the crystal structure of a conserved domain of cytoplasmic dynein LIC from a thermophilic fungus. Interestingly, this domain has a similar fold to GTPases. Despite having a Ras-like fold, the fungal LIC was crystallized without nucleotide bound and does not appear to bind nucleotide in solution. Based on this structure, we examined whether the human LIC would bind nucleotide since its sequence includes canonical G protein motifs unlike the fungal LIC. Interestingly the human LIC does in fact co-purify with a guanine nucleotide. Work is currently focused on conducting an *in vivo* mutational analysis of the LIC guided by the structure. Not only does our data render a more complete structural picture of dynein, but it also suggests a potentially interesting evolved biological function.

1780-Pos Board B510
Lis1 Regulates Dynein as a Molecular Wedge
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Cytoplasmic dynein walks along microtubules by coupling cycles of ATP hydrolysis in its motor domain with cycles of microtubule binding and release at its microtubule-binding domain. Unlike the other cytoskeletal motors–kinesin and myosin–that have achieved functional diversity through molecular diversity, a single isoform of cytoplasmic dynein is responsible for a host of cellular functions. These range from transport of macromolecular cargo to the construction of the mitotic spindle. How is dynein tuned to perform these different tasks? Regulation is a likely source of functional versatility and understanding it is an area of intense focus in the motors field.
Lis1 is one of dynein's essential and ubiquitous regulators. We have previously shown that Lis1 acts as a molecular ''clutch'', disengaging the cycles of ATP hydrolysis from the cycles of microtubule binding and release. This results in a dynein-Lis1 complex that remains bound to a microtubule despite continuing ATP hydrolysis. We also showed, using negative stain EM and 2D image analysis, that Lis1 binds to dynein's motor domain at AAA3/4.
We have now used a combination of three dimensional electron microscopy, biochemistry and single molecule methods to characterize the mechanism by which Lis1 regulates dynein. A cryo-negative stain structure of the dynein-Lis1 complex (21Å resolution) shows that Lis1 physically blocks dynein's linker domain from docking at its normal AAA5 site under no nucleotide conditions. Instead, the N-terminal half of the linker takes an alternative path around Lis1. Our single molecule data show that a shortened linker, short enough to bypass Lis1 but long enough to preserve its interaction with AAA5, renders dynein insensitive to Lis1. Taken together, our data suggest that Lis1 achieves its uncoupling activity by acting as a molecular wedge.

1781-Pos Board B511
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1782-Pos Board B512
Illuminating the Cooperative Action of Kinesin-II and OSM-3-Kinesin in the Chemosensory Cilia of *Caenorhabditis Elegans*
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Intraflagellar transport (IFT) is indispensable for the assembly and maintenance of cilia. In the chemosensory cilia of *C. elegans*, two motors of the kinesin-2-family, heterotrimeric kinesin-II and homodimeric OSM-3, collaborate to drive anterograde IFT. It is known that both kinesins associate with IFT-trains that move along the middle segment, and that OSM-3 alone drives transport in the distal segment. However, many questions remain concerning how these kinesins cooperate, how this is regulated and why both motors are required. To address this problem, we have improved the fidelity of IFT-assays by using single transgenes encoding for fluorescently-labeled kinesins. In combination with ultrasensitive, quantitative fluorescence microscopy and dynamic photoactivated localization microscopy allowing observation of single motors in living nematodes, we find that the motor composition of IFT-trains is highly dynamic. Kinesin-II undocks gradually from IFT-trains close to the ciliary base, and not suddenly upon reaching the middle segment tips as previously thought, whereas OSM-3 docks gradually resulting in accelerating IFT-trains. Undocked kinesin-II is transported back to the ciliary base by dynein-driven retrograde trains. Consequently, the IFT-system ensures that kinesin-II stays close to the ciliary base, where, it is responsible for the loading of IFT-trains onto the axoneme, and that OSM-3 stays around the distal segment, where it drives fast long-distance transport. Our work on motor cooperation and dynamics provides new insight into how IFT drives the proper development and functioning of cilia and, more broadly, how kinesin motors work together to generate intracellular transport pathways within cells.

1783-Pos Board B513
BicD2 and Dynactin Convert a Non-Processive Cytoplasmic Dynein to an Ultra-Processive Directional Motor
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Cytoplasmic dynein is the predominant minus-end directed microtubule motor in metazoan cells. Dynein transports diverse cargoes over long distances in neurons, and the motor is thought to be adapted for a myriad of cellular functions through the use of several accessory protein factors that impinge on its basic biophysical characteristics. One of these accessory factors is the multisubunit dynactin complex, which has been implicated in dynein-based cargo transport and the modulation of dynein processivity and directionality. While isolated dynein from Saccharomyces has been shown to be a strongly processive motor, dynein from other organisms displays weakly processive, bidirectional or diffusive motility. Here we show that, on its own, cytoplasmic dynein from humans and other metazoans is not a processive motor. Previous attempts to study dynein-dynactin co-complexes have found relatively modest effects on dynein processivity and directionality by dynactin. We utilize the evolutionarily conserved coiled-coil adapter protein BicD2 to strongly induce the formation of a stable dynein-dynactin-BicD2 (DDB) supercomplex that is over 2MDa in size. Using multicolor single-molecule microscopy, we have found that, remarkably, the purified DDB supercomplex is unidirectional and ultra-processive, displaying run-lengths that greatly exceed the previously observed enhancement of single dynein run-lengths by dynactin. The DDB supercomplex accumulates at microtubule minus-ends and displays characteristics expected of a processive cargo transport motor. Our data suggest that the dynein motor is more plastic that previously thought, able to transition from a non-processive motor to an ultra-processive mode of motility upon association with external regulatory factors.

1784-Pos Board B514
New Folding Pattern of P150 and "Anntena" for Dynein Binding
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Dynactin is known to be a regulator of cytoplasmic dynein and play a part in intracellular transport. Dynactin is a large complex composed of multiple subunits including p150, p50 and Arp1, and has a characteristic architecture. Sidearm domain of dynactin is essential for binding to dynein and microtubule, but the structural detail of the domain is poorly understood due to its intricate organization. Here, by electron microscopy of the nanogold-labeled or the truncated mutants of human dynactin complex, the location of each domain of the largest subunit p150 was identified. We rediscovered the