Phosphorylation in the accessory domain of yeast histone chaperone protein 1 exposes the nuclear export signal sequence

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March 11, 2021

Abstract

Histone is a scaffold protein that constitutes nucleosomes with DNA in the cell nucleus. When forming histone, hetero octamer is assisted by histone chaperone proteins. As a histone chaperone protein, the crystal structure of yeast nucleosome assembly protein (yNap1) has been determined. For yNap1, a nuclear export signal/sequence (NES) has been identified as a part of the long ?-helix. Experimental evidence via mutagenesis on budding yeast suggests the NES is necessary for transport out from the cell nucleus. However, the NES is masked by a region defined as an accessory domain (AD). In addition, the role of the AD in nuclear transport has not been elucidated yet. To address the role of the AD, we focused on phosphorylation in the AD because proteome experiments have identified multiple phosphorylation sites of yNap1. To computationally treat phosphorylation, we performed all-atom molecular dynamics (MD) simulations for a set of non-phosphorylated and phosphorylated yNap1 (Nap1-nonP and Nap1-P). As an analysis, we addressed how the NES is exposed to the protein surface by measuring its solvent-access surface area (SASA). As a result, there was a difference in the SASA distributions between both systems. Quantitatively, the median of the SASA distribution of Nap1-P was greater than that of Nap1-nonP, meaning that phosphorylation in the AD exposed to the NES, resulting in increasing its accessibility. In conclusion, yNap1 might modulate the accessibility of the NES by dislocating the AD through phosphorylation.

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A short running title: Phosphorylation effect on yNap1

Keywords: Histone chaperone, Phosphorylation, Molecular dynamics, Nuclear export signal/sequence

Total number of manuscript pages: 17 (including References section)

Number of figures: 4

Number of supplementary figures: 1

Abstract

Histone chaperone proteins assist in the formation of the histone octamers, the scaffold proteins that facilitate the packing of DNA into nucleosomes in the cell nucleus. One such histone chaperone protein is yeast nucleosome assembly protein 1 (yNap1), the crystal structure of which has been determined and found to have a nuclear export signal (NES) sequence within its long α-helix. Experimental evidence obtained from mutagenesis studies of the budding yeast suggests that the NES is necessary for the transport of yNap1 from the cell nucleus to the cytosol. However, the NES sequence is masked by an accessory domain, the exact role of which has not yet been elucidated, especially in nucleocytoplasmic transport. To clarify the role of the accessory domain, we focused on its phosphorylation because proteomic experiments have identified multiple phosphorylation sites on yNap1. To study this phenomenon computationally, all-atom molecular dynamics simulations of the non-phosphorylated yNap1 (Nap1-nonP) and phosphorylated yNap1 (Nap1-P) systems were performed. Specifically, we addressed how the NES sequence is exposed to the protein surface by measuring its solvent-accessible surface area (SASA). It was found that the median of the SASA distribution of Nap1-P was greater than that of Nap1-nonP, indicating that phosphorylation in the accessory domain exposes the NES, resulting in its increased accessibility. In conclusion, yNap1 might modulate the accessibility of the NES by dislocating the accessory domain through its phosphorylation.

1. Introduction

Histones are structural scaffold proteins made up of multiple subunits (four sets of the dimers H3–H4 and H2A–H2B) and form a hetero-octamer. They facilitate the packing and ordering of DNA into nucleosomes by allowing the double-helical molecules to wrap around their hetero-octamer structure. These nucleosomes in turn assemble into chromatin, which further condenses into a chromosome and regulates transcriptional activity by allowing certain parts of the DNA to become accessible to transcription factors. When chromatin is highly packed, forming a structure known as heterochromatin, transcription is repressed owing to the inaccessibility of the nucleic acid sequences to transcription factors. By contrast, in the loosened form of chromatin known as euchromatin, transcription is actively carried out.

Histone chaperone proteins assist in the formation of nucleosomes by associating with the various histone subunits during the different steps of hetero-octamer assembly. One such histone chaperone protein, nucleosome assembly protein 1 (Nap1), is widely conserved among eukaryotes. Aside from depositing the histone subunits in nucleosomes, Nap1 has been implicated in the transportation of histones across the nuclear membrane.[1] Generally, the entry and exit of cargo proteins across the nuclear pore are facilitated by karyopherins known as importin and exportin, respectively. Proteins need to bind to these karyopherins to pass through the nuclear pore. When a cargo protein is to be transported from the nucleus to the cytosol, the karyopherin acting as an exportin recognizes and binds to the nuclear export signal (NES), a leucine-rich sequence on the cargo protein. GTP-bound Ran, a low-molecular-weight GTPase, regulates the direction of transport by binding to and activating an exportin in the nucleus. Once the cargo protein has moved out of the nucleus into the cytosol, the complex dissociates and Ran hydrolyzes its bound GTP into GDP.

A study of the crystal structure of yeast nucleosome assembly protein 1 (yNap1) has revealed that it is a homodimer, with the NES sequence being part of the long α-helix.[2] Furthermore, mutagenesis experiments on the budding yeast have suggested that the NES is necessary for the transport of yNap1 from the nucleus to the cytosol.[3] However, when yNap1 forms a homodimer, each protein interface has a masking region, known as the accessory domain, which masks the NES mutually.[2] Although the accessory domain is generally conserved among eukaryotes, the Nap family members Yeast VPS75, human SET, and human TSPY lack this domain as they do not have an NES sequence, suggesting an evolutionary link between them.[4-6] In terms of its biological function, the accessory domain might allow exportin to access the NES to properly transport the complex from the nucleus. However, the exact role of this domain in nucleocytoplasmic transport has yet to be elucidated.

To shed some light on this subject, in this study, we focused on phosphorylation in the accessory domain because several proteomic studies have identified multiple phosphorylation sites on yNap1.[7-11] For example,

in vitro experiments on the budding yeast and on *Drosophila melanogaster* have confirmed that multiple residues in the accessory domain are phosphorylated by casein kinase 2 (CK2).[8,12] One yeast study revealed that non-phosphorylatable (alanine) and phosphomimetic (aspartic acid) mutations in the accessory domain could lead to elongation of the S phase (the cell cycle during which DNA is replicated and nucleosomes are assembled),[8] suggesting that proper yNap1 transport requires nucleosome assembly. To investigate the phosphorylation effect in the accessory domain, we performed all-atom molecular dynamics (MD) simulations of the following systems: (1) non-phosphorylated yNap1 (Nap1-nonP) and (2) phosphorylated yNap1 (Nap1-P). In both systems, we quantitatively measured the solvent-accessible surface area (SASA) of the NES to address how it is exposed to the protein surface to increase its accessibility. By determining a difference in the SASA distribution between both systems, the phosphorylation effect on the NES recognition by an exportin could be surmised.

2. Methodology

To model the all-atom structure of Nap1-nonP, an experimentally determined structure of yNap1 (PDB ID: 2Z2R)[13] was solvated with the TIP3P water model[14] in a rectangular box. The dimeric yNap1 molecule consists of 417 residues, of which residues 74–365 were used for the modeling of Nap1-nonP. The experimentally undetermined loop regions were modeled with a homology modeling tool (Phyre2),[1515] with the N- and C-terminal coils omitted to reduce the system size. Additionally, because each nuclear localization sequence (NLS) region contains a long β-sheet protruding from the protein surface, the size of the periodic boundary box would need to be increased when these regions are modeled explicitly. Therefore, to reduce the system size, we omitted the NLS regions by replacing them with Gly, Gly, and Ser. Figure 1 shows the modeled all-atom structure of Nap1-nonP.

To address the phosphorylation effect on the accessory domain, an all-atom system of Nap1-P was also prepared. To model Nap1-P, Nap1-nonP was phosphorylated using PyTMs (a PyMol plugin) by employing a set of phosphoserine parameters proposed in a previous study.[16] The Ser 140, Ser 159, and Ser 177 residues were considered as the set of phosphorylation sites. Finally, the total number of atoms, including the water molecules, was 894,611 for the Nap1-nonP system and 894,527 for the Nap1-P system.

To extend the MD simulation time step to 2.0 fs, we constrained the chemical bonds of the proteins and water molecules with the LINCS[1717] and the SETTLE algorithms,[18] respectively. The temperature and pressure of the systems were controlled using the modified Berendsen thermostat[1919] and the Parrinello–Rahman method,[20,21] respectively. The electrostatic interactions were evaluated with the particle mesh Ewald method[22] using a real-space cutoff of 10.0 Å. The cutoff value for van der Waals interactions was set to 10.0 Å. All MD simulations were performed with the GPU version of the GROMACS 2019 package,[23] using the AMBER 14SBonlysc force field.[24] To equilibrate each solvated system, 10 ps of the NVT ensemble ($T = 300$ K) and 10 ps of the NPTensemble ($T = 300$ K and $P = 1$ bar) were performed sequentially after short energy minimization, where the last snapshots of the NPT simulations were employed as the starting structures of the production runs. Finally, five trials of 100-ns MD simulations were started from the relaxed structures of both systems to obtain statistically reliable trajectories.

3. Results and discussion

3.1 Phosphorylation in the accessory domain exposes the nuclear export signal sequence

Five trials of 100-ns MD simulations for each system (Nap1-nonP and Nap1-P) were performed to evaluate the phosphorylation effect in the accessory domain. Through these MD simulations, we observed that the accessory domain fluctuated more heavily than the other regions on yNap1. In terms of their root-mean-square fluctuation (RMSF) profiles (Figure 2), the two systems had no obvious differences between them, indicating that phosphorylation in the accessory domain did not change the overall fluctuation of yNap1.

Next, we focused on the location of the accessory domain in relation to the NES before and after phosphorylation. Figure 3 shows the representative structures of Nap1-nonP and Nap1-P. In Nap1-nonP, the accessory domain clearly masked the NES (Figure 3A), whereas in Nap1-P, it was dislocated from the se-

quence (Figure 3B). To evaluate this dislocation quantitatively, we determined the exposure of the NES as a measure of the SASA (Figure 4). The median of the SASA distribution of Nap1-P was greater than that of Nap1-nonP, indicating that phosphorylation in the accessory domain increases the exposure of the NES and its accessibility to exportin, thereby promoting the export of yNap1 from the nucleus.

3.2 Analyses of the serine sites essential for phosphorylation in the accessory domain

To elucidate why the phosphorylation of the accessory domain would lead to its dislocation from the NES, the contribution of the three phosphorylation sites were examined separately by changing the phosphorylation patterns in additional MD simulations. The two phosphorylation scenarios studied were (1) single-site phosphorylation at Ser 140 and (2) double-site phosphorylation at Ser 159 and Ser 177. For scenario (1), Ser 140 was identified through searches of multiple phosphorylation sites.[7-11] For scenario (2), Ser 159 and Ser 177 were identified experimentally, where they were phosphorylated by CK2 as reported in a previous study.[8] The validity of the phosphorylation sites was confirmed using a phosphorylation prediction server (NetPhos).[25] According to the MD simulations, neither the single phosphorylation (p-Ser 140) nor the double phosphorylation (p-Ser 159 and p-Ser 177) resulted in any significant increase in the SASA of the NES (Figure S1 in Supporting Information), indicating that the phosphorylation of all three sites at once will contribute equally to the increase in the SASA; that is, all the serine residues (Ser 140, Ser 159, and Ser 177) might play an important role in exposing the NES via their phosphorylation.

3.3 Spatiotemporal regulatory mechanism for the nuclear transportation of Nap1

Our computational results indicated that a spatiotemporal regulatory mechanism was involved in the nuclear transportation of Nap1, as supported by evidence from several previous studies. For example, it was reported that CK2 phosphorylates Ser 159 and Ser 177 and contributes to the regulatory mechanism depending on the cell cycle.[7]In the mutagenesis study of that report, the prevention of Nap1 phosphorylation resulted in a shortened G1 phase and a prolonged S phase.[8] Taking these results together, it can be surmised that the phosphorylation of Nap1 might increase its turnover rate between the nucleus and cytosol to respond to nucleosome formation in the S phase. However, any thorough determination of the spatiotemporal regulation of Nap1 is difficult at the present time because there are currently no experimental data to support when/where Nap1 is phosphorylated in yeast. With further research progress in this area, it is expected that measurements of phosphorylation at a high spatiotemporal resolution will provide valuable evidence to reveal the function of Nap1.

4. Conclusion

In this study, we addressed how phosphorylation in the accessory domain affects the accessibility of the NES to an exportin. To study the phenomenon computationally, all-atom MD simulations of the non-phosphorylated and phosphorylated yNap1 systems were performed, with specific address of how the NES is exposed on the protein surface. To evaluate the exposure quantitatively, the SASAs of the NESs of Nap1-nonP and Nap1-P were measured. Interestingly, the median of the SASA distribution of Nap1-P was larger than that of Nap1-nonP, suggesting that phosphorylation in the accessory domain exposes the NES to increase its accessibility to an exportin. In conclusion, the accessibility of the NES of yNap1 is modulated by dislocation of the accessory domain via phosphorylation. The present computational results shed light on the mediation of molecular recognition on protein surfaces via the phosphorylation of essential residues on other proteins.

Acknowledgements

The MD simulations were partially performed using the computational resources of Cygnus provided by the Multidisciplinary Cooperative Research Program (project code: LSC) at the Center for Computational Sciences (CCS), University of Tsukuba, Japan. This work was supported in part by the Japan Agency for Medical Research and Development (AMED) (Grant No. JP20ae0101047h0001), the Japan Agency for Science and Technology (JST) CREST program (Grant No. JPMJCR20B3), and a grant-in-aid for innovative research in “Hydrogeomics” and “Biometal Sciences” (Grant Nos. 19H05047 and 20H05497) by the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

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Figure legends

Figure 1 Modeled all-atom structure of Nap1 homodimer. A pair of molecules (cyan and green) is shown with their NESs (red) and accessary domains (blue).

Figure 2 RMSF of Nap1-P (red, the dashed line) and Nap1-nonP (bule, the solid line) calculated from the five trials of 100-ns MDs. The accessary domain is highlighted with the red area. It is noted that the blank from the 290 to the 310 residues corresponds to the omitted region when modeling the all-atom structures of Nap1-nonP and Nap1-P.

Figure 3 Structures of yNap1 by focusing the NES (red) and the AD (blue). (a) The initial structure of Nap1-P in carton mode. (b) One of the representative structures of Nap1-P with the AD dislocated from the NES in carton mode. The three phosphoserines (orange) are shown as balls.

Figure 4 Distribution of SASA of the NES for Nap1-nonP (blue) and Nap1-P (red). Their medians are shown with the dashed line for Nap1-nonP and the sold line for Nap1-P.

Figure 1

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Figure 2

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Figure 3

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Figure 4

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