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Plasma membrane cholesterol depletion disrupts prechordal plate and affects early forebrain patterning

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ABSTRACT

Cholesterol-rich membrane microdomains (CRMMs) are specialized structures that have recently gained much attention in cell biology because of their involvement in cell signaling and trafficking. However, few investigations, particularly those addressing embryonic development, have succeeded in manipulating and observing CRMMs in living cells. In this study, we performed a detailed characterization of the CRMMs lipid composition during early frog development. Our data showed that disruption of CRMMs through methyl-β-cyclodextrin (MβCD) cholesterol depletion at the blastula stage did not affect Spemann's organizer gene expression and inductive properties, but impaired correct head development in frog and chick embryos by affecting the prechordal plate gene expression and cellular morphology. The MβCD anterior defect phenotype was recapitulated in head anlagen (HA) explant cultures. Culture of animal cap expressing Dkk1 combined with MβCD-HA generated a head containing eyes and cement gland. Together, these data show that during *Xenopus* blastula and gastrula stages, CRMMs have a very dynamic lipid composition and provide evidence that the secreted Wnt antagonist Dkk1 can partially rescue anterior structures in cholesterol-depleted head anlagen.

Introduction

Over the past few years, it has become evident that the classical "mosaic fluid" model for plasma membrane structure has acquired an intrinsic specialization, with the myriad of reports concerning the identification of cholesterol-rich membrane microdomains (CRMMs), also called detergent-resistant membranes (DRMs), lipid rafts or caveolae (Jacobson et al., 2007; Liu and Anderson, 1995; Luria et al., 2002; Parton and Simons, 2007; Sargiacomo et al., 1993; Vereb et al., 2003). CRMMs are described as small (10–200 nm), heterogeneous, highly dynamic, cholesterol- and sphingolipid-enriched domains that compartmentalize cellular processes, participating in cell signaling events. The term caveolae designates caveolin-rich CRMMs, since caveolin was the first integral protein identified as a lipid raft modifier and also a marker protein of these CRMMs (Lisanti et al., 1994; Rothberg et al., 1992), possessing a scaffold domain where different proteins associate to initiate signaling cascades (Parton and Simons, 2007). In the plasma membrane, cholesterol is a major component of lipid rafts (Brown and London, 1998; Kurzchalia and Parton, 1999; Maxfield, 2002; Simons and Toomre, 2000). The removal of cell surface cholesterol by methyl-β-cyclodextrin (MβCD) results in disorganization of these domains, affecting a diverse range of activities, such as signaling, adhesion, motility and membrane trafficking (Pike and Miller, 1998; Roper et al., 2000). Therefore, cholesterol has a structural/stabilizing role in the membrane microdomain assembly, which imparts, together with sphingolipids, a "liquid-ordered" state that is more rigid than the non-raft plasma membrane, which exists in a fluid "liquid-disordered" condition since it is mostly composed by phospholipids (Prinetti et al., 2000; Razani et al., 2002). In addition, certain transmembrane domain proteins might favor a particular lipid microenvironment over another, leading to preferential partitioning into one or another microdomain (Bauer and Pelkmans, 2006). It is now clear that CRMMs play an essential role in cell membrane subcompartmentalization, mediating signaling, as well as particle/pathogen anchoring/internalization events (Lingwood and Simons, 2010). Current knowledge of the biophysics and functional role of CRMMs is based mostly on indirect methods and *in vitro* studies of artificial membranes and cells, and the scarcity of *in vivo* studies, particularly during embryonic development, is notable.

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Although lipids are present in the CRMM, few reports concern the lipid composition of the microdomain in embryonic cells. The lack of *in vivo* models for developmental studies could be partially explained by the highly dynamic behavior of CRMMs and lack of reliable tools to access CRMMs in living organisms.

In amphibians, it has been clearly demonstrated that signaling pathways such as Wnt/β-catenin and chordin/noggin/BMP are involved in early embryo axis formation in a coordinated fashion, and explain the regional specificities of head, trunk and tail organizers (De Robertis et al., 2000; Niehrs, 2004). At the early blastula stage of the frog *Xenopus laevis*, a signal inducing dorsal mesoderm emanates from the dorsal-vegetal blastomeres that constitute the Nieuwkoop center (Nieuwkoop, 1973). The Nieuwkoop center induces axis-forming properties in the Spemann's organizer (SO) center, which arises on the dorsal equator at the early gastrula stage (De Robertis and Kuroda, 2004; Harland and Gerhart, 1997). SO morphogenetic and inductive properties were demonstrated in an outstanding experiment, in which this region was grafted to the ventral side of a salamander embryo and induced the formation of a secondary axis (Spemann and Mangold, 1924, reviewed by Niehrs, 2004). It has been proposed that the amphibian SO contributes to three parts along the anteroposterior axis: the anterior endoderm, the prechordal plate (PcP) and the chordamesoderm (Glinka et al., 1998; Niehrs, 2004; Spemann, 1938), which strongly supports the existence of head and trunk embryonic organizers. As the PcP arises from the SO in amphibians, Hensen's node originates the PcP in the chick, and in both organisms, the PcP is required for forebrain induction.

Although much attention has been directed toward uncovering the signaling network involved in SO/PcP inductive properties, the intriguing question remains, of how embryonic cells compartmentalize signals during these events.

Plasma membranes contain most of these signaling components and play a determining role in the capture and transduction of these signals. Therefore, it seemed reasonable to hypothesize that CRMMs participate in early embryonic patterning. In addition, it has been reported that cholesterol is important for proper forehead and limb development. Low cholesterol levels result in holoprosencephaly

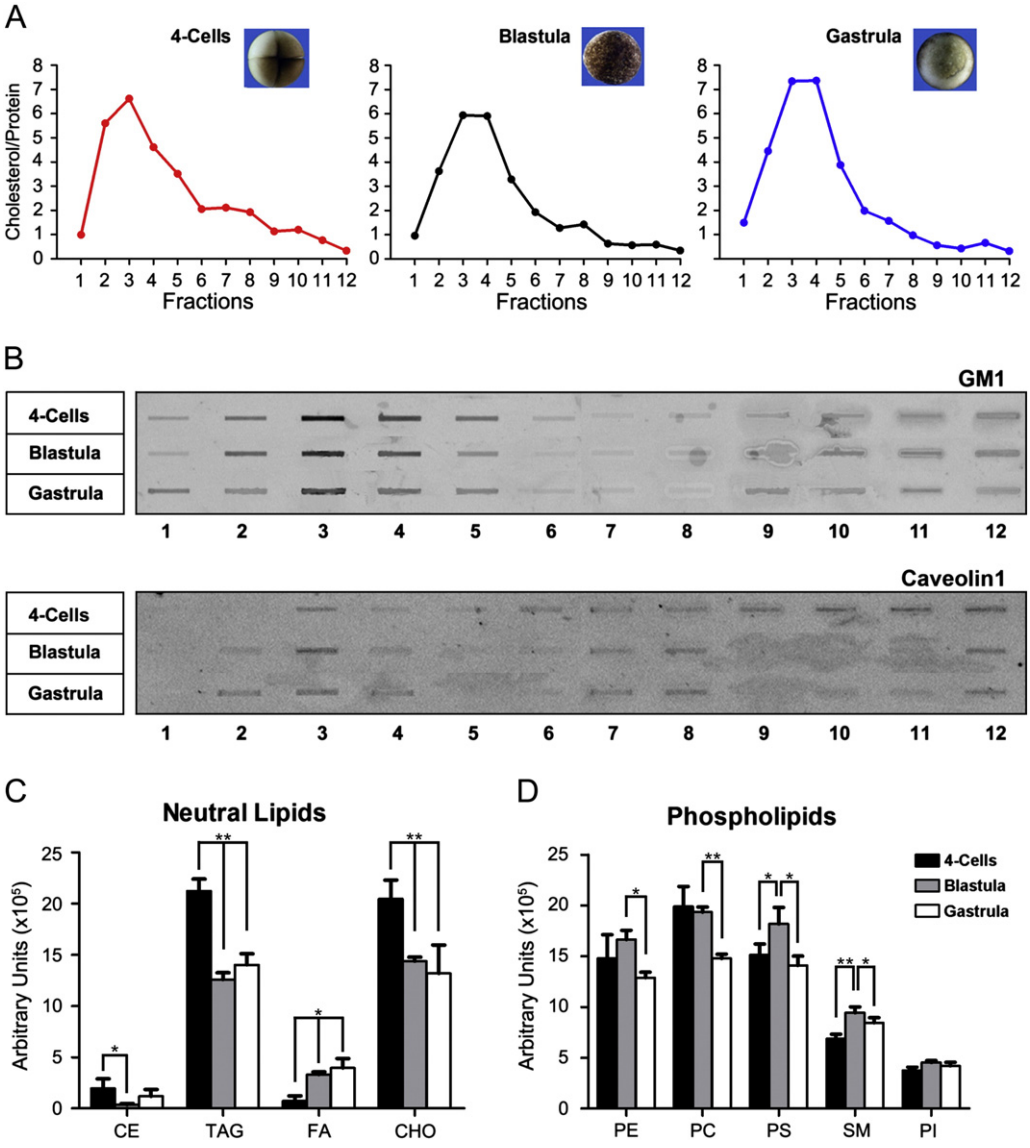

Fig. 1. CRMMs are present and have a dynamic composition during development. (A) Cholesterol content assays of the fraction obtained by the sucrose gradient showed a peak in low-density fractions at the 4-cell, blastula and gastrula stages. (B) Dot blot analysis revealed the presence of GM1 ganglioside and Caveolin1 protein in the low fractions. (C) Lipid characterization showed cholesterol (CHO) and triacylglycerol (TAG) as the most abundant among the neutral lipids, while phosphatidylethanolamine (PE), phosphatidylcholine (PC) and phosphatidylserine (PS) were the predominant phospholipids in the CRMM fraction. (CE) cholesterol ester, (FA) fatty acid, (SM) sphingomyelin, (PI) phosphatidylinositol. (*$P<0.05$; **$P<0.01$).