











Edited by Keiko Hirose Linda Amos

Published by

Pan Stanford Publishing Pte. Ltd. Penthouse Level, Suntec Tower 3 8 Temasek Boulevard Singapore 038988

Email: editorial@panstanford.com Web: www.panstanford.com

British Library Cataloguing-in-Publication Data

A catalogue record for this book is available from the British Library.

Handbook of Dynein

Copyright © 2012 Pan Stanford Publishing Pte. Ltd.

All rights reserved. This book, or parts thereof, may not be reproduced in any form or by any means, electronic or mechanical, including photocopying, recording or any information storage and retrieval system now known or to be invented, without written permission from the publisher.

For photocopying of material in this volume, please pay a copying fee through the Copyright Clearance Center, Inc., 222 Rosewood Drive, Danvers, MA 01923, USA. In this case permission to photocopy is not required from the publisher.

ISBN 978-981-4303-33-0 (Hardcover) ISBN 978-981-4303-34-7 (eBook)

Printed in the USA

Contents

-	neins: <i>A</i> eir Secr	Ancient Protein Complexes Gradually Reveal	1
			1
		os and Keiko Hirose	
1.1		luction	1
1.2	Dynei	n Molecular Structure Coming into View	2
		Axonemal Arms	2
		Dynein Molecules	4
		Lighter Components of the Complexes	11
		Effect on the Heavy Chain of Vanadate and UV	11
		Motility of Dynein	11
1.3	Evolu	tion—Where did Dyneins Come From?	12
	1.3.1	AAA+ Proteins	12
	1.3.2	Ancestors and Relatives of the Dynein Heavy Chain	13
	1.3.3	CD-2 Is Probably Similar to the Common Ancestor	15
	1.3.4	Almost All Eukaryotic Organisms Express Dynein	15
1.4	Summ	nary	16
2. Tw	o Deca	des of Cytoplasmic Dynein: From Fast to Forceful	27
Rich	ard J. Mo	cKenney and Richard B. Vallee	
2.1	Introd	luction	27
2.2	Cytop	lasmic Dynein Functions	28
2.3	Comp	ositional and Regulatory Diversity	29
	2.3.1	Cytoplasmic Dynein Isoforms	29
	2.3.2	Accessory Complexes	30
2.4	Dynac	etin	30
2.5	LIS1 a	and NudE/NudEL	32
	2.5.1	Effects of LIS1 and NudE on Dynein Activity in vitro	33
	2.5.2	LIS1/NudE Enhancement of Multiple Motor Transport	34
2.6	Cytop	lasmic Dynein Regulation by Dynactin vs. NudE-LIS1	35
3. Fur	ıctiona	l Analysis of the Dynein Motor Domain	43
Ton	ohiro Sh	nima, Kazuo Sutoh, and Takahide Kon	
3.1	Introd	luction	43
3.2	The D	ynein Motor Domain	45
3.3		se Cycles in the Head Domain	46
3.4	Move	ment of the Linker	49

3.5	Microtub	ule Binding at the Stalk	52
3.6	Perspecti	ives	55
4. Str	uctural Stu	udies on the Dynein Heavy Chain	63
Anti	nony J. Rober	rts and Stan A. Burgess	
4.1	Introduct	tion	63
4.2	The Form	and Function of the Heavy Chain	64
	4.2.1 Th	ne Tail Domain	64
	4.2.2 Th	ne Stalk Domain	66
	4.2.3 Th	ne Head Domain	68
	4.	2.3.1 The AAA+ ring	68
	4.	2.3.2 The C-terminal sequence	70
		2.3.3 The linker domain	70
4.3		ational Changes	72
4.4	Conclusio	ons and Outlook	75
5. Str	uctural An	alysis of Dynein Bound to Microtubules	81
Keik	o Hirose		
5.1	Introduct	tion	81
5.2	Formatio	n of a Dynein-Microtubule Complex	83
	5.2.1 M'	T-Binding of Outer-Arm Dynein Purified from	
	Ch	nlamydomonas Axoneme	84
		T-Binding of Outer-Arm Dynein Purified from	
		etrahymena Axoneme	85
		T-Binding of Outer-Arm Dynein Purified from	0.5
		a Urchin Axonemes	85
		T-Binding of Cytoplasmic Dynein	87
F 2		T-Binding of Recombinant Dynein Motor Domains	88
5.3	-	of the Dynein-Microtubule Complex	88
		O Structural Analysis of the Dynein-MT Complex	88
	5.	3.1.1 3D structural analysis of recombinant dynein stalk regions bound to MTs	88
	5.	3.1.2 3D structural analysis of recombinant dynein	
		bound to MTs	89
	5.	3.1.3 3D structural analysis of axonemal dynein	
		cross-bridging MTs	89
		ngles of Dynein Stalks Bound to MTs	90
		ovement of the Heads in MT-Bound Axonemal Dynein	91
5.4	Motile Me	echanism of Dynein	93
5.5	Future Or	utlook	94

Contents	vii
----------	-----

6.				Atomic Structure and Roles in the Dynein Motor	99
	Andr	ew P. Ca	rter		
	6.1	Introd	uction		99
	6.2	Struct	ural Feat	cures of the Dynein Stalk	101
		6.2.1	Historic	al Background	101
		6.2.2	Microtu	bule-Binding Domain	102
		6.2.3	Stalk Co	oiled Coil	103
		6.2.4	Junction	n of Stalk and AAA Ring	104
	6.3	Comm	unicatio	n Along the Dynein Stalk	105
		6.3.1	Models	for Communication	105
			6.3.1.1	Large scale conformational changes	106
			6.3.1.2	Communication via changes in stalk	
				binding angle	106
			6.3.1.3	Relative sliding of helices in the coiled coil	107
		6.3.2	Structur	ral Changes During Communication	108
			6.3.2.1	Half-heptad movement	109
			6.3.2.2	Smaller shifts in the coiled coil	110
			6.3.2.3	Conformational changes around the kink	
				in the stalk	111
	6.4	Role o	f the Stal	k in the Dynein Powerstroke	112
		6.4.1	Models	of Stalk Involvement in the Powerstroke	113
			6.4.1.1	Stalk acts as a paddle	114
			6.4.1.2	Structural change near MTBD rotates stalk	
				(stalk lever arm model)	115
			6.4.1.3	Stalk is rigid and holds AAA ring so that	
				conformational change is directed	116
			6.4.1.4	Stalk acts as a tether, preferentially binding	
				toward the MT minus end	116
				lk and Directionality	118
	6.5	Concl	ision		118
7.	Mot	ile and	Enzyma	atic Properties of Native Dynein Molecules	123
	Yoko	Y. Toyos	hima and	Hideo Higuchi	
	7.1	Introd	uction		123
	7.2	Prepar	rations o	f Native Dynein Molecules for Functional Assays	124
		7.2.1	Cytopla	smic Dynein from Mammalian Brain	124
		7.2.2	Axonem	al Dyneins from Tetrahymena Cilia	126

7.3	Meası	rement of Dynein ATPase Activity	127
	7.3.1	Method for Steady-State ATPase Assaying of Dyneins	127
	7.3.2	ATPase Activity Properties of Native Dyneins	128
7.4	In vitr	o Motility of Native Dynein Molecules	129
	7.4.1	In vitro Motility Assay for Dynein	129
	7.4.2	Motile Properties of Native Dynein Molecules	130
7.5	In vitr	o Motility of Single Native Dynein Molecules	132
	7.5.1	Processivity of Single Dynein Molecules	132
	7.5.2	Processivity and Step Size as Determined with FIONA Method	133
	7.5.3	Step Size and Force Measurement of Single Dynein	
		Molecules by Optical Trapping	134
	7.5.4	Model of Dynein Walking	135
7.6	Vesicl	es Transport Driven by Dynein in Cells	137
	7.6.1	Step Size of Dynein During Vesicle Transport in Cells	137
	7.6.2	Force Generation by Dynein in Cells	139
	7.6.3	In vivo Mechanical Measurement of Dynein	139
7.7	Summ	nary	140
8. Mo	tile Pro	perties of Cytoplasmic Dynein	145
Sam	ara L. Re	eck-Peterson, Ronald D. Vale, and Arne Gennerich	
8.1	Introd	luction	145
8.2	Source	es of Cytoplasmic Dynein	147
	8.2.1	Native Dynein	147
	8.2.2	Recombinant Dynein	148
8.3	Cytopl	lasmic Dynein Motility in the Absence of Load	149
	8.3.1	Cytoplasmic Dynein is a Processive Motor	150
	8.3.2	Stepping Behavior of Cytoplasmic Dynein	
		Under Unloaded Conditions	151
	8.3.3	Directionality of Cytoplasmic Dynein	153
8.4	Respo	nse of Cytoplasmic Dynein to Load	155
8.5	Regula	ation of Cytoplasmic Dynein Motility	158
	8.5.1	AAA+ Domains	159
		8.5.1.1 AAA1	159
		8.5.1.2 AAA2, AAA4	159
		8.5.1.3 AAA3	159
	8.5.2	Dynein Cofactors	161
		8.5.2.1 Dynactin	161
		8.5.2.2 LIS1 and NudE	163

			Contents ix
8.6	In vivo	Studies of Dynein Motility	164
8.7		isions and Future Directions	165
9. Mo	tility of	Inner-Arm Dyneins	173
Yuji	Shitaka,	Hiroaki Kojima, and Kazuhiro Oiwa	
9.1	Introd	uction	173
	9.1.1	Description of Inner Arms	173
	9.1.2	In vitro Motility Assays	178
	9.1.3	Single-Molecule Measurements	178
9.2	Solutio	ons	179
9.3	Protei	n Preparations	179
	9.3.1	Inner-Arm Dynein Subspecies	179
	9.3.2	Preparation of Microtubules and their	
		Fluorescent Labeling	181
	9.3.3	In vitro Motility Assays	182
		9.3.3.1 Overview	182
		9.3.3.2 Preparation of dynein-coated beads for	
		optical trap nanometry	184
		9.3.3.3 Preparation of a flow cell	185
		9.3.3.4 Preparation of patterned surface for	
		bio-nano device development	186
		9.3.3.5 <i>In vitro</i> motility assay of inner-arm dyneins	187
		9.3.3.6 Evaluation of the processivity	188
		9.3.3.7 Optical trap nanometry	189
9.4	Result		190
		In vitro Motility Assays	190
		Optical-Trap Nanometry Studies on Dynein Motility	192
	9.4.3	The Directional Control of MT Movement on	102
0.5	C	Dynein-Coated Surfaces	193
9.5		ary and Outlook	194
	9.5.1	Levels of Coordination that Need to be Studied	194
10. Dy	nein M	otility in Cilia and Flagella	203
Ava	nti Gokh	ale, Maureen Wirschell, Winfield S. Sale, and David R. Mitcheli	'
10.1	Introd	uction	203
10.2		me Structural Organization and Dynein Subforms	204
	10.2.1	Basic Axonemal Structure, Axis of Bending	
		and the 96 nm Repeat	205

	10.2.2	Delillillo	if of the Dynams and their Functions	207
		10.2.2.1	The outer dynein arms, beat frequency,	
			and force	208
		10.2.2.2	The inner dynein arms and control of ciliary	
400		1.0	waveform	209
10.3			cures that Regulate Dynein	210
	10.3.1		ral Pair–Radial Spoke Network	210
		The DRC		212
			ein Arm Linkers	213
10.4	_		ule/Direction Switching Model	213
			ng Microtubule Model	213
	10.4.2	A "Switc	hing" Model for Forward and Reverse Bending	215
	10.4.3	Inherent	Regulation of Microtubule Sliding	217
10.5	Mecha	nical Feed	back and Calcium Regulation	218
	10.5.1	Mechani	cal Feedback Mechanisms	218
		10.5.1.1	Central pair orientation	218
		10.5.1.2	Radial spoke tilting	219
		10.5.1.3	Central pair asymmetry	221
	10.5.2	Signaling	g Mechanisms in Bend Propagation	222
		10.5.2.1	Central pair as control center	222
		10.5.2.2	Control via bend-plane doublets	222
	10.5.3	Calcium	Sensors and Control of Dynein	223
		10.5.3.1	Asymmetry in metazoan spermatozoa	223
		10.5.3.2	Waveform control in algal flagella	224
		10.5.3.3	Arm-associated calcium-binding proteins	224
10.6	Regula	tion of Mo	otility by Phosphorylation	225
	10.6.1	Regulati	on of Outer Arms by Calcium and	
		Phospho	rylation	225
		10.6.1.1	Control of beat frequency	226
		10.6.1.2	Activation of motility	227
	10.6.2	Regulati	on of Inner Dynein Arms by Phosphorylation	227
		10.6.2.1	Protein kinase inhibition	228
		10.6.2.2	IC138 mutants	228
		10.6.2.3	Inhibition of CK1	230
		10.6.2.4	Role of IC97	230
10.7	Future	Challenge	es and Opportunities	231

				Contents
l1.3D	Structu	ires of Ax	conemes	245
Tak	ashi Ishik	rawa		
11.1	Introd	uction		245
11.2	Single-	Particle A	nalysis and Electron Tomography	246
	11.2.1		n Preparation for Electron Tomography/	
		Microsco	рру	248
			Ice-embedding by plunge freezing (cryo)	248
		11.2.1.2	Freeze-fracture deep-etch replica and	2.10
		44.04.0	rotary shadowing	249
		11.2.1.3	Negative staining, cryo-negative, and	249
		11 2 1 1	positive staining Plastic embedded sections	250
	11 2 2		puisition and Reconstruction in Electron	230
	11.2.2	Tomogra	•	250
	11.2.3	_	nging from Tomogram, Classification,	200
	11.2.0	and Mod		251
11.3	In situ		of Dynein Arms Revealed by Electron	
	Tomog	raphy		252
	11.3.1	3D Struc	ture of Outer Dynein Arms	253
	11.3.2	3D Struc	ture of Inner Dynein Arms	255
	11.3.3	-	tric Dynein Arrangement in	
		-	omonas Flagella	256
	11.3.4		de-Induced Structural Change of Flagellar	
		Dynein A		257
11.4			ner Components in Flagella	258
			d Other Interdoublet Linkers	258
	11.4.2		l lc . lp :	260
11 5		-	pokes and Central Pairs	260
11.5	Future	Outlook		261
12. Fu	nctiona	l Diversit	y of Axonemal Dyneins	267
Rits	su Kamiya	1		
12.1	Introd	uction		267
12.2	Bioche	mical Ana	llyses	268
	12.2.1	Separati	on of Individual Dyneins	268
	12.2.2	Subunit	Composition	269
		12.2.2.1	Total gene assignment of axonemal	
			dynein heavy chains	271
		12.2.2.2	Phylogeny of axonemal dyneins	272

хi

12.3	Function	onal Diver	sity Assessed by the Mutant Motility	272
	12.3.1	Available	e Mutants	272
	12.3.2	Mutant P	Phenotypes	273
		12.3.2.1	Mutants lacking any single type of dynein	
			can swim slowly	273
		12.3.2.2	Outer- and inner-arm dynein	274
			Specific functions of certain types of dyneins	274
		12.3.2.4	Function of individual heavy chains	275
12.4	In Vitro	Motility .	Assays	275
	12.4.1	General I	Features	275
		ADP Sens	-	276
	12.4.3		al Interactions Between Outer-Arm	
		Heavy Ch		277
	12.4.4		enomena in Inner-Arm Dyneins	278
			Microtubule bending	278
			Ratchet-like properties	279
12.5	Conclu	sions and	Outlook	279
13. As	sembly	and Regu	lation of Dynein Light Chains	285
Afu	a Nyarko	and Elisar	Barbar	
13.1	Introd	uction		285
13.2	The Re	gulatory S	Subunits: Structure, Functions, and	
		ation State		286
13.3	Interac	tions Bety	ween the Intermediate and Light	
	Chain S	Subunits		289
	13.3.1	Mapping	Interactions	289
	13.3.2	Identifyi	ng Conformational Changes	291
	13.3.3	High-Res	solution Complex Structures	294
13.4	Regula	tion by Di	sorder-to-Order Transitions	294
	13.4.1	Disorder	-to-Order Transition at the Sites of Binding	294
	13.4.2	Disorder	-to-Order Transition Distant from Binding	295
	13.4.3	Regulatio	on by Subunit Phosphorylation	296
13.5	Poly-B	ivalency ir	n Assembled IC	297
13.6	The Lig	ght Chains	-Intermediate Chain Assembled Subcomplex	298
14. Re	gulator	y Subunit	s of Axonemal Dynein	303
Kaz	uo Inaba			
14.1	Introd	uction		303
				304

				Contents xiii	
	14.2.1	Heavy Cl	hains	304	
	14.2.2	Interme	diate Chains	304	
	14.2.3	Light Ch	ains	304	
		14.2.3.1	LC1: leucine-rich repeat light chain	305	
		14.2.3.2	LC2: Tctex2-related light chain	305	
		14.2.3.3	LC3 and LC5: thioredoxin homolog		
			associated with dynein HCs	305	
		14.2.3.4	LC4: Ca ²⁺ -binding light chain	305	
		14.2.3.5	LC7a and LC7b: <i>Drosophila</i> roadblock homologs	306	
		14.2.3.6	LC8, LC6, and LC10: highly conserved		
			protein LC8 and its homologs	306	
	14.2.4	Dynein I	Oocking Complex	306	
	14.2.5	Proteins	Associated with Outer-Arm Dynein	307	
14.3	Outer-	Arm Dyne	ein from Sperm Flagella	308	
	14.3.1	Heavy Cl	hains	308	
	14.3.2	Intermed	diate Chains	308	
	14.3.3	Light Ch	ains	309	
	14.3.4	Proteins	Associated with Outer-Arm Dynein	309	
14.4	Chlamy	ydomonas	Inner-Arm Dynein	309	
	14.4.1	Subunits	s of Dynein-f/I1	310	
	14.4.2	Subunits	of Other Inner-Arm Dyneins	310	
14.5	Inner-A	Arm Dyne	in from Sperm Flagella	311	
14.6	_	-	tions of Dynein Subunits in Ciliary		
		agellar Mo	-	311	
			Phosphorylation and Dephosphorylation	312	
		-	pendent Regulation	312	
		Redox Po		313	
	_		de Metabolism	314	
14.7	_	rison and Compon	Evolutionary Aspects of Axonemal	314	
	-	-			
		-	in Yeast Nuclear Segregation	325	
Mel	issa D. Sti	uchell-Brer	eton, Jeffery K. Moore, and John A. Cooper		
15.1	Introd	uction		325	
15.2	Mecha	nisms tha	t Position Nucleus and Spindle	326	
	15.2.1	The Kar	9 Pathway	327	
	1522	The Dyn	ein Pathway	327	

		15.2.2.1	Discovery of dynein in yeast	328
		15.2.2.2	Dynein localizes to MT plus ends and the	
			cell cortex in <i>S. cerevisiae</i>	329
		15.2.2.3	, ,	
			cMTs and the cortex	329
15.3	Compo	nents of t	he Yeast Dynein Complex and Conservation	
		Species		330
	15.3.1	Heavy Cl	nain/DYN1	331
	15.3.2	Intermed	diate Chain/PAC11	331
	15.3.3	Light Int	ermediate Chain/DYN3	332
	15.3.4	Light Ch	ain/DYN2	333
15.4	Regula	tory Com	ponents Contributing to Dynein Function	333
	15.4.1	Dynactin	l	334
		15.4.1.1	Nip100/p150 ^{Glued}	334
		15.4.1.2	Jnm1/dynamitin	336
		15.4.1.3	Yll049w/Ldb18/p24	336
		15.4.1.4	Arp1	337
		15.4.1.5	Arp10/Arp11	338
	15.4.2	Bik1/CL	IP-170 and the Kinesin Kip2	338
	15.4.3	Pac1/LIS	S1 and Ndl1/NudE(L)	340
	15.4.4	Num1		342
	15.4.5	Cell Pola	rity and Cell Cycle Regulators	343
15.5	Offload	ling Mode	ıl	345
15.6	Metho	ds for Stu	dying Dynein Function in Yeast	347
	15.6.1	Assaying	the Position of the Nucleus and Spindle	
		as a Mea	sure of Dynein Activity	347
		15.6.1.1	Examining nuclear segregation by	
			DAPI-stain	347
		15.6.1.1	Examining the position of the mitotic	
			spindle using GFP-labeled MTs	349
Index				363