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Phospholipid Membranes as Regulators of Localized Activity

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In a recent article, Tsuji and Yoshikawa (2010) report that the interaction of long DNA with phospholipids on the surface of aqueous microdroplets can lead to changes of the DNA conformation, and as consequence, to a remarkable alteration of the DNA's transcriptional activity.

The experiments carried out by Tsuji and Yoshikawa (2010) demonstrate that the interaction of a molecule with a lipidic interface not only can lead to a change of the conformation of the molecule but also—and this as a direct consequence—to a substantial change in the molecule's activity and function. Under some of the experimental conditions used, the molecule is inactive if it is away from the interface, i.e., the molecule is "turned off," while if it is bound to the interface the molecule is in an active state, i.e., "turned on." The particular system studied by the authors consisted of small water droplets with diameters between about 20 and 60 μm, so-called microdroplets, dispersed in an apolar medium (mineral oil) with the help of a phospholipid, DOPE (1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine). In such system, the amphiphilic DOPE molecules are localized on the surface of the droplets with the polar zwitterionic head groups facing toward the aqueous interior of the droplets, while the hydrophobic acyl chains are in contact with the bulk oil. The aqueous microdroplets are coated with a thin phospholipidic interfacial layer that ideally is a single monolayer, sufficient to keep the microdroplets dispersed and stable for at least the desired period of time. Aqueous microdroplets, which are coated with amphiphilic lipids, can be considered as cell-sized, cell-mimicking systems in which the lipid monolayer on the surface of the droplets mimics the inner lipidic monolayer of the plasma membrane (Fiordemondo and Stano, 2007; Hase et al., 2007).

In the work of Tsuji and Yoshikawa (2010), the microdroplets were prepared in the presence of a long DNA (about 43 kilo base pairs) and various amounts of the polyamine spermine (*N,N′*-bis (3-aminopropyl)butane-1,4-diamine) and Mg^{2+} ions. While the polycationic spermine is a DNA-condensing agent and promotes folding of the DNA, high Mg^{2+} ion concentrations lead to binding of the DNA to the DOPE interface, which is accompanied by an unfolding of the DNA into a less compact extended coil conformation, which is similar to the DNA conformation inside the droplets in the absence of spermine. With a series of elegant and convincing experiments in which fluorescence spectroscopy, confocal fluorescence microscopy, and fluorescently labeled oligonucleotides were used, Tsuji and Yoshikawa (2010) showed that, in the presence of spermine and Mg^{2+}, DNA acted as a template for the T4 RNA polymerase-catalyzed transcription of a particular gene sequence contained in the DNA into the corresponding mRNA only if the DNA was adsorbed onto the DOPE coated droplet surface, i.e., if the DNA was in the extended coil conformation and not in the condensed folded state. Therefore, the interaction of the DNA with DOPE in presence of spermine and Mg^{2+} resulted in localized transcriptional activity of the DNA.

In these microdroplet experiments, the phospholipid interface acted as a kind of regulator, responsible for turning the DNA on at the droplet surface. Therefore, the phospholipid interface in this in vitro system served in two ways: (1) as physical boundary that allows separating the microdroplets from the bulk organic phase (compartmentalization), and (2) as regulator of the localized activity of DNA via specific interactions with the DNA.

Tsuji and Yoshikawa (2010) mention that their finding is interesting in the context of protocell model research. Protocells are considered here as chemical compartment systems that are thought to have arisen before the first living cells were formed at the origin of life (Morowitz et al., 1988). Although nobody knows how living systems originated from the nonliving form of matter, it is likely that cell-like systems preceded the first cell(s), and that the formation of dynamic molecular assemblies and the interaction within and with molecular compartments were essential for the formation of more and more complex chemical systems on the way to the first cells (Luisi, 2006; Walde, 2006; Deamer, 2009, Schrum et al., 2010). The interaction of DNA with a lipid interface as regulator of the DNA's function, as described in the work of Tsuji and Yoshikawa (2010), illustrates this line of thinking.

The role of lipid membranes as regulators of localized activity and function is well known in biological cells and essential for all living systems. For example, an enzyme, protein kinase C, specifically binds to negatively charged phosphatidylserine-rich domains of membranes. Through this specific interaction, as well as through interactions with other molecules, the enzyme is activated, i.e. "turned on," at specific sites within the cell. Therefore, biological membranes not only compartmentalize and regulate functions but also localize them, conceptually similar to the phospholipid monolayer in the cell-mimicking microdroplet system studied by Tsuji and Yoshikawa (2010).

Another well-studied biological example is the interaction of inactive pancreatic lipase and colipase with lipidic interfaces, leading to conformational changes of the lipase and as a consequence to a turning-on of its activity. Again, the

activation of the lipase is localized, at the lipidic interface, analogous to the DNA activation demonstrated by Tsuji and Yoshikawa (2010), although the two cases are chemically very different. They share, however, the properties of turning on an activity at localized sites in the systems, conceptually clearly different from nonlocalized regulation of the activity of molecules. As an example for the latter case, one can consider the change of the pH of an enzyme solution from an inactive state of the enzyme to the enzymes' active state (pH optimum); for example, by increasing the pH of an α-chymotrypsin solution from pH = 3 (inactive) to pH = 8 (active). Such activation occurs throughout the solution and is not localized.

There may be many examples with simple biomimetic in vitro systems in which the interaction of "turned off" molecules with lipidic interfaces lead to localized changes of the activity of the molecules, although this seems not to have been reviewed and brought into a broader context. In one reported case, for example, the activity of fragmented, and thus inactivated, superoxide dismutase was recruited upon interaction with phospholipid bilayers (Tuan le et al., 2008). The inactive fragments interacted with the lipidic interface and partially recovered the activity of the native enzyme.

This last example is based on the fact that lipid interfaces can accumulate different molecules, depending on their lipid-binding properties, to bring them in close proximity. If the different compounds are prone to self-assemble within the lipid membrane, the lipid interface can promote the formation of an active complex, while the individual components are inactive (Umakoshi et al., 2010). In this case, the lipidic interface again acts as a regulator for turning on an activity of a molecular complex, localized on or within the membrane. For such systems, the term LIPOzyme was coined (Umakoshi et al., 2010), an interesting concept that even goes beyond turning on a "silent" molecule. The close proximity of the assembled molecules in the membrane environment leads to novel properties that are not present in the individual components (Luisi, 2006). Such emergent properties are relevant for gaining a deeper molecular understanding of the origin of the first living systems (Luisi, 2006) and certainly deserve further investigations in connection to protocell research.

There is no doubt that many more properties of lipid membranes in cell-mimicking systems as soft interfaces will be explored and discovered in the future. The work of Tsuji and Yoshikawa (2010) is a stimulating contribution and should be taken as motivation for further developments of more and more complex cell-mimicking chemical systems, which may, in the end, also be relevant for biotechnological applications (Umakoshi et al., 2010).

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