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Histones Bind, Aggregate and Fuse Phosphoinositides Containing Bilayers

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1. Introduction

Phosphoinositides (PIPns) are negatively charged phospholipids mainly found at the cytosolic surface of membranes. They are considered as minor components of cell membranes because they represent less than a 15 % of the total phospholipids found in eukaryotic cells. However, phosphoinositides are recognised as direct signalling molecules, which can act as second messengers by interacting with effector proteins either electrostatically or via specific phosphoinositides binding domains. This family of lipids is formed from seven members, phosphorylated in different positions, which are constantly being turned over by an array of kinases and phosphatases. Each of them has a unique subcellular distribution (1).

Moreover, the existence of a nuclear pool of phosphoinositides (2) has been described, whose physical state and location has been a matter of controversy, but they are part of the nucleoplasm and perhaps located on invaginations of the nuclear envelope (NE) that penetrate the nucleus. These invaginations are known as the nucleoplasmic reticula.

The roles of phosphoinositides inside the nucleus are unclear but in DNA and RNA polymerase activity upon the addition of phospholipids changes have been observed (3). Lately, it has also been demonstrated that they play important roles in membrane dynamics (4). Recent studies have shown that the NE formation is also dependent on these phospholipids (5). In order to investigate the detailed mechanism of the NE assembly, cell-free systems, which mimic the steps of the NE assembly have been used. These systems have shown that there is a NE precursor membrane vesicle population (MV1), that does not derive from the endoplasmic reticulum (ER), but is essential to the NE assembly and is highly enriched in PIPns (up to a 60 mol %) (5). The NE formation is a vital process that occurs in every mitotic cycle and during fertilisation, and defects induce diseases, such as specific cancers or premature aging diseases (6).

The nuclear envelope encapsulates chromatin, which is highly condensed by histones, the most abundant basic proteins present within the nucleus. Histones contain 25 to 35 % basic amino acid residues. Given their positive net charge, interactions with PIPn is expected. Histones are commonly seen as static molecules that pack DNA but they are highly mobile and dynamic proteins (7).

We used an *in vitro* biophysical approach to obtain a more detailed understanding of the mechanism of interaction between histones and the PIPn.

As a first approach, given the presence and location of phosphoinositides in the eukaryotic cell nucleoplasm, we studied the interaction of these lipids with histones, mainly linker histone H1. Using model membranes, turbidity measurements were performed, revealing that a variety of histones caused a dose-dependent aggregation of phosphatidylcholine (PC) vesicle containing negatively-charged phospholipids. 5 mol % PtdIns(4)P was enough to cause extensive aggregation, while with PtdIns at least 20 mol % (Figure 1) was necessary to obtain a similar effect. With confocal microscopy we were able to visualise H1 binding to vesicles and vesicle aggregation (Figures 2). In order to compare

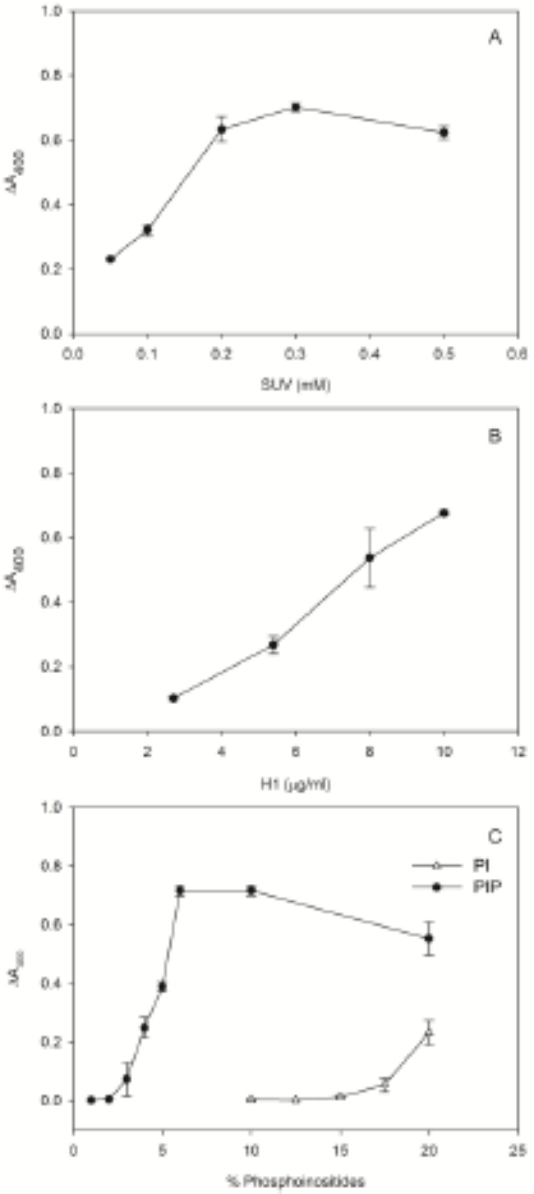

Figure 1. H1-induced aggreation of PIPns contining vesicles. Extents of vesicle aggregation measured as changes in turbidity (ΔA_{400}). **(A)** Increasing concentrations of Small Unilamellar Vesicles (SUV) containing 10 mol % of PtdIns(4)P were treated with 10 µg/ml H1. **(B)** Increasing concentrations of H1 added to 0.3 mM of vesicles containing 10 mol % of PtdIns(4)P. (C) SUV with increasing concentrations of PIPns at 0.3 mM total lipid concentration treated with 10 µg/ml H1. Each point corresponds to the mean (n = 3) ± S. E.

the binding affinities of H1 for vesicles containing PtdIns or PtdIns(4)P, ITC studies were performed, and revealed that the PtdIns(4)P-H1 association constant was one order of magnitude higher than that of PtdIns-H1, and the corresponding lipid/histone stoichiometries were ~ 0.5 and ~ 1.0, respectively (Table 1). This indicated that the two negative charges of PtdIns(4)P are involved in histone binding.

Although these *in vitro* studies indicate that these molecules interact by electrostatic interactions, the fact that both the PIPns and histones are present in the nucleoplasm may suggest also a specific biological function.

Table 1. ITC parameters for the interaction of histone H1 with vesicles of two different lipid compositions. Results in cal/mol injectant. Average values ± S.D. (n = 3)

	PC:PI (9:1)	PC:PIP (9:1)
n	0.99 ± 0.17	0.53 ± 0.13
K_a	1.08 ± 0.3·10⁴	9.31 ± 0.44·10⁴
ΔH°	-4.25 ± 1.3·10⁶ cal/mol	-1.41 ± 0.27·10⁶ cal/mol
ΔS°	-1.42 ± 0.45·10⁴ cal/K·mol	-4.71 ± 0.91·10³ cal/K·mol
ΔG°	-2.99 ± 0.32·10³ cal/mol	-7.9 ± 0.35·10³ cal/mol

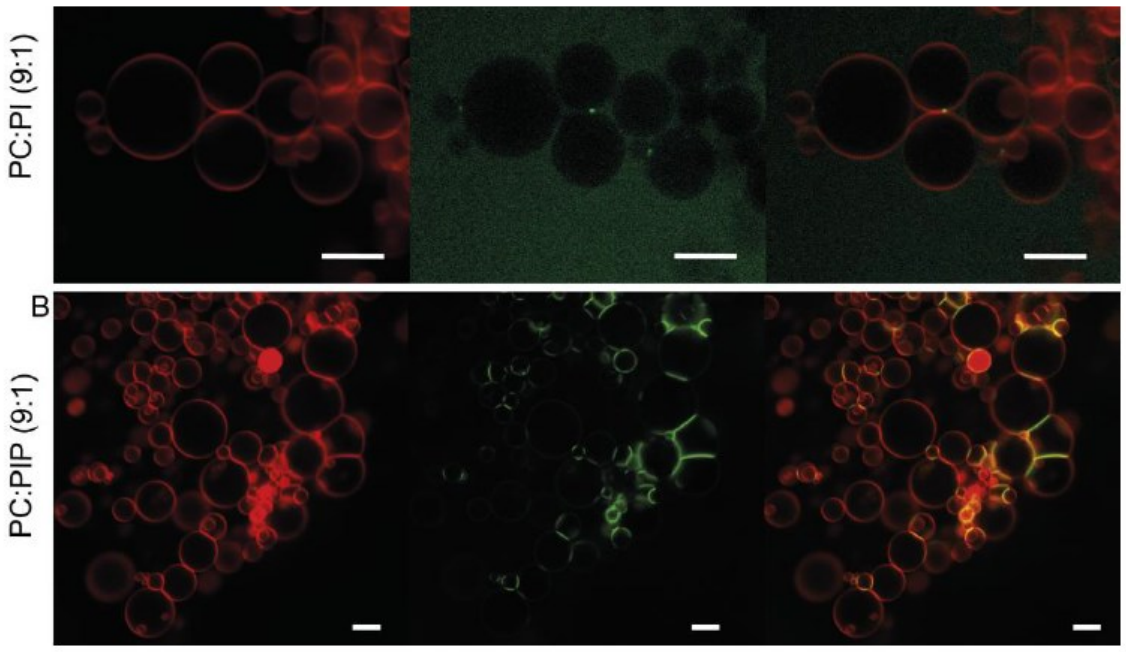

Figure 2. Histone binding to PIPns containing membranes. Representative Giant Unilamellar Vesicles (lipid composition at the left-hand side) imaged by confocal microscopy. (i) Rho-PE for membrane labelling, (ii) H1-Alexa488, and (iii) colocalization of both fluorescent probes. Scale bars 10 µm.

Due to the fact that over every mitotic cycle the NE disassembles and reassembles, fission and fusion processes are required. Therefore we explored whether histones could participate in these events by inducing fusion of phosphoinositides enriched vesicles. This part of our investigation was based on

our observation of vesicle-vesicle aggregation, a requisite for *in vitro* vesicle fusion events. To test whether, in addition to aggregation, fusogenic events were taking place, we used fluorescent probes and monitored the change in their spectroscopic properties. We have shown that, in the presence of PtdIns(4)P, histones induce not only intervesicular lipid mixing, but also inner monolayer lipid mixing (Figure 3), which is a diagnostic for *in vitro* membrane fusion.

Even if these results cannot directly demonstrate *in situ* that histones are promoting fusion events at the nuclear membrane, they point towards the likelihood of being involved during nuclear membrane fusion. Our *in vitro* studies corroborate with work performed in the studies by Garnier-Lhomme *et al.* (8) and Byrne *et al.* (5) where the importance of elevated levels of PIPns in NE assembly was demonstrated.

In conclusion, the findings demonstrate that phosphoinositides can interact *in vitro* with the nuclear proteins, histones

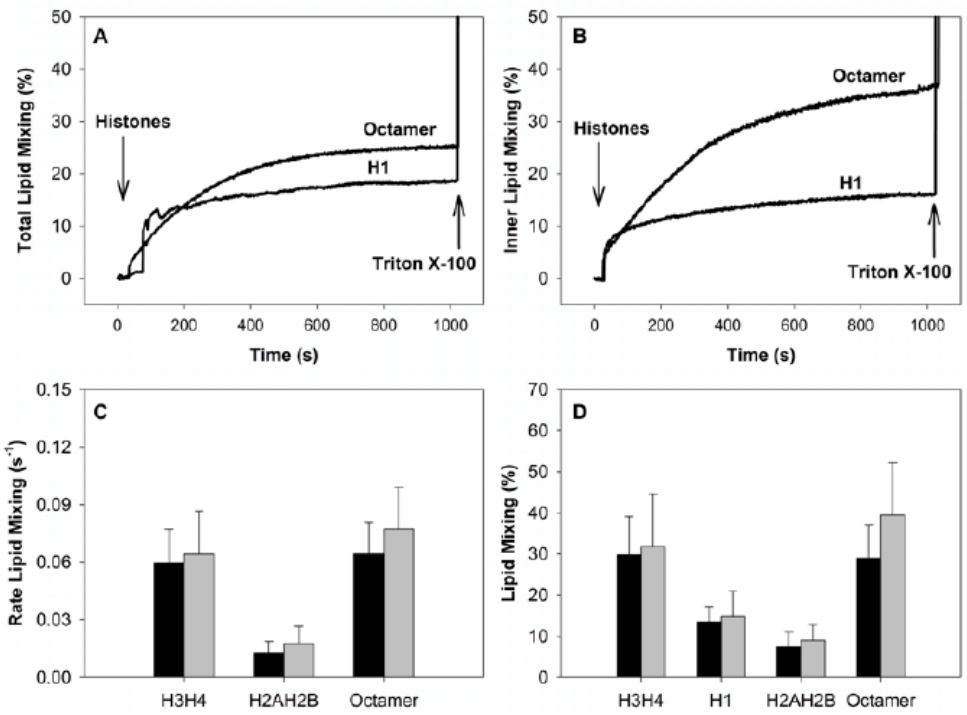

Figure 3. Membrane fusion measured as lipid mixing. Histones induce intervesicular lipid mixing of the inner and outer monolayer. **(A)** Representative total lipid mixing time course. **(B)** Representative inner lipid mixing time course. Arrows indicate the protein or detergent addition. **(C)** Initial rates of histone-induced lipid mixing. **(D)** Extent of histone-induced lipid mixing at equilibrium (20 min). Black corresponds to total lipid mixing and gray correspond to inner lipid mixing. Average values + S.E.M (n =3).

(specifically H1), not only producing Both, vesicle aggregation and fusion.

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