Photocontrol of chromatin remodelers as an ATP driven molecular motor using photoresponsive molecules

Chromatin Remodeler Research Group
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1. Background and Aim of Study

The structural changes of chromatin are essential for the regulation of gene expression and chromosomal function. Chromatin which consists of DNA and histone usually takes packaged form in the unit of nucleosome core. In nucleosome core, DNA is wrapped in nucleosome which is octamers of histone. Therefore, during DNA is replicated, it should change its form. Various kinds of chromatin remodellers accomplish different roles in regulation of chromatin remodelling. Interestingly, the catalytic subunits of those share were conserved ATPase - translocase domain (Tr). Snf2 is a catalytic subunit of the SWI/SNF chromatin remodelling complex in yeast and BRG1 is the catalytic subunit of SWI/SNF chromatin remodelling complexes in human.

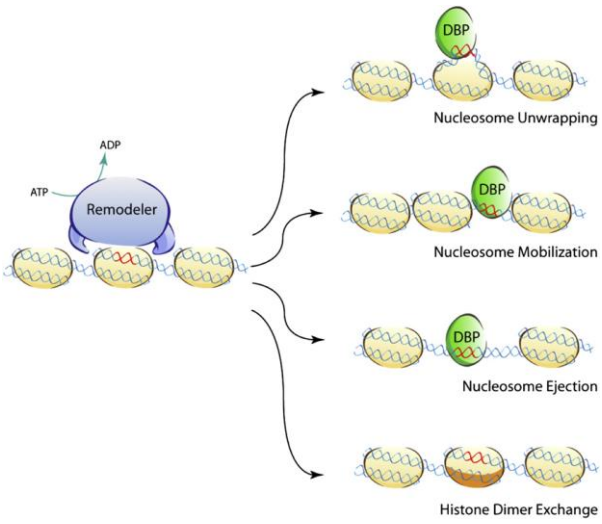

Fig. 1 Different effects of the ATP-dependent chromatin remodeling activity of remodelers on nucleosomal DNA

Dronpa is a fluorescent protein which shows on and off switching for its emission by 400 nm and 500 nm light irradiation. Dronpa variant 145N and 145K forms heterodimer upon 400 nm light irradiation and dissociate upon 500 nm light. Therefore, it is expected that the Dronpa variants are applicable to mechanical photoswitching molecule device to control the function of chromatin remodellers. In this study, we prepared novel photochromic chromatin remodellers subunit fused with Dronpa 145K and 145N.

It has been demonstrated that fused photochromic molecule Dronpa worked as a regulatory nanodevice into the Tr domain which enables to control the SNF2 and BRG1 photoreversibly.

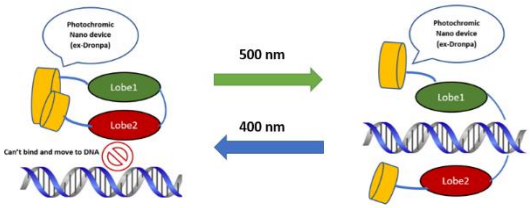

Fig. 2 Proposed possible mechanism for regulation of SNF2 and Brg1 with Dronpa

2. Materials and Methods

Preparation of Dronpa-fused SNF2 and Dronpa-fused BRG1 The Dronpa-fused SNF2 and Dronpa-fused BRG1 was prepared by collaborator professor Shinya Watanabe.

ATPase assay The effect of Dronpa variants bound to the SNF2 and Brg1 on the ATPase activity in the presence of DNA has been examined. The transition between monomer and multimer form of Dronpa 145N-145K was achieved by 400 nm and 500 nm light irradiation at 25°C with a F-2500 spectrofiuorometer (Hitachi).

3. Results and Discussion

3-1. Continual comparison light irradiation ATPase assay of Dronpa-SNF2

To compare the ATPase activity of Dronpa-SNF2 at 400 nm and 500 nm, we conducted continual light irradiation ATPase assay. 0.25uM Dronpa-Snf2, 2mM ATP, 0.1mg/ml of Salmon DNA (Sperm), 30mM Tris-HCl (pH 7.5), 150mM NaCl, 5mM $MgCl_2$, 0.5mM DTT were measured at 25 °C.

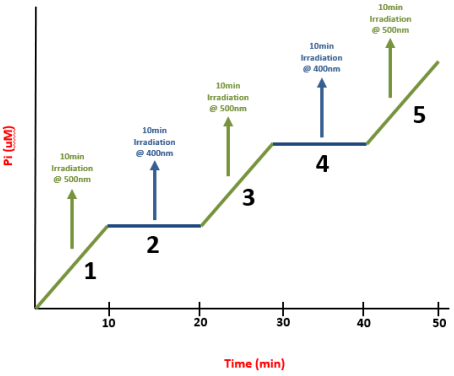

Fig. 3 Expected model of light irradiation ATPase assay of Dronpa-SNF2

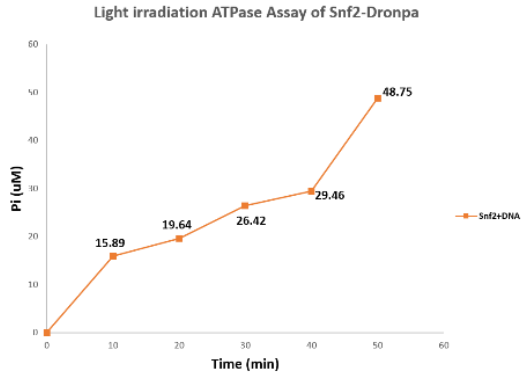

Fig. 4 Continual light irradiation ATPase assay of Dronpa-SNF2

At stage 1 and 5, which Dronpa-SNF2 irradiated 500 nm light it showed increase in its ATPase activity. At stage 2 and 4, which Dronpa-SNF2 irradiated 400 nm light it showed decrease in its ATPase activity.

3-2. Light irradiation ATPase assay of Dronpa-SNF2

0.25uM Dronpa-Snf2, 2mM ATP, 0.1mg/ml of Salmon DNA (Sperm), 30mM Tris-HCl (pH 7.5), 150mM NaCl, 5mM $MgCl_2$, 0.5mM DTT were measured at 25 °C. Significant decrease of ATPase activity of Dronpa-SNF2 were observed at 400 nm compared to 500 nm.

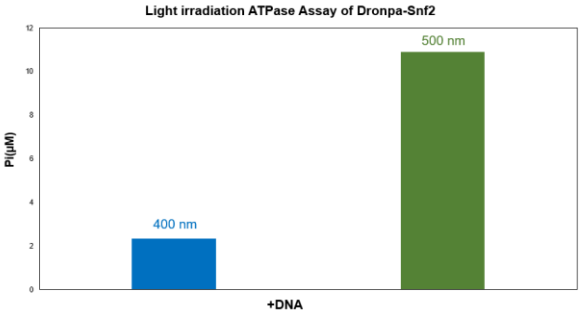

Fig. 5 Light irradiation ATPase assay of Dronpa-SNF2

2-3. Light irradiation ATPase assay of Dronpa-Brg1

The ATPase activity of Dronpa-Brg1 was measured at 25 °C. Due to its complex structure, ATPase assay was conducted at two different conditions which were 0.25uM Dronpa-Brg1, 2mM ATP, 30mM Tris-HCl (pH 7.5), 150mM NaCl, 5mM $MgCl_2$, 0.5mM DTT and 0.25uM Dronpa-Brg1, 2mM ATP, 0.1mg/ml of Salmon DNA (Sperm), 30mM Tris-HCl (pH 7.5), 150mM NaCl, 5mM $MgCl_2$, 0.5mM DTT.

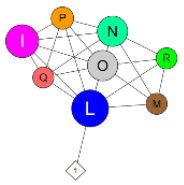

Fig. 6 Structure of BAF complex

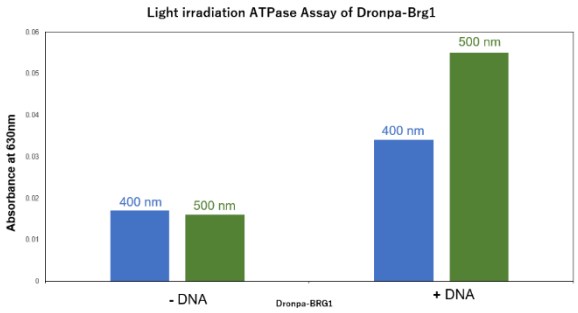

Fig. 7 Light irradiation ATPase assay of Dronpa-BRG1

Dronpa-Brg1 showed decrease of ATPase activity of Dronpa-SNF2 were observed at 400 nm compared to 500 nm and also showed DNA dependent ATPase activity.

Conclusion

We have successfully demonstrated that incorporating Dronpa 145N145K into the catalytic site in chromatin remodellers, enables the reversible control of ATPase activity by 400 nm and 500 nm light irradiation.

References

(1) Clapier, C., Iwasa, J., Cairns, B. et al. Mechanisms of action and regulation of ATP-dependent chromatin-remodelling complexes. Nat Rev Mol Cell Biol 18, 407–422 (2017).