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Review

Gap junction proteins: Master regulators of the planarian stem cell response to tissue maintenance and injury ☆

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ABSTRACT

Gap junction (GJ) proteins are crucial mediators of cell–cell communication during embryogenesis, tissue regeneration and disease. GJ proteins form plasma membrane channels that facilitate passage of small molecules across cells and modulate signaling pathways and cellular behavior in different tissues. These properties have been conserved throughout evolution, and in most invertebrates GJ proteins are known as innexins. Despite their critical relevance for physiology and disease, the mechanisms by which GJ proteins modulate cell behavior are poorly understood. This review summarizes findings from recent work that uses planarian flatworms as a paradigm to analyze GJ proteins in the complexity of the whole organism. The planarian model allows access to a large pool of adult somatic stem cells (known as neoblasts) that support physiological cell turnover and tissue regeneration. Innexin proteins are present in planarians and play a fundamental role in controlling neoblast behavior. We discuss the possibility that GJ proteins participate as cellular sensors that inform neoblasts about local and systemic physiological demands. We believe that functional analyses of GJ proteins will bring a complementary perspective to studies that focus on the temporal expression of genes. Finally, integrating functional studies along with molecular genetics and epigenetic approaches would expand our understanding of cellular regulation *in vivo* and greatly enhance the possibilities for rationally modulating stem cell behavior in their natural environment. This article is part of a Special Issue entitled: The communicating junctions, roles and dysfunctions.

Contents

1. Introduction

Effective cell–cell communication is a hallmark of multicellular organisms, which allows for proper embryonic development, growth and continued tissue renewal as adults. An illustration of this phenomenon is observed in long-lived organisms (*e.g.* humans), which maintain the form and function of differentiated tissues over years. This extended process of tissue maintenance relies on stem cells that are activated to proliferate and migrate in order to precisely replace aged or damaged cells. Physiological turnover is not restricted to one tissue type and is simultaneously accomplished in many tissues; in humans it involves the daily renewal of billions of cells [1,2]. Thus, in order to integrate local and systemic signals that consistently satisfy physiological demands, efficient mechanisms for cellular communication are instrumental.

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Gap junction (GJ) proteins contribute to cell communication that integrates different tissues and organs throughout the body [3–5]. This type of cell communication has been illustrated in a wide range of invertebrates and vertebrates, which suggests a conserved mechanism throughout evolution [3–7]. Traditionally, the function of GJ proteins was known to be associated with the formation of membrane channels coupling multiple cell layers to enable the passage of physiological signals such as ions, second messengers and small metabolites to pass from one cell to another. However, recent advances have revealed and assigned additional functions to GJ proteins, including roles as sensors of the extracellular environment, cell–cell adhesion factors that facilitate cell migration, and modulators of endocrine, pain, signal transduction pathways and programmed cell death [3,8–15]. Significantly, the intercellular information mediated through gap junctions has been implicated to regulate the local and systemic physiological demands associated with embryonic development, growth, differentiation, regeneration, and tissue homeostasis [3,8].

Gap junctions are composed of proteins called connexins and pannexins in the vertebrates, and innexins in most invertebrates [4,6]. Though the molecular topology between connexins and innexins remains similar, phylogenetic analyses show dissimilarities in the primary sequences of the two types of proteins [16]. Evolutionary analyses, based on the conservation of motifs group the innexins and pannexins into one superfamily, suggesting that these proteins might have evolved from the same precursor protein [17]. Furthermore, the conservation of primary sequences and motifs also suggests the notion that innexins probably evolved prior to connexins, while connexins evolved independently at a later time [16–18].

In vertebrates and invertebrates GJ proteins form channels that mediate cell–cell communication. Disruption of this cellular communication can lead to abnormalities in both embryonic and adult stages, ranging from embryonic lethality and cardiac failure to cancer and epilepsy [19–24]. For example, deficiency in connexin 26 leads to embryonic lethality in mice starting with abnormalities at day 10-post coitum and death at day 11 [22]. Connexin 45 is also embryonically lethal, probably due to deformities caused during cardiac development [19,25]. However, extensive data suggest that GJ proteins are not simply housekeeping components but are also critical modulators of morphogenesis and axial patterning during embryonic development and adult tissue maintenance [3,26,27]. The former is illustrated in the context of left–right asymmetry determination in vertebrate embryonic development, where GJ proteins establish long-range communication among cells influencing gene expression and consistent organ formation on the left or right side [3,5,28–30]. Inhibition of this direct cellular communication leads to the inconsistent placement of organs during development in a process known as left–right visceral randomization [3,5,28–31]. GJ proteins also modulate cellular behavior in adult tissues, controlling cell cycle progression, growth, apoptosis and cellular response during injury and inflammatory response [3,32–38]. Altogether, gap junction-dependent signals play fundamental roles in cell communication at many levels and their functions can be essential for the cell survival and behavior. In many cases, these signals act as intermediaries capable of spatiotemporal regulation of morphogenetic patterning and cellular response, providing an excellent paradigm for the rational modulation of cell behavior during therapeutic intervention.

Studies demonstrate the existence of functional differences between normal and cancer cells based on the presence of various GJ proteins [39–43]. This phenotypic feature also extends to stem cell populations that can be defined based on gap junction signatures [35,44–48]. Thus, gap junction communication (GJC) encompasses a physiological phenomenon that modulates cellular behavior at the local and systemic level. Despite the physiological and biomedical relevance of GJC, the mechanisms by which this cellular crosstalk is accomplished *in vivo* remain largely elusive.

Experimental evidence accumulated over the last 40 years has advanced our understanding of GJC and its regulatory aspects, especially those associated with tissue specificity, permeability and the way GJC relates to homeostasis and disease [3,39,42,43,45,49–51]. However, in many cases, deletion of genes encoded for GJ proteins leads to embryonic lethality, highlighting the central role played by GJC during embryonic development [19,22,25]. Although this limits the analysis of GJC in adult tissues, introducing tissue-specific genetic deletions that remove one or more genes encoding for GJ proteins has proved a powerful molecular tool. Nonetheless, functional disruption of a GJ protein in many cases tends to be compensated by others, which can potentially mask the study of its specific systemic roles [3,10–14,24,43,52]. The possibility for simultaneously disrupting multiple gap junction proteins represents an attractive alternative to avoid compensatory/redundant mechanisms; however this approach is often difficult to perform, and its effect can be complicated to analyze in the adult organism. To understand how tissues integrate to satisfy physiological cell turnover and repair, GJC role at the systemic level (*i.e.*: in the complexity of the whole adult organism), needs to be defined, and the above difficulties provide a barrier towards this purpose.

In this review, we discuss recent attempts to study GJC in the complexity of the whole adult organism during tissue maintenance and regeneration. The approach [38,46,53] capitalizes on a classic model organism, the flatworm planaria, which provides an excellent system in which to analyze GJC-mediated stem cell regulation during the processes of tissue renewal and regeneration in adults. Planarians are also amenable to genetic manipulation, in particular loss-of-function studies allowing simultaneous downregulation of multiple GJ proteins. The opportunity to study gap junction-mediated signaling *in situ* in the adult animal, (by using state-of-the-art molecular genetic technology along with electrophysiology and biochemical tools) offers a fresh approach to further our understanding of cell regulation mechanisms.

2. Planarians: a model to elucidate systemic cell turnover and regeneration

Planarians are multicellular organisms and members of the phylum platyhelminthes (flatworms). These organisms contain derivatives of all three germ layers (ectoderm, mesoderm and endoderm), and this review will mainly focus on the most common species of freshwater planarians used in laboratory settings (*i.e.*: *Dugesia japonica* and *Schmidtea mediterranea*) [54–61]. Key features of these organisms include bilateral symmetry, cephalization, and dorsoventral and anteroposterior polarities [54–61]. Planarians also possess multiple tissues and organs; for example, recent research highlights the complexity of the planarian nervous system, which includes multiple types of neurons, receptors and neurotransmitters similar to those in vertebrates [54,62–64]. Altogether, planarians display tissue complexity and developmental features that are evolutionarily conserved among metazoans.

The best-known feature of planarians is their capacity to regenerate an entire organism from small tissue fragments. This extraordinary plasticity in the adult organism relies on a population of stem cells known as neoblasts. Scattered throughout the animal, neoblasts are the only mitotic cells in the worm, giving rise to all tissues and supporting of physiological cell turnover in the adult [65–67]. During injury, undifferentiated neoblasts respond with increased proliferation, giving rise to progeny that migrate to the damaged site to re-establish form and function [57–59,66,67]. Neoblasts respond to damage quickly and can rebuild any part of the body (including neuronal connections within the brain and sensory system, and other tissues such as muscle and the digestive system), within a week [54,56,59–62,64]. Tightly coordinated neoblast proliferation in response to damage indicates that these stem cells process information regarding their local and systemic environment. In order to