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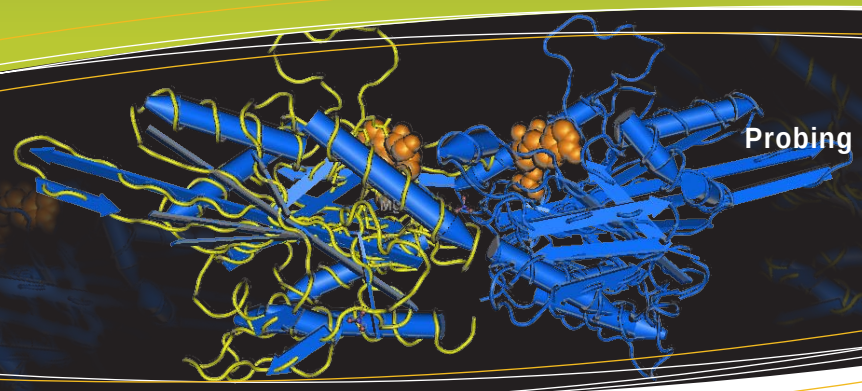

this issue

Meetings

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Probing Sub-domains of Kinesin

Recent reports have highlighted a new phase of research for molecular motors. In contrast to five or more years ago, when macromolecular approaches were used[1], recent research has focused on resolving how kinesin is regulated by intramolecular dynamics. There are two main technical approaches that have allowed measurement of these dynamic mechanisms; these are 1. total internal reflection fluorescence (TIRF) microscopy to observe single kinesin motors and 2. sub-domain swapping from one motor to another.

Interestingly, kinesin motor proteins are relatively flexible in accepting substitutions of small amino acid stretches from one partner to another. Kinesins are comprised of two general domains: 1. the catalytic core and 2. the non-catalytic regions which include, the neck linker (NL), coiled-coil (CC) and cargo binding sub-domains. The NL sub-domain is 14-22 amino acids in length (see Figure 1) and Hoeprich et al.[2] reported this sub-domain can be swapped from kinesin 1 (K-1) to kinesin 2 (K-2) with measurable function differences being transferred to the new partner. Hoeprich et al.[2] used single kinesin tracking to show that the length of the NL sub-domain regulates the ability to move across the microtubule (MT) landscape when MAPs (e.g., tau) are coating its surface. K-2 hardly misses a step when transversing these obstacles, whereas K-1 stutters and progresses more slowly compared to an uncoated MT. In a parameter called processivity or run length, which is a measure of the distance that a motor travels before dissociating from the MT, K-2 travels on average the same distance whether the MT is coated or uncoated with different Tau isoforms (3RS or 4RL), whereas K-1 travels shorter distances on Tau-coated MTs before dissociating. When the NL is genetically swapped between these motors, the characteristic processivity is also swapped.

Similar analyses by Düselder et al.[3] highlighted the importance of the NL length in determining processivity of K-5 (also named Eg5 or KIF11). Their results indicate that with a longer NL, e.g., 13-22 amino-acids, processivity is not altered, whereas 11 amino acids and shorter stretches cause decreased processivity. In contrast, velocity and force generation are much less affected by change in length of the NL. However, Shastry and Hancock find dissimilar

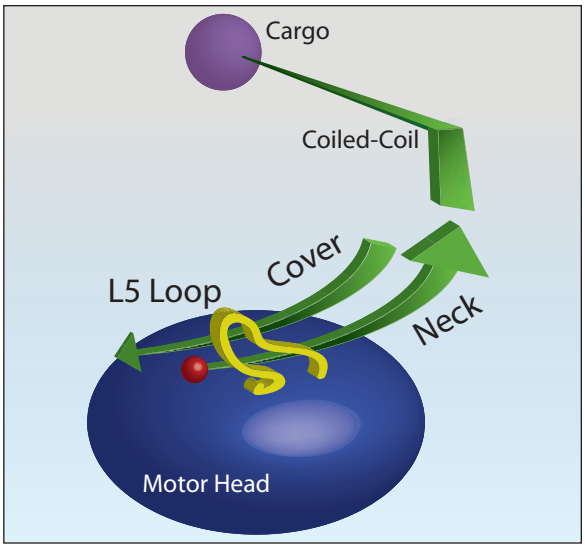

Figure 1. Schematic diagram of kinesin indicating the position of Loop 5, neck, and cover neck relative to the motor domain, coiled-coil, and cargo. Note they are in close proximity to each other and hence interact during the mechanochemical transduction of energy to movement and direction.

Table 1

Motor	Kinesin Family	ID seq before NL	NL	ID seq post-NL	NL aa length	Processivity (µm)	Ref
HsKIF5C (KHC)	K-1	LDFGRRA	KTVKNVVCVNEELT-----	AEEWKRR	14	1.5	4
HsKIF3A	K-2	LRYANRA	KNIKNKARINEDPKDAL	LRQFQKE	17	1.0	4
HsKIF1A	K-3	LRYADRT	KQIRCNAVINEDPNNKL	IRELKDE	17	0.5*/9.2**	6
HsKIF7	K-4	LNYASRA	QNIRNRATVNWRPE---	AERPPEE	14	0/nd	this article
HsKIF11(Eg5, KSP)	K-5	LEYAHRA	KNILNKPEVNQKLTKKAL	IKEYTEE	18	0.3	8

Note: *monomer; **dimer. NL = neck linker; aa = amino acid

Continued from Page 1

results with an optimal NL length of 14 amino acids for processivity[4,5]. The discordant results might be due to different buffers being used, which is noted by Shastry and Hancock as an important determinant of affinity and run length. Interestingly, NL regions have highly specific sequences and lengths for each motor, indicating that this area is optimized for the particular motor's function. In Table 1, we see that NLs vary from 14 to 22 amino acids and may contain prolines. Prolines are predicted to form a kink in the structure which may cause auto-inhibition of motor activity by bending the coiled-coil back on to the motor domain, or create a different direction of travel for the motor, i.e., enable protofilament hopping or circumferential travel.

Analysis of K-3 family members (e.g., KIF1A, KIF13A, KIF131B, KIF16B) by Soppina et al.[6] showed that the coiled-coil 1 sub-domain (CC1) folds back on the NL which caused not only very low processivity but also inhibited dimerization. The typical classification of K-3 members as having low processivity seemed to be contrary to their function as vesicle motors which transport vesicles to distal parts of the cell. Soppina et al.[6] showed that replacing the NL-CC1 subdomains with a coiled-coil resulted in a highly processive dimeric construct, hence demonstrating that, in fact, the motors are highly processive when dimerized, which occurs *in vivo* when they bind cargo[7].

Finally, a complex interplay of kinesin sub-domains was reported by Hesse et al.[8] K-1 is thought to contain three sub-domains: Cover, NL, and L13 (analogous to L5 in other kinesins)[9,10] (Figure 1) which coordinate to generate force from Pi release to the NL, and hence contribute to motor domain movement. By swapping these three sub-domains between K-1 and K-5 family members, Hesse et al.[8] discovered some individual features that relate to the specific function of those motors. For example the K-5 members' domains, when transferred to K-1, conferred the characteristic lower resilience to force representative of K-5 members.

These studies indicate a new level of understanding of kinesin sub-domains, demonstrating that they are interchangeable but also carry specific information about the function of their original motor. These sub-domains are short stretches of amino acids that appear to have a high impact on the motor's specific functions. These sites are also potential targets for molecular intervention with respect to drug development and molecular biology tools.

Custom Motor Proteins

Product	Cat. #	Amount
Myosin - cardiac S1 fragment Native source - bovine heart. Suitable for soluble sarcomere assay	CS-MYS03	inquire
Myosin - smooth muscle S1 fragment Native source - chicken gizzard muscle. Suitable for soluble sarcomere assay	CS-MYS05	inquire
Myosin - skeletal muscle S1 fragment Native source - rabbit leg muscle. Suitable for soluble sarcomere assay	CS-MYS04	inquire
KIF7 motor domain Recombinant protein 6xHis tagged	CS-KF51	inquire
Dynein (cytoplasmic) Native porcine brain source	CS-DN01	inquire

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Proteins and Reagents

Product	Cat. #	Amount
Paclitaxel 2mM	TXD01	10x100µl
Kinesin heavy chain motor domain protein GST tagged, *Homo sapiens* recombinant	KR01-A KR01-XL	2x25µg 1x1mg
CENP-E kinesin motor domain protain GST tagged, *Homo sapiens* recombinant	CP01-A CP01-XL	2x25µg 1x1mg
Eg5 kinesin motor domain protein GST tagged, *Homo sapiens* recombinant	EG01-A EG01-B EG01-XL	2x25µg 10x25µg 1x1mg
Tubulin protein pre-formed microtubules, porcine brain	MT002-A MT002-XL	4x500µg 1x10mg
Tubulin protein >99% pure, porcine brain	T240-A T240-B T240-C T240-DX	1x1mg 5x1mg 20x1mg 1x10mg

Kits and Assays

Product	Cat. #	Amount
Kinesin ATPase Endpoint Assay Biochem Kit™ HTS applications, colorimetric format	BK053	1000 assays
Kinesin ELIPA Biochem Kit™ Kinetic absorbance format	BK060	96 assays