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Review

# The modulation of gap-junctional intercellular communication by lipid rafts ☆

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ABSTRACT

Lipid rafts are specific microdomains of plasma membrane which are enriched in cholesterol and sphingolipids. These domains seem to favour the interactions of particular proteins and the regulation of signalling pathways in the cells. Recent data have shown that among the proteins, which are preferentially localized in lipid rafts, are connexins that are the structural proteins of gap junctions. Since gap junctional intercellular communication is involved in various cellular processes and pathologies such as cancer, we were interested to review the various observations concerning this specific localization of connexins in lipid rafts and its consequences on gap junctional intercellular communication capacity. In particular, we will focus our discussion on the role of the lipid raft–connexin connection in cancer progression. This article is part of a Special Issue entitled: The Communicating junctions, composition, structure and characteristics.



**Contents**



## 1. Introduction

### 1.1. Lipidic composition of the plasma membrane

Historically, the plasma membrane has been described for long as a fluid lipid bilayer, exhibiting a uniform lipid distribution in which are inserted moving proteins. However, more recently, it appeared that the lipid bilayer is organized as a much more complex structure, exhibiting a large variety of lipids, composed of hydrocarbon tails which are variable in length and saturation, that are associated or not with proteins. Such an organization is achieved through non-covalent bonds and allows the establishment of several different physical states (or phases) because of thermodynamic considerations [1]. Therefore, according to its composition, the lipid bilayer can be structured in three major phases (solid-gel phase, fluid-liquid-crystalline phase, and liquid-ordered phase, Fig. 1). The solid-gel phase ($L\beta$) is a consequence of immobile and tightly packed membrane phospholipids that contain mainly long saturated acyl chains. Under physiological conditions, when membrane phospholipids are enriched with polyunsaturated fatty acids, the membrane is as a fluid-liquid crystalline (or liquid-disordered) phase ($L\alpha$). Such a structure exhibits acyl chains that have a disordered liquid organization and are characterized by high lateral mobility. Finally, an intermediate liquid-ordered phase ($Lo$) was also observed in mixtures containing phospholipids enriched with saturated fatty acid (sphingolipids) associated with cholesterol [2]. $Lo$ microdomains are also called lipid rafts, floating in the $L\alpha$ phase like rafts on the ocean. Initially, these microdomains, or lipid rafts, have been highlighted due to their low density and insolubility in the presence of non-ionic detergents at low temperature [3]. Now, it appears that the specific interactions between lipids and membrane proteins in lipid rafts allow the creation of dynamic platforms which

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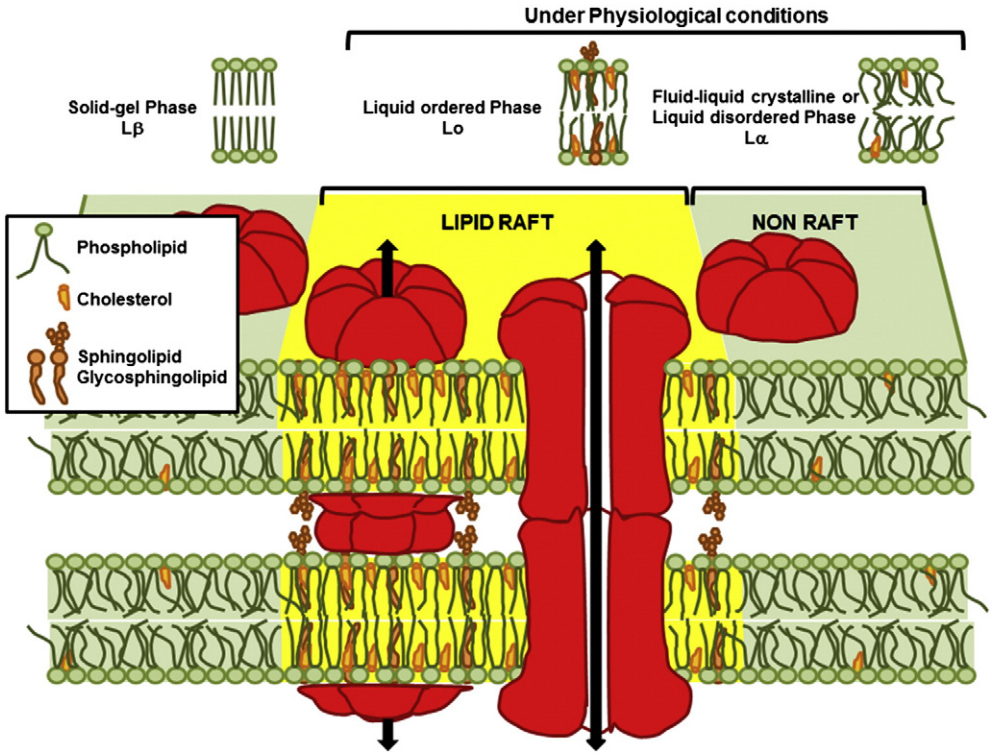


**Fig. 1.** The three major phases of lipid bilayer organization in biological membranes.

include functional complexes of proteins involved in various cellular processes [4]. The presence of proteins inserted in lipid rafts is the consequence of specific protein interactions or post-translational modifications (palmitoylation, myristoylation or glycosyl phosphatidyl inositol moieties known as "GPI anchors") that target them to these microdomains of the plasma membrane [5]. In addition, lipid rafts containing these clusters of proteins are highly dynamic permitting inclusion or exclusion of proteins and allow protection against enzymes (like phosphatases) that could decrease their activity.

The lipid rafts actively participate in signal transduction, local cytoskeletal remodeling events and targeted traffic of associated proteins [6–8]. The functional role of lipid rafts was first observed in caveolae that are specialized invaginated membrane structures enriched in cholesterol and sphingolipids and involved in endocytosis [9]. These particular lipid rafts are characterized by the presence of a small cholesterol binding protein, caveolin, which constitutes a family of membrane proteins including three members. These members are caveolin 1 (Cav-1, an ubiquitous caveolin), caveolin 2 (in most cases, coexpressed with caveolin 1) and caveolin 3 (replacing caveolin 1 in striated muscles) [10].

### *1.2. Connexins and gap junctions*

The gap junctional intercellular communication (GJIC) is mediated by the superfamily of connexins (Cxs), that is encoded by about twenty different genes in humans [11]. Such a fundamental intercellular communication is mediated by gap junctions that are intercellular plasma membrane channels allowing direct intercytoplasmic exchange of ions and small molecules (PM<1000 Da) between adjacent cells [12]. GJIC and their structural proteins, the connexins, have been involved in the regulation of various biological aspects such as cell homeostasis, proliferation and differentiation. These gap junctions play an important role in tissue function but also in progression of diseases such as cancer [13]. GJIC is tightly regulated by mechanisms including changes in connexin expression, regulation of connexin trafficking, the assembly and the degradation of the intercellular channel and their functional status [14–16]. Several reports suggested that Cxs involved in GJIC could be localized in lipid raft microdomains. Moreover, it was also shown that the assembly of gap junction and its activity could be regulated by the lipid composition of the membrane [17–19].

In this review, we will summarize the various observations concerning the localization of connexins in lipid rafts and its consequences on GJIC regulation. Finally, we will discuss the role of the lipid raft–connexin connection in cancer progression.

## 2. Involvement of lipid rafts in the regulation of gap junctional intercellular communication

### *2.1. Evidence for connexin localization in lipid rafts*

In lipid rafts, the localization of membrane proteins and their partners can be observed by fluorescence imaging approach [20–22]. Biochemical techniques, like co-immunoprecipitation or co-immunolabelling, are also used to study the tight interactions of proteins with specific markers of the lipid rafts such as caveolins. One limitation of the use of lipid raft protein markers is that they may be specific for particular types of lipid rafts such as Cav-1 which is a marker for caveolae. In addition, since lipid rafts are tightly packed with cholesterol and sphingolipids, non-ionic detergents are generally not sufficient for solubilizing the anchored proteins. Nevertheless, the lipid rafts can be isolated according to their light density by using sucrose gradient [23]. Following ultracentrifugation, the lipid rafts are enriched at the lower concentrations or light fraction of the gradient contrary to the non-raft proteins that are in the heavy fraction. Finally, another way to study the biological importance of lipid rafts is to prevent their formation by depleting cholesterol from the plasma membrane by using methyl-β-cyclodextrin (MβC) [24,25].

Different techniques permitted to localize connexins in lipid rafts and in particular in those containing caveolin-1 (caveolae). From such approaches, it appeared that some of them are able to interact or co-localize specifically with Cav-1 (Cx43, Cx32, Cx46 and Cx36) while others cannot (Cx26 and Cx50) [26]. Cav-1 interacts with the carboxyl tail of Cx43 between residues 244 and 256 [27]. However, interactions may depend on