlined and BindN+ (specificity: 85%) in bold. (C) Amino acid residue alignment between the CTD of Cx43 of *Rattus norvegicus* and *Homo sapiens*. Amino acid substitutions are shown in red. Gaps are shown in green. (D) Topological diagram of the CTD of Cx43 of *R. norvegicus*. RNA binding propensity (amino acid sequence) predicted by the three computational methods is shown in red. Below, sequence alignment between part of the CTD of rat and human with the predicted representation of three computational methods. aaRNA in blue and red, PPRint underlined and BindN+ in bold (E) Ribbon model of the CTD structure (top) and hydrophobicity surface (bottom) according to the Kyte and Doolittle scale with values ranging from blue (hydrophilic) to orange red (hydrophobic) [115]. The red dashed circles highlight the predicted RNA-binding domains.

and homeostasis in tissues, among other functions. A hallmark of the broad functional spectrum of Cx43 is the rare disease oculodentodigital dysplasia, caused by mutations in the Cx43 gene (GJA1), which affects the shape and function of many different parts of the body [9].

2. Horizontal transfer through membrane vesicles

The transmission of information between cells in the body occurs through several mechanisms of communication, such as the secretion