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Heterotrimeric kinesin exhibits a two-step cargo binding mechanism

Xuguang JIANG[1]†, Tadayuki OGAWA[1, 2, 3]†, Kento YONEZAWA[4, 5], Nobutaka SHIMIZU[4], Sotaro ICHINOSE[1, 6], Takayuki UCHIHASHI[7, 8], Wataru NAGAIKE[7], Toshio MORIYA[4], Naruhiko ADACHI[4], Masato KAWASAKI[4], Naoshi DOHMAE[3], Toshiya SENDA[4], Nobutaka HIROKAWA[1,9]

[1] Department of Cell Biology and Anatomy, Graduate School of Medicine, The University of Tokyo, Bunkyo, Tokyo 113-0033, Japan

[2] Research Center for Advanced Medical Science, Dokkyo Medical University, Mibu, Shimotsuga-gun, Tochigi 321-0293, Japan

[3] Biomolecular Characterization Unit, RIKEN Center for Sustainable Resource Science, Wako, Saitama 351-0198, Japan

[4] Structural Biology Research Center, Photon Factory, Institute of Materials Structure Science, High Energy Accelerator Research Organization, Tsukuba, Ibaraki 305-0801, Japan

[5] Center for Digital Green-innovation, Nara Institute of Science and Technology, Ikoma, Nara 630-0192, Japan

[6] Department of Anatomy, Graduate School of Medicine, Gunma University, Gunma 371-8511, Japan

[7] Department of Physics, Nagoya University, Nagoya 464-8601, Japan

[8] Exploratory Research Center on Life and Living Systems (ExCELLS), National Institutes of Natural Sciences, Okazaki, Aichi 444-8787, Japan

[9] Juntendo Advanced Research Institute for Health Science, Juntendo University, 2-1-1, Hongo, Bunkyo-ku, Tokyo, 113-0033, Japan

1 Introduction

Molecular mechanisms that produce the remarkable dynamics of the motor protein-mediated intracellular transport have been a focus of extensive research in the past decades. Mammalian KIF3/KAP3 is one of the most ubiquitously and abundantly expressed kinesins that forms a kinesin complex consisting of heterodimeric KIF3 motors (KIF3A/KIF3B or KIF3A/KIF3C) and the armadillo repeat (ARM)- containing cargo-binding adaptor KAP3. KIF3/KAP3 has been revealed to play diverse critical roles in cells, however, little is known of how KIF3/KAP3 specifically recognizes its diverse cargos.

Here, we identified the distinct structural profiles of the KIF3/KAP3 and KIF3/KAP3-APC complexes by small-angle X-ray scattering (SAXS), which suggesting the molecular bases of its specific cargo binding. More importantly, by performing further high-speed atomic force microscopy (HS-AFM) and cryo-electron microscopy (cryo-EM) analysis, the dynamic complex structure of KIF3/KAP3 with its specific cargo was unveiled, suggesting stepwise cargo recognition dynamics composed of cargo loading, locking, and release [1].

2 Experiment

KIF3A/B/KAP3 (ABK) and KIF3A/B/KAP3/APC (ABK-APC$_{ARM}$) complexes were bacterially expressed and purified, which were then applied onto a Superdex^{TM} 200 Increase 10/300 column and examined on the RI-MALS system to evaluate their molecular weights. SEC-SAXS data were collected at beamlines 10C and 15A2 in KEK-PF at 293K. The final model calculation using *DAMMIN* slow mode was performed using the filtered model derived from *DAMAVER* as the initial starting model. Deposition IDs of SASBDB are SASDMV5 for ABK and SASDMW5 for ABK-APC$_{ARM}$, respectively. High-speed AFM data were collected using a home-built high-speed AFM apparatus at Kanazawa University. Cryo-EM data were acquired with a Talos Arctica in PF-KEK operating at 200 kV using the EPU software for automated data collection, which were subsequently processed and analyzed using *RELOIN3.1*.

3 Results and Discussion

The specific cargo binding of KIF3/KAP3 was validated in vitro, and the biochemical basis of the KIF3-cargo interaction was revealed by the integrated analysis via SEC-SAXS, HS-AFM and cryo-EM (Fig. 1). Remarkably, the results revealed a binding cleft responsible for their specific cargo docking, which was further verified by analysis of ABK-APC$_{ARM}$ (Fig. 1). Considering that a scaffold structure was shared by different kinesins, the specificity and difference mainly resulted from the C-terminal tail region that form distinguishable docking cavities with adaptor

proteins. Interestingly, the coiled-coil region was also found to contribute to the APC_{ARM} binding of KIF3/KAP3 according to our results (Fig. 1), suggesting a new mechanism by which kinesin–cargo interaction can be modulated through stabilization by the coiled-coil stalk. More importantly, HS-AFM tapping-induced dissociation of ABK-APC_{ARM} indicated the temporal roles of the KIF3A tail, KIF3 coiled-coil region, and KAP3 in APC_{ARM} binding, suggesting a stepwise KIF3/KAP3 cargo-binding mechanism after cargo recruitment: (i) Form D, cargo docking into the binding cleft of KIF3-tail/KAP3; (ii) Form T, transitional states; and (iii) Form L, subsequent locking and stabilization by wound and attached coiled coils (Figs. 1 and 2). This binding mechanism might be implicated in the precise regulation of kinesin-mediated cargo transport by upstream signals such as kinases. Since motor proteins, including kinesin, dynein and myosin, share a similar elongated C-terminal structure with a coiled-coil stalk and a cargo-binding tail region, this two-step cargo-binding mechanism might be conserved among motor proteins, and the cargo specificity may depend on their distinct tail regions and mediators. However, this needs to be further investigated on other motor proteins. Moreover, we cannot exclude the possibility that intermediate conformations in the cargo association of kinesin-2 are different from what we observed in cargo dissociation by AFM, which needs to be further investigated and monitored by advanced techniques in future.

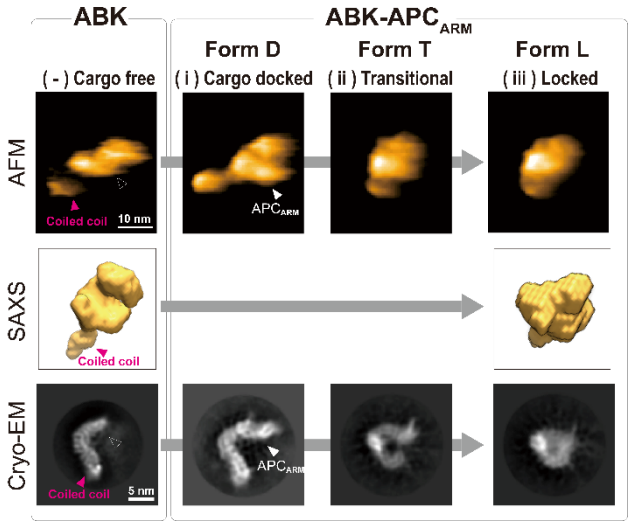

Fig. 1: Integrated structural analysis of kinesin-2 complex

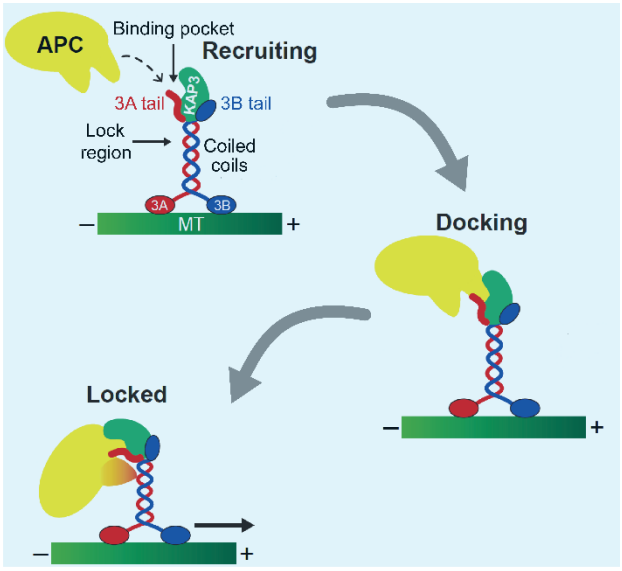

Fig. 2: The two-step cargo binding model of kinesin-2

References

[1] X. Jiang and T. Ogawa *et al*., EMBO Reports (2023) e56864 (advanced online), https://doi.org/10.15252/embr.202356864

† t-ogawa700@dokkyomed.ac.jp