the FEBS Journal
MINIREVIEW

DYNLL/LC8: a light chain subunit of the dynein motor complex and beyond

Péter Rapali, Áron Szenes, László Radnai, Anita Bakos, Gábor Pál and László Nyitray

Department of Biochemistry, Eötvös Loránd University, Budapest, Hungary

Keywords
dynein; hub protein; intracellular transport; linear motif; protein–protein interactions

Correspondence
L. Nyitray, Department of Biochemistry, Eötvös Loránd University, Pázmány P.s. 1/c, H-1117 Budapest, Hungary
Fax: +36 381 2172
Tel: +36 1381 2171
E-mail: nyitray@elte.hu

(Received 8 February 2011, revised 11 May 2011, accepted 15 June 2011)

doi:10.1111/j.1742-4658.2011.08254.x

The LC8 family members of dynein light chains (DYNLL1 and DYNLL2 in vertebrates) are highly conserved ubiquitous eukaryotic homodimer proteins that interact, besides dynein and myosin 5a motor proteins, with a large (and still incomplete) number of proteins involved in diverse biological functions. Despite an earlier suggestion that LC8 light chains function as cargo adapters of the above molecular motors, they are now recognized as regulatory hub proteins that interact with short linear motifs located in intrinsically disordered protein segments. The most prominent LC8 function is to promote dimerization of their binding partners that are often scaffold proteins of various complexes, including the intermediate chains of the dynein motor complex. Structural and functional aspects of this intriguing hub protein will be highlighted in this minireview.

Introduction

Dynein light chains (molecular mass 10–20 kDa) are accessory subunits of the large dynein motor complexes. The LC8 family of light chains (DYNLL1 and DYNLL2 in vertebrates; abbreviated here as LC8), together with the Tctex (DYNLT1 and DYNLT3) and LC7 (Roadblock; DYNLRB1, DYNLRB2) light chains, bind as homodimers to the dimeric cytoplasmic dynein intermediate chains (DYNC1I1, DYNC1I2; abbreviated here as DIC), which are scaffold subunits for cargo binding to the motor complex (for recent reviews see [1–3]; a schematic view of the subunit structure of dynein is shown in this minireview series [3]). LC8 was first described as a subunit of *Chlamydomonas* axonemal dynein [4], and was subsequently found to bind to all cytoplasmic and most axonemal dyneins [2]. The LC8 genes are present in all sequenced eukaryotic genomes [1] and code for an extremely conserved 10 kDa protein. The *Chlamydomonas*, *Caenorhabditis elegans*, *Drosophila* and mammalian LC8 orthologs share more than 90% identity. The two mammalian paralogs DYNLL1 and DYNLL2 differ only in six out of 89 residues, and they are fully conserved as orthologs. Based on genetic studies, it is clear that at least in metazoans LC8 is an essential protein; knocking out or knocking down of its gene in *Drosophila* and *C. elegans* either is embryonically lethal or causes severe pleiotropic phenotypes [5,6].

Since LC8 was identified as tail-binding light chain of not only dyneins but also of myosin 5a [1,7], and was also found to interact with many proteins that

Abbreviations
DIC, dynein intermediate chain; DYNLL/LC8, dynein light chain LC8; ERα, estrogen receptor α; HTP, high-throughput; MYO5A, myosin 5a heavy chain; nNOS, neuronal nitric oxide synthase; Pak1, p21-activated kinase; PSD, postsynaptic density.

were shown to be transported either on microtubules or on the actin filaments, it was widely assumed that LC8 could function as a cargo adapter being simultaneously associated with both the motor and the cargo protein [8–13]. However, recent structural and thermodynamic studies challenged this hypothesis: the two identical binding sites of LC8 and the homodimeric nature of both DIC and myosin 5a heavy chain (MYO5A) make it unlikely that LC8 can bridge the cargo to either motor complex [14–17]. The facts that LC8 interacts with proteins that are not associated with intracellular transport and that it is present in plants that are entirely devoid of dynein motors [2] point to a more general role of LC8. Recently, it has been recognized by analyzing the sequences of LC8 interaction partners that the short linear LC8 binding motifs are located in intrinsically disordered protein segments [18,19]. Moreover, the LC8 binding motif is often located close to coiled-coil or other dimerization domains of the interacting partners. Accordingly, the current view is that LC8 is an essential hub protein that functions as a 'molecular velcro': it promotes dimerization and structural stabilization and hence it could allosterically regulate its binding partners in diverse protein complexes and networks, the dynein motor complex being only one of them [18].

Structure of DYNLL/LC8 alone and in various complexes

The solution and crystal structures of LC8 in apo form [8,20–23] and in complex with peptides from six binding partners [DIC, neuronal nitric oxide synthase (nNOS), Bim, Swallow, p21-activated kinase (Pak1), EML3] have been determined [8,14,22,24–26]. Moreover, models with three additional binding peptides have recently been published [27,28]. LC8 has a unique fold (Fig. 1A, B): two-five-stranded, antiparallel β-sheets are responsible for dimerization; each β-sheet contains four strands from one monomer and a fifth strand from the other monomer. These sheets are flanked by two pairs of α-helices at the opposite faces of the dimer. Interestingly, the Tctex/DYNLT light chain is a structural homolog of LC8/DYNLL with no apparent sequence similarity [29,30]. Despite their structural similarity there is no overlap in known targets of the two light chains [1]. The bound ligands of LC8 lie in two identical parallel grooves formed at the two edges of the dimerization interface. The bound peptides form an extra antiparallel β-strand and therefore augment the central β-sheets [8,14,22,24–26] (Fig. 1A, B). Practically all non-identical residues of LC8 paralogs and orthologs in metazoans are located

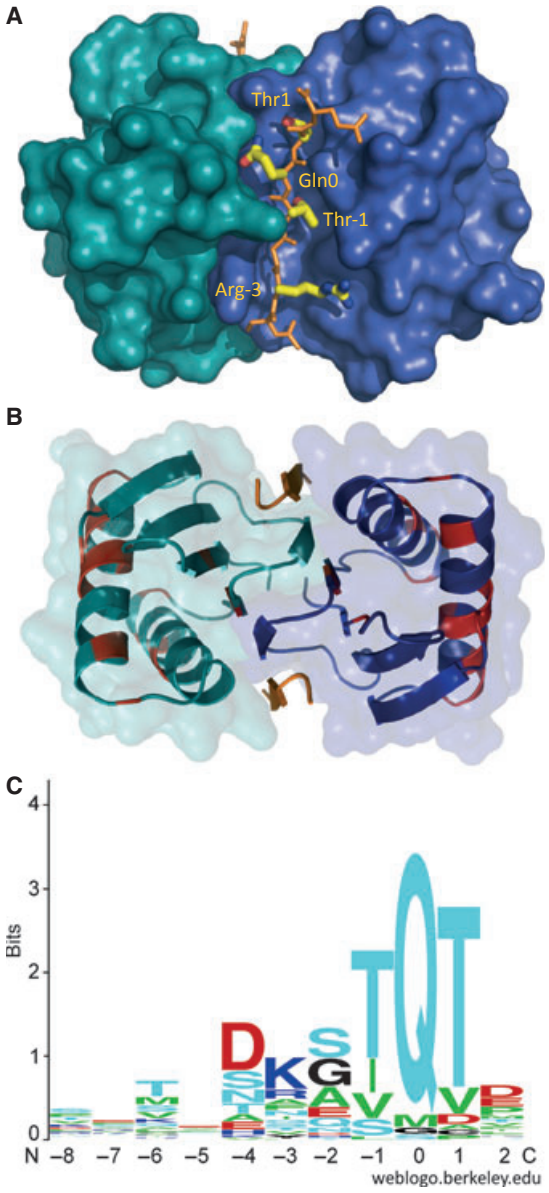

Fig. 1. (A) Structure of DYNLL/LC8 complexed with a peptide ligand. Crystal structure of human DYNLL2 in complex with the binding motif of EML3 at 1.3 Å resolution (PDB ID: 2XQQ) [26]. DYNLL2 is a homodimer with the monomers related by a twofold axis (cyan and blue). Two peptides (orange) corresponding to the binding motif of EML3 lie in the binding grooves formed at the edge of the dimerization interface of DYNLL2. Side-chains with a key role in the interaction (Arg–3, Thr–1, Gln0, Thr1) are highlighted. (B) Two five-stranded β-sheets are formed at the dimerization interface, each containing four strands from one monomer and the fifth from the other. Residues not fully conserved in LC8 paralogs and orthologs are shown in red. Most of the natural diversity clearly occurs in α-helices and loops far from the ligand, while the binding grooves are highly conserved. The bound peptides augment the five-stranded β-sheets by an antiparallel β-strand. (C) Sequence logo of the binding motif of all hitherto determined and verified LC8 binding motifs. Similar colors indicate similar chemical properties [135].

on the outer surface of the homodimer protein and are not involved in binding of target proteins (Fig. 1B).

LC8 binding motifs were originally divided into two classes: ($K_{-3}X_{-2}T_{-1}Q_0T_1X_2$) and [$X_{-3}G_{-2}(I/V)_{-1}Q_0V_1D_2$] [8,12,31]. In both classes the central Gln (position 0) caps the N-terminal end of the second α-helix, while the side-chains of residues at positions +1, −1 and −3 interact with the interior of the binding groove. A few LC8 partners contain non-canonical binding motifs that lack the most conserved Gln residue (e.g. MYO5A [32,33], Pak1 [22], GRINL1A [27]), but the overall binding topology of these peptides is similar to that of the canonical ones. In the DYNLL1–Pak1 complex a specific H-bond network compensates for lack of the conserved Gln [22], and similar compensatory interactions are seen in the docking model of DYNLL1–GRINL1A [27] and the very recently determined crystal structure of DYNLL2–MYO5A (L. Radnai, P. Rapali and L. Nyitray, unpublished results).

How can a highly conserved binding site promiscuously interact with such a diverse motif set? Available data indicate that conformation of the binding grooves of LC8 is highly dynamic. Binding groove residues of apo-LC8 show conformational exchanges on multiple time scales in NMR spectroscopic experiments, while ligand binding reduces the conformational breathing of these regions [34,35]. Evidence for structural plasticity can also be obtained by comparing crystal structures of apo-LC8 and LC8 complexes [20]. Ligand binding is coupled to a slight opening of the grooves and in this way LC8 can accommodate to different interaction patterns [20]. Finally our detailed kinetic studies on ligand binding of LC8 with different partners also provided evidence of conformational changes required for complex formation. The biphasic binding transients, observed by using a stopped-flow method, can best be described by a conformational selection model [36].

The affinity of LC8 to several monomeric binding peptides was determined and found to be moderately weak (K_d between 0.1 and 40 μM) (Table 1). However, most identified LC8 partners are known or predicted to be dimeric (Tables 1 and 2). These interacting proteins, as bivalent ligands, can form dimer-to-dimer complexes with LC8. Bivalent interactions are known to produce significant gains in binding affinity, specificity and functionality due to the avidity effect [37]. Indeed, compared with the monomeric peptides, a two to three order of magnitude increase in the apparent affinity was measured with bivalent DIC, MYO5A and an artificially dimerized target peptide of LC8 [15,26,29]. DIC is a poly-bivalent scaffold for binding of the three classes of dimeric dynein light chains; the implication of poly-bivalency on dynein function will be discussed later. Interaction partners that contain tandem LC8 binding motifs (p53BP1, Nup159, GKAP, Bassoon, U19, ATMIN; Table 1) are also poly-bivalent ligands. Hitherto only a few 3D structures of dimer-to-dimer LC complexes have been published: a short DIC fragment containing the binding sites for LC8 and Tctex1 in complex with these two light chains [15,29], and LC8 in complex with an artificially dimerized binding motif of EML3 [26].

The dimerization constant of *Drosophila* LC8 was reported to be moderately weak [38]; however, a more careful measurement showed higher affinity ($K_d \sim$ 200 nM) indicating that under cellular conditions the LC8 pool is mostly dimeric [22]. Nevertheless, at low pH (due to protonation of His55 at the dimerization interface [38–41]) or by phosphorylation of Ser88, the dimers readily dissociate to stable monomers [42,43]. The structure of the monomers, which are unable to bind target proteins, has also been determined [39,41]. As expected, tight binders shift the apparent equilibrium back towards the dimers and hence form complexes even with the Ser88Glu phosphomimetic mutants [24,36]. Such a monomer–dimer transition could have regulatory roles in the interaction network of LC8. Originally, LC8 was identified as a substrate of Pak1 and LC8 phosphorylation on Ser88 was implicated in cancer development, metastasis and triggering macropinocytosis [44,45]; however, recent studies ruled out that Pak1 is able to phosphorylate LC8 [22,36]. Consequently protein kinases involved in the potential dimer–monomer regulatory switch have not yet been identified. Instead of being a substrate, LC8 seems to be involved in the nuclear translocation of Pak1 [46] a function shared with several other LC8 partners (see below). Alternative ways of regulating LC8–target complexes could be the phosphorylation of LC8 binding motif or LC8 binding groove residues. Indeed, binding of the pro-apoptotic Bim is abolished upon JNK-kinase phosphorylation of its LC8 binding motif [47]. Phosphorylation of the LC8 residue Tyr65 by a hitherto unknown kinase [48] could also interfere with target binding.

What are the structural consequences of LC8 binding to the target proteins? Characterization of LC8 complexes indicated that LC8 binding facilitates the folding and increases the α-helical content of DIC, Swallow, MYO5A and synthaphilin [32,33,49–51]. The stabilized coiled-coils could provide additional binding platforms in various complexes (Fig. 2A). This structure/folding promoting 'chaperon-like' activity is consistent with the high percentage of potential coiled-coil forming sequences near the LC8 binding motifs (Tables 1 and 2) and could be one of the major

Table 1. DYNLL/LC8 interaction partners with verified binding motifs.

Protein name	Organism	Uniprot	Paralog/ ortholog	Sequence	First residue	K_d (μM)	PDB	Disorder	CC/dimer	Reference
Adenain (ADE41)	Adenovirus	P11826	DYNLL1	CITLVKST**Q**TV	104			–	–	[110]
AIBC1 (BCAS1)	Human, rat	O75363/Q3ZB98	DYNLL1	KRMLDAQV**Q**TD	563			D, I	C	[31,111,112]
ATMIN[a]	Human	O43313	DYNLL1	LESLDIET**Q**TD	665	1.7		D, I	–	[b]
p54 (E183L)	ASF virus	Q4TWM2	DYNLL1	VTTQNTAS**Q**TM	139			D, I	–	[28,113]
Bassoon (Bsn)	Rat	O88778	DYNLL1/2	PTTANYGS**Q**TE	1423			D, I	C	[70]
				SPMVAQGT**Q**TP	1527					
				RATAEFST**Q**TP	1499					
BimEL (BCL2L11)	Human	O43521	DYNLL1	PMSCDKST**Q**TP	107	0.8	1F95	D, I[c]	–	[13,31,58]
Bmf	Human	Q96LC9	DYNLL2	TSQEDKAT**Q**TL	63	0.7 0.004[d]		D, I	–	[12,36,114]
BS69 (ZMYND11)	Human	Q15326	DYNLL1	PRMLHRST**Q**TT	408			D, I	C	[31]
DIC (sw)	Fruit fly	Q24246	ddlc1	TLVYTKQT**Q**TT	125	8 0.2[d]	2P2T, 3FM7, 2PG1	D, I[c]	C[c]	[14,29,51]
DIC1 (Dyncli1)	Mouse	O88485	DYNLL1	VVSYSKET**Q**TP	146			D, I	C[c]	[31,58]
DIC2 (Dyncli2)	Rat	Q62871	DYNLL1	IVTYTKET**Q**TP	153			D, I	C	[99]
DNMT3A	Human	Q9Y6K1	DYNLL1	LVLKDLGI**Q**VD	648			–	–	[12,62]
Egalitarian	Fruit fly	P92030	ddlc1	VKLVDAES**Q**TL	947			D, I	C	[11]
EML3	Human	Q32P44	DYNLL1/2	PSLVSRGT**Q**TE	78	0.1 0.05[d]	2XQQ, 3P8M	D, I	C	[26]
Gephyrin (Gphn)	Rat	Q03555	DYNLL1/2	KQTEDKGV**Q**CE	216			D, I	C	[12,28,58,85,87]
GKAP (DLGAP1)	Human, rat	O14490/P97836	DYNLL2	NRCLSIGI**Q**VD	672	2.4		D, I	C	[12,58,88,89]
				SKFQSVGV**Q**VE	647			D, I		
Grinl1A (GCOM1)	Human	P0CAP1	DYNLL1	TEVETREIGVG	423			D, I	C	[28]
E4	Papilloma virus	P06425	DYNLL1	DHHQDKQT**Q**TP	18			D, I	–	[110]
Hsc73 (Hspa8)	Rat	P63018	DYNLL1	TTIPTKQT**Q**TF	418			–	C	[62]
KID-1 (Znf354a)	Rat	Q02975	DYNLL1	SHRTTKST**Q**TQ	94			D, I	–	[12,62]
MAP4	Human	P27816	DYNLL1	SRSGSKST**Q**TV	797			D, I	–	[12]
Mark3	Rat	Q8VHF0	DYNLL1	VVAYPKRS**Q**TS	429			D, I	–	[62]
METT-10	*Caenorhabditis elegans*	Q09357	dlc-1	LNAWDNAS**Q**AY	416			D	E	[73]
Myosin Va (MYO5A)	Human	Q9Y4I1	DYNLL2	QPKDDKNTMTD	1281	8.8 0.04[d]		D, I[c]	C[c]	[32,33,36]
NEK9	Human	Q8TD19	DYNLL1	VGMHSKGT**Q**TA	940			D, I	C	[26,61,115]
nNOS (NOS1)	Human	P29475	DYNLL1	AEMKDTGI**Q**VD	226	7	1F96, 1CMI	D, I[c]	E	[12,36,58,116]
NRF1	Human	Q16656	DYNLL1	MEEHGVT**Q**TE	1			D, I	C	[58,117]

Table 1. (Continued).

Protein name	Organism	Uniprot	Paralog/ ortholog	Sequence	First residue	K_d (μM)	PDB	Disorder	CC/dimer	Reference
NUP159	Yeast	P40477	DYN2	SASADFDV**Q**TS	1102			D, I	C	[71]
				DNYAESGI**Q**TD	1115			D, I		
				VETCNFSV**Q**TF	1164			D, I		
				IPVKHNST**Q**TV	1140			D, I		
				KEAVDNGL**Q**TE	1152			D, I		
p53BP1 (TP53BP1)	Human	Q12888	DYNLL1	PSQNNIGI**Q**TM	1147	4.5		D, I	C	[10,61]
				ETVVSAAT**Q**TI	1164			D, I		
PAK1	Human	Q13153	DYNLL1/2	TPTRDVATSPI	212	42	3DVT	D, I[c]	E	[22,36,44]
P protein	Rabies virus	P15198	DYNLL1	RSSEDKST**Q**TT	142			D, I	–	[12,118,119]
P protein	Mokola virus	O56780	DYNLL1	KSTEDKST**Q**TP	139			D, I	C	[12,119]
RACK1 (GNB2L1)	Human	P63244	DYNLL1	LGVCKYTV**Q**DE	135			–	–	[120]
RASGRP3	Human	Q8IV61	DYNLL1	RATTSQAT**Q**TE	607			D, I	–	[121]
U19	HHV-7	Q69501	DYNLL1	TILVSRST**Q**TG	225			D, I	C	[110]
				LGHFTRST**Q**TS	237					
UL9	HHV-1	P10193	DYNLL1	GVQMAKST**Q**TF	744			D, I	C	[110]
Spice1	Mouse	Q8K3I7	DYNLL1	QDVLRRTV**Q**TR	555			D	C	[62]
Swallow	Fruit fly	P40688	ddlc1	SATSAKAT**Q**TD	286	1	3E2B	D	C[c]	[12,14,16,67,68]
Syntaphilin (Snph)	Rat	B5DF41	DYNLL1	SCMQERAI**Q**TD	307			D	C[c]	[49]
TRPS1	Human	Q9UHF7	DYNLL1	TEKVDRST**Q**DE	1202			D	E	[61,92]
UNC-83	*C. elegans*	Q23064	dlc-1	DSISDRHI**Q**TM	643			D, I	C	[103]
VP35	Ebolavirus	Q05127	DYNLL1	PKTRNSQT**Q**TD	66			D, I	C	[122]

[a] ATMIN has at least five verified motifs. [b] Unpublished (P. Rapali, L. Nyitray and I. Rodriguez-Nacho). [c] Experimentally verified disordered or coiled-coil region. [d] Determined with a bivalent ligand. D, I, Binding motif in predicted disorder region determined by DISPROT and IUPRED [132,133]. C, Predicted coiled-coil by COILS [134]. E, Experimentally determined dimer, trimer or oligomer.

Table 2. DYNLL/LC8 interaction partners with unknown or predicted binding motifs. D, I, Binding motif in predicted disorder region determined by DISPROT and IUPRED [132,133]. C, Predicted coiled-coil by COILS [134]. E, Experimentally determined dimer, trimer or oligomer.

Protein name	Organism	Uniprot	Paralog/ortholog	Sequence	First residue	Disorder	CC/dimer	References
AMBRA1	Human	Q9C0C7	DYNLL1	TSVTSQGTQTL	1097	D, I	–	[26,61]
Astrin (SPAG5)	Human	Q96R06	DYNLL1/2	PETQDSSTQTD	467	D, I	C	[61,75]
C20orf117	Human	O94964	DYNLL1	VGLASVGTQTI	1135	D, I	C	[26,61]
p26CP	BIV	P19558	DYNLL	VQLIESKVREKENA	214	D, I	–	[123]
CIZ1	Human	Q9ULV3	DYNLL1	KLQKQAQTQTS	340	D, I	C	[61,72]
Dazl	Mouse	Q64368	DYNLL1	KKSVDRSIQTV	245	D	E	[9]
ERα (ESR1)	Human	P03372	DYNLL1			D[a]	E	[93]
Ewg	Fruit fly	Q24312	ddlc1	LSDVDYTTQTV	536	D, I	E	[117]
CHICA (FAM83D)	Human	Q9H4H8	DYNLL1	LSVSEVGTQTS	402	D, I	–	[26,61]
Gag	HFV	P14349	DYNLL1			D, I	–	[124]
Gag	Rice Sirevirus	Q109L4	LC8	IDVGISCDLLD	467	D	C	[125]
GLCCI1	Human	Q86VQ1	DYNLL1	SSTRSIDTQTP	343	D, I	C	[26,61]
gurken mRNA	Fruit fly		ddlc1	n/a		n/a	n/a	[102]
IκBα (NFKBIA)	Human	P25963	DYNLL1			D, I[a]	–	[81,82,117]
KIAA0802	Human	Q9Y4B5	DYNLL1	NGSRTMGTQTV	1622	D, I	C	[26,61]
KIBRA	Human	Q8IX03	DYNLL1	KQYLDVSSQTD	275	D	C	[94]
MORC3	Human	Q14149	DYNLL1	DQGNTAATQTE	633	D, I	C	[26,61]
NudE	Mouse	Q9CZA6	DYNLL1			D, I	C[a]	[104–106]
Phototropin	*Vicia*	Q8H934	LC8			–	–	[126]
PKIα	Human	P61925	DYNLL1			D, I[a]	–	[127]
PTH mRNA	Human		DYNLL1	n/a		n/a	n/a	[128]
RSP3	*Chlamy.s*	P12759	LC8	KYREDETTQTL	26	D, I	C	[129]
				LPADATQTQTK	58	D, I		
				VPEADTSTQTD	112	D, I		
Spn-F	Fruit fly	Q9V9Y9	ddlc1			–		[130,131]
TPR	Human	P12270	DYNLL1/2	TATVMPTTQVE	1714	D, I	C	[61,77]
UHRF1BP1L	Human	A0JNW5	DYNLL1	QRSVTQATQTS	1392	D, I	C	[26,61]
ZMYM3	Human	Q14202	DYNLL1	VEMKSKGSQTE	848	D, I	–	[26,61]

[a] Experimentally verified disordered or coiled-coil region.

physiological functions of LC8 [18,19]. Because of the mutual avidity effect, LC8 molecules inside the cell are expected to be bound to their numerous and (mostly) homodimeric partners rather than being in free, uncomplexed state. The molecular glue function of LC8 does not necessarily induce structural stabilization of the target protein; if LC binding causes spatial constraints in the target, it could lead to dissociation of pre-existing dimeric domains or destabilization of binding platforms preventing additional interactions, and/or could inhibit enzymatic or other activities of the partner proteins (Fig. 2B). LC8-induced dissociation of a binding platform has not been described yet. Besides stabilization or destabilization of homodimeric partners we envisage one situation in which an LC8 dimer can bind two different ligands and cause heterodimerization: if the two targets have an independent (even weak) interaction domain/motif near the LC8 binding motifs (Fig. 2C). Hitherto no such LC8 interaction or complex has been described.

The DYNLL/LC8 binding peptide is a short linear motif

LC8 binds to a loose consensus sequence, a short linear motif. Such motifs are usually localized in disordered segments and the implied plasticity and versatility in their molecular recognition is a clear advantage in eukaryotic interactomes [52–54]. These properties must also contribute to the promiscuous interaction network around LC8. The segment containing the binding motif in DIC was experimentally shown to be disordered by biochemical and NMR studies [34,51,55], and the same results was obtained with a MYO5A fragment [32]. Bim and Bmf, two LC8 binding pro-apoptotic proteins, are intrinsically disordered along almost their entire length [56]. Among the LC8 interactors, DISPROT database [57] also lists estrogen receptor α (ERα), IκBα and PKIα as proteins that have experimentally verified intrinsically disordered domains.

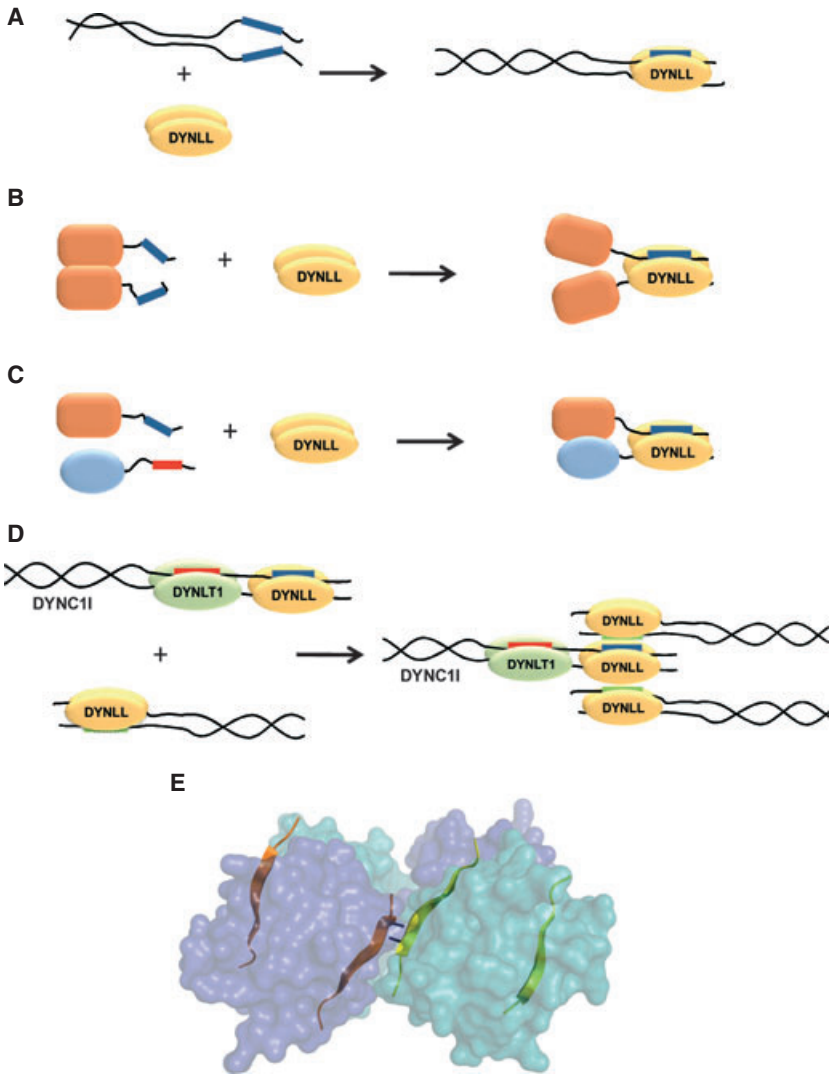

Fig. 2. (A) Possible interaction modes of DYNLL/LC8 with its targets. Interaction of LC8 with a partner that contains a potential or preformed coiled-coil domain near the LC8 motif could lead to homodimerization or coiled-coil stabilization. This is the only experimentally proved model of LC8 complex formation. The newly formed structure could act as a platform for further interactions. (B) If the LC8 binding motif is localized near interacting globular domains, LC8 binding could pry apart the domains by steric constraints and might destroy further interaction sites or inhibit other activities. (C) The same destabilizing effect may occur if the LC8 binding site is located within a coiled-coil domain (not shown). Heterodimerization of two targets could occur if two LC8 binding motifs are located near two weakly interacting domains. (D) Heterodimeric coiled-coils could also form by this mechanism (not shown). LC8 could function as a direct cargo adapter on dynein if one assumes that two homodimeric LC8–target complexes interact via their ligands. (E) Such an interaction between two ligand-bound LC8 complexes via antiparallel β-strands of the ligands has been observed hitherto only as a crystal contact in the crystal structure of the LC8–EML3 complex. Subunits of LC8 are colored as in Fig. 1; the interacting EML3 peptide ligands are brown and green (PDB 3P8M). There are additional although less likely theoretical interaction modes that are not depicted here.

Close inspection of the most recent list of validated binding peptides (Table 1) indicates that several motifs represent a mix of residues from the previously described two motif classes (see above). It suggests that even though the two putative motif types show some distinctive thermodynamic differences [14,36] their physiological significance is rather questionable. A sequence logo visualization of all known binding motifs (50 in 41 proteins) shows that the most frequently occurring and therefore likely key binding determinants of the motif are the most conserved Gln_0, the flanking Thr+1 and Thr−1 residues and Asp−4 (Fig. 1C). We depict 11 residues both on the logo and in Tables 1 and 2 since the binding site on

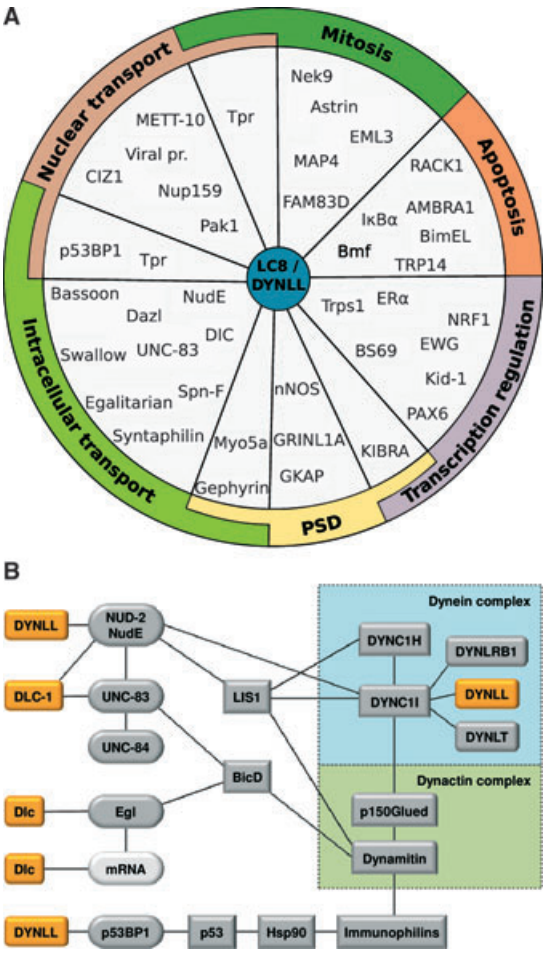

Fig. 3. (A) Partial interaction networks of selected DYNLL/LC8 binding partners. Functional categories that involve at least five LC8/DYNLL interactors are shown with different colors. More details of the interactions, including a full reference list, is found in Tables 1 and 2. (B) Egalitarian (Egl, in *Drosophila*), NudE (in mammals)/NUD-2 (in *Caenorhabditis elegans*), UNC-83 (in *C. elegans*) were originally suggested to function as dynein regulatory proteins associated to the motor complex via LC8 bound to the DIC (DYNC1I). An alternative scenario is shown here. The LC8 orthologs do not directly link the above proteins to the motor complex; instead the dimerizing/stabilizing effect of LC8 allows the scaffold proteins to bind to different adaptors/regulators (e.g. NudE to LIS1), to dynein subunits (NudE to DIC) or to the dynactin complex (Egl and UNC-83 to the dynamitin subunit of dynactin) via separate domains/linear motifs. UNC-84 is a membrane protein attaching the dynein motor to the nucleus for transport in *C. elegans* in complex with DLC-1/UNC-83/Bicaudal D and the *C. elegans* ortholog of Egl. The Dlc/Egl/BicD complex is responsible for effective mRNA transport in *Drosophila*; Egl is a direct mRNA binding protein [101]; Dlc may also bind specifically *gurken* mRNAs [102]. p53BP1 was shown to associate with the dynein motor complex in dynein-mediated p53 translocation. Based on experimental results the cargo adapter for p53 is not LC8 but the Hsp90–immunophilin complex that is associated to dynactin. Only experimentally verified physical interactions are shown.

LC8 could accommodate up to 11 residues. Nevertheless, only seven residues form the core binding motif as noted earlier [58]. The above mentioned four residues were found critical for binding in mutational studies, structure comparisons and a semi-quantitative pepscan analysis [8,22,58]. In Table 1 we also list all affinity values (measured with monovalent and/or bivalent peptide ligands) that have been determined hitherto. The only clear correlation between binding strength and sequence is that lack of Gln0 decreases the overall affinity of the peptide. A recent high-throughput (HTP) *in silico* analysis of the contribution of key residues and their context to the global binding energy of all known eukaryotic linear motifs including the LC8 recognition motif also highlighted the importance of the central three residues plus Lys−3 (instead of Asp−4) as key binding determinants for LC8 binding. The so-called 'contextual residues' of short linear motifs, in general and in LC8 complexes as well, seem to contribute more to the binding specificity than to the binding affinity of the interaction [59]. The same tendency was observed in a pepscan analysis of LC8 binding motifs [58].

Recently, we have conducted the first comprehensive and quantitative experimental analysis of the binding motif preference of LC8 using a directed evolution approach, phage display. The *in vitro* evolved and thermodynamically selected pattern (from $\sim 10^9$ sequences) resembles the natural one except at positions −4 and −5. Directed evolution identified position −5 as a significant contributor to the binding energy. A monovalent form of the consensus peptide (based on 25 selected individual sequences) binds to LC8 with a K_d of 0.08 μM (which is an affinity an order of magnitude higher than the previously known tightest binding Bmf peptide) and in a bivalent format with sub-nanomolar dissociation constant. Interestingly, the selected consensus is present in EML3, a human microtubule binding protein involved in mitosis. The crystal structure of the LC8–EML3 peptide revealed how the affinity-enhancing Val_{-5} is accommodated in a shallow binding pocket on LC8 [26].

DYNLL/LC8 as a hub protein: an ever-increasing interaction network

A thorough literature mining revealed 66 proteins and two mRNAs that are reported to interact with LC8 with relatively high confidence (Tables 1 and 2). In 42 of these proteins 55 LC8 binding motifs were identified and verified by small-scale experimental methods (Table 1). The very recently identified LC8 interacting protein ATMIN has at least five binding motifs. However, they have not been unequivocally assigned to the

potential 11 consensus-like motifs (P. Rapali, L. Nyitray and I. Rodriguez-Nacho, unpublished results), and therefore only one representative site is shown in Table 1. In the rest of the LC8 partners the binding motif is either unknown (eight proteins, e.g. ERα, IκBα, NudE) or was predicted by us based on the consensus motif. We did not include the more than 400 additional proteins that were deposited in protein–protein interaction databases (available through the INTACT database [60]). Either these interactions were collected by low confidence HTP methods or the experiments did not prove that the interaction is direct, i.e. binary. We made exceptions only with eight proteins that were identified as LC8 binding partners by an innovative HTP approach providing low false-negative and false-positive detection rates [61], because these were also identified by our very recent high-confidence *in vitro* evolution based prediction method [26]. The latter prediction revealed at least 100 additional novel LC8 binding partners in the human proteome (not included here). Each contains a binding sequence in the intrinsically disordered region of the putative interactor [26]. Further studies are needed to verify that these are indeed genuine components of the LC8 interaction hub.

The known binding motifs are almost exclusively (94%) located within disordered regions of the LC8 partner protein, as judged by two independent disorder predictors. In Table 2 only those predicted motifs are shown that are located in intrinsically disordered regions of the partner protein. The majority of the LC8 partner proteins contain coiled-coil predicted sequences in close proximity to the known or putative binding motif (78%) or it was shown experimentally that they form dimers or higher oligomers (in nine proteins). The above two characteristics are intimately associated with the LC8 interaction network as previously proposed based on a much smaller set of LC8 binding proteins [18,19].

There are only two reported exceptions in which the LC8 binding linear motif is more probably located in an ordered domain of the interactor. Hsc73 was identified in rat brain lysate by a low-throughput proteomic method [62]. The KXTQT-type binding motif is located in a well-defined 3D structure; the surface exposed β-strand might fit into the binding pocket of LC8 [63]. RACK1 was identified as an LC8 target by the yeast two-hybrid method. It forms a ternary complex with LC8 and BimEL upon apoptotic stimulation [64] and the putative LC8 binding motif is located in a modeled β-propeller WD domain of RACK1 [65]. DNMT3A, a DNA methyltransferase, is the only LC8 partner in our list that we now consider as false-positive. It was identified as an LC8 partner from rat brain by affinity chromatography and by pepscan analysis [12,62]; however, the crystal structure of the enzyme [66] shows that the binding motif is located within a globular domain and it is highly unlikely that this sequence would be accessible for LC8 interaction.

What are the functions of the LC8 binding partners and what could they tell us about the cellular role of LC8? Apparently, LC8 is involved in a wide variety of functions. Nevertheless, its already identified binding partners appear to represent only a few, often overlapping, essential cell functions and/or protein complexes (Fig. 3A). In the following paragraphs we select a few examples from these clusters and describe their functions and the possible roles of LC8 in the complexes.

Intracellular transport

Dynein and LC8 were proposed to be involved in targeting the Swallow protein and the *bicoid* mRNA in *Drosophila* oocytes [67]. Intensive studies on the structural aspects of the LC8–Swallow interaction revealed that it is unlikely that LC8 directly links the Swallow–mRNA complex to the dynein motor complex [14,68]. Very recent results ruled out the role of Swallow in *bicoid* mRNA transport; instead, it was found to be localized to the plasma membrane, where it functions indirectly in *bicoid* mRNA anchoring [69].

Syntaphilin is targeted to axonal mitochondria and to microtubules as well. A model was proposed in which LC8 serves as the 'stabilizer' of a coiled-coil structure in syntaphilin for facilitating its docking/anchoring to a mitochondrial receptor. Such a physical coupling between LC8 and syntaphilin may control mitochondrial mobility and density in axons and at synapses [49].

Drosophila Dazl is an RNA-binding protein essential for gametogenesis. It was proposed that Dazl travels along the microtubule network in association with the dynein complex and controls the subcellular distribution of a specific set of mRNAs [9].

Bassoon is an LC8 interactor linking the complex to retrograde transport of Golgi-derived vesicles in neurons. It was convincingly shown that Bassoon and LC8 are co-transported by the dynein complex [70]; however, it is still not clear how Bassoon is associated with the motor complex.

Nuclear transport

The yeast LC8 ortholog Dyn2 dimerizes and stabilizes the Nup82–Nsp1–Nup159 complex, a module of the nuclear pore filaments. Dyn2 binds to five tandem motifs located between a disordered Phe-Gly repeat and a coiled-coil domain of Nup159 forming a rigid

'beads-on-a-string' structure. Dyn2 could play a role in organizing the disordered Phe-Gly repeats within the NPC scaffold to facilitate nucleocytoplasmic transport [71].

LC8 interacts with the replication factor Ciz1 [61,72]. In a proposed model LC8 brings Ciz1 to the nucleus, where it binds Cdk2 and p21. These complexes may play a regulatory role in cell cycle progression of cancer cells [72].

METT-10 is a *C. elegans* nuclear protein, a putative methyltransferase that acts to inhibit germ cell proliferation. Interaction of METT-10 with LC8 promotes its nuclear accumulation [73].

ATMIN is an ATM-interacting protein the association of which with LC8 might prevent nuclear accumulation of ATMIN or regulate its association with other nuclear proteins involved in detecting DNA damage (P. Rapali, L. Nyitray and I. Rodriguez-Nacho, unpublished results).

Mitosis

NEK9 is a pleiotropic regulator of mitotic progression, participating in the control of spindle dynamics and chromosome separation [31,74].

LC8 binding probably stabilizes the dimeric Astrin which in complex with SKAP is targeted to bioriented kinetochores [75].

EML3 could have a role in correct metaphase chromosome alignment [76]. The above three LC8 partners were found associated with LC8 in a HTP screen to characterize chromosome segregation protein complexes and were also predicted to have LC8 binding motifs based on our *in vitro* evolution assay [26].

The nucleoporin Tpr functions during mitosis as a spatiotemporal regulator of spindle checkpoints and it is involved in recruitment of checkpoint proteins to dynein [77].

Apoptosis/autophagy

BimL and Bmf are BH3-only pro-apoptotic proteins thought to be normally sequestered to dynein and MYO5A motor complexes via DYNLL1 and DYNLL2, respectively [13,78]. Specific apoptotic stimuli liberate them from the cytoskeleton, in complex with the respective LC8 isoforms, allowing them to translocate to Bcl-2 proteins thereby activating apoptosis. Surprisingly, it was found that the *in vivo* target specificity of the two highly similar LC8 isoforms is determined by a single surface residue (Tyr41 in DYNLL1 and His41 in DYNLL2) [21]. *In vitro* the two LC8 isoforms bind the targets with the same affinity. The molecular surface around the 'specificity residue' might make contacts with other components of their respective motor or cytoskeletal complexes. The contribution of additional binding motifs of the intrinsically disordered Bim and Bmf [56] in their specific localization cannot be ruled out either.

AMBRA1 is a component of a multiprotein complex that regulates autophagy and development of the nervous system in mammals [79].

The *Drosophila* ortholog of LC8 is required for the regulation of autophagy and cell death; however, its interaction partner(s) has not been identified yet [80].

Binding of DYNLL1 inhibits TNFα-induced NFκB activation by interacting with IκBα, thereby preventing its phosphorylation by IκBα kinase, its nuclear translocation and its regulatory role in apoptosis [81,82]. Very interestingly this interaction is redox regulated: TNFα induces the production of reactive oxygen species, which in turn oxidize DYNLL1 (on Cys2 which is an isoform-specific residue) resulting in the dissociation of the complex and NFκB activation. A novel disulfide reductase, TRP14, contributes to the NFκB inhibitory activity by maintaining LC8 in its reduced state [82,83].

Postsynaptic density (PSD)

PSD is a dynamic complex crucial for receptor immobilization at both excitatory and inhibitory synapses. Scaffold proteins constitute one major group of proteins present at the PSD and two of them are LC8 binding partners.

Gephyrin is critical for glycine- and GABA-receptor clustering and also interacts with many other proteins, including several cytoskeletal components [84]. LC8 binds to a disordered linker domain between two globular dimerization/oligomerization domains [85,86]. It was shown that the gephyrin–LC8 complex together with the Gly-receptor is involved in transport processes by the dynein complex [87].

GKAP is an important scaffold molecule involved in the assembly of a multiprotein complex at excitatory synapses. Only the DYNLL2 paralog was identified as an interactor of GKAP *in vivo*, and it was suggested that this interaction is involved in recruiting nNOS to the PSD [88] and in the trafficking of PSD-95/GKAP complex by the MYO5A motor [89]. An alternative model could be that these three LC8 interactors bind independently to PSD components and to the motor protein; nNOS is indeed able to interact with PSD-95 [90]. However, the interaction domain or motif responsible for GKAP/PSD-95 binding to the myosin motor still needs to be identified.

Transcription regulation

BS69, identified by a yeast two-hybrid screen [31], is a multifunctional scaffold protein involved in transcription repression in association with various transcription factors in cellular senescence through the p53–p21 and JNK pathways [91].

LC8 interacts with the transcription factor TRPS1 and suppresses its transcriptional repression activity [92].

LC8 binds to the N-terminal disordered domain of ERα and facilitates its nuclear accumulation. In the nucleus the recruitment of the LC8–ERα complex to the chromatin of estrogen-receptor-targeted genes is assisted by the LC8–KIBRA–histon H3 complex [93,94].

The last category of functionally organized LC8 partners is of viral proteins which are discussed in the accompanying minireview [95].

Role of DYNLL/LC8 on the dynein motor complex

Originally it was suggested that LC8 acts as a cargo adapter that links the cargo to the motor complex. However, DIC and Swallow, a cargo protein, occupy the very same binding grooves in their LC8 complexes. Therefore it is unlikely that LC8 could bind to DIC and a potential cargo simultaneously [14–16]. The cargo adapter function of LC8 on MYO5A is also questionable for the same reason [32,36]. So what is the function of LC8? Based on recent results, LC8 together with the other two classes of light chains is involved in regulating dynein complex assembly and hence indirectly affecting cargo binding. Detailed studies of Barbar and colleagues [51,55] have shown that LC8 promotes self-association of the disordered N-terminal domain of DIC to form a coiled-coil or other interchain helical structure. Extension of the binding studies to the other LCs showed that LC8-induced DIC dimerization enhances the binding affinity of the dimeric DYNLT and the resulting complex will be rather stable [15,29]. On the other hand, binding of DYNLRB to the preformed complex does not cause further binding enhancement; instead it reverses local self-association [96]. The assembled DIC is an elongated poly-bivalent scaffold. Its flexibility is modulated by long-range coupling between DIC dimerization and the binding of light chains and other components of the motor complex including the heavy chain and $p150^{Glued}$ subunit of dynactin [97]. Poly-bivalency of DIC is a very important property of the complex; it provides high stability with associated flexibility since it also retains some disorder. Nyarko *et al*. proposed that many of the LC8 binding partners could also be poly-bivalent scaffold proteins [98].

An ingenious *in vivo* molecular trap was designed by Varma *et al*., based on a chemically inducible dimeric LC8 binding peptide from DIC. It was shown that LC8 occupancy of the motor complex directly affects some, but not all, dynein-mediated processes. The results suggest that LC8 (and also Tctex for which a similar trap was made) are essential for minus-end-directed lysosomal and endosomal transport [99].

The ultimate question remains: how are proteins shown to be co-localized to and/or co-transported by dynein recruited to the motor complex if not via LC8? We speculate that they bind directly or indirectly to the genuine adaptor dynactin or dynein subunit (dynamitin, $p150^{glued}$, intermediate or the light intermediate chains), i.e. LC8 is not a cargo adapter as suggested originally and the co-localization of two LC8–partner pairs in the complex (LC8–DIC and LC8–cargo) is merely coincidental (Fig. 3B). However, even if the two complexes are interdependent, stabilization of both LC8–partner complexes is a requirement for effective cargo transport. Figure 3B illustrates a few interaction networks that explain previous findings without assuming a direct cargo bridging role of LC8 on the dynein complex. We also speculate that the direct cargo adapter function of LC8, though thermodynamically not favored, cannot be fully ruled out; since the LC8 ligand extends an existing β-sheet it is conceivable that this sheet is further extended by a β-strand coming from an LC8 motif of a cargo bound to another LC8 dimer as depicted in Fig. 2D. Such an antiparallel dimer-to-dimer interaction, between two identical LC8 ligands, was observed in a crystal structure of the LC8–EML3 complex [26] (Fig. 2E); however, there is no experimental evidence yet of any ligand–ligand interaction between the LC8–DIC complex and any of a putative LC8–cargo complex.

Bicaudal-D (BicD) and Egalitarian (Egl) function together with dynein to regulate cargo transport during *Drosophila* development and it was proposed that the Egl–BicD complex is loaded through LC8 onto the motor complex [11]. Since mammalian BicD is able to bind to the dynactin complex [100], it is more likely that LC8 has only a dimerizing/stabilizing role on Egl which in turn is able to provide a scaffold for simultaneous binding of BicD and of developmentally important mRNAs (including *gurken*) recruited for cytoplasmic sorting by the dynein motor [101]. Whether the *Drosophila* LC8 ortholog binds directly the *gurken* transcript [102] or is linked to the motor

through Egl awaits further investigations. We envisage a similar scenario in the case of the *C. elegans* UNC-83 and NUD-2 scaffold proteins that are involved in dynein-powered nuclear migration and interact with LC8 [103]; LC8 assists dimerization/stabilization of the scaffolds that in turn are able to recruit the motor to nuclear membrane via UNC-84. Mammalian NudE together with LIS1 has important functions in nuclear and centrosomal transport in migrating neurons powered by dynein [61,104,105]. LC8-stabilized NudE interacts directly with DIC and recruits LIS1 to regulate the dynein motor activity [106]. Finally, we propose the same explanation for the role of LC8–p53BP1 interactions [10] in dynein-mediated p53 translocation. p53BP1 is a large multidomain scaffold protein, an activator of p53-dependent gene transcription that is thought to be responsible for recruiting/assembling various proteins in the ATM/ATR and Rad3-related signaling pathways [107].

Perspective

LC8 is an essential hub protein of eukaryotic metazoan cells that probably interacts with hundreds of proteins, but as yet almost nothing is known about the spatiotemporal dynamics of its interaction network. Based on the dimerizing effect of LC8 on its partners and the ensuing avidity it is reasonable to assume that most LC8 complexes are quite stable (K_d in the nanomolar range) in the cell. A large number of LC8 interaction partners are large multidomain/modular proteins targeting and regulating many proteins in complex ways, i.e. they are scaffold proteins according to the definition in [108]. Poly-bivalency of the scaffold, as observed with DIC [98], could be another general property of many LC8 interactors. LC8 acts on the scaffolds as a 'molecular velcro' holding two chains in close proximity. This can facilitate formation of additional binding platforms and promote assembly and functioning of molecular machines such as motor protein complexes or the postsynaptic density. LC8 could also be involved in specific sequestration of certain proteins to the cytoskeleton or to other cellular structures. Interestingly, LC8 appears to be preferentially involved in certain cellular functions such as intracellular and nuclear transport, mitosis, presynaptic and postsynaptic processes, and transcription regulation.

In conclusion we foresee that a large number of additional LC8 binding interactions will be described in the near future. It remains to be seen how such an extended interaction network is regulated and how LC8 'selects' and recognizes its targets in a cellular environment where many potential interactors are simultaneously available for binding. The future of LC8 studies will certainly bring surprises but we hope they will also bring more understanding of the intricacy of this intriguing small protein which is equally a 'party hub' functioning inside preformed complexes and a 'date hub' connecting diverse biological modules [109].

Acknowledgements

This work was supported by the Hungarian Scientific Research Fund (OTKA) K81784, NK81950 (L.N.) and K68408 (G.P.), as well as by the European Union and the European Social Fund (TÁMOP) 4.2.1./B-09/KMR-2010-0003. G.P. is supported by a János Bolyai Research Fellowship.

References

1 Pfister KK, Shah PR, Hummerich H, Russ A, Cotton J, Annuar AA, King SM & Fisher EMC (2006) Genetic analysis of the cytoplasmic dynein subunit families. *PLoS Genet* **2**, e1.
2 Wickstead B & Gull K (2007) Dyneins across eukaryotes: a comparative genomic analysis. *Traffic* **8**, 1708–1721.
3 Sakakibara H & Oiwa K (2011) Molecular organization and force-generating mechanism of dynein. *FEBS J* **278**, 2964–2979.
4 Pfister KK, Fay RB & Witman GB (1982) Purification and polypeptide composition of dynein ATPases from Chlamydomonas flagella. *Cell Motil* **2**, 525–547.
5 Dick T, Ray K, Salz HK & Chia W (1996) Cytoplasmic dynein (ddlc1) mutations cause morphogenetic defects and apoptotic cell death in *Drosophila melanogaster*. *Mol Cell Biol* **16**, 1966–1977.
6 Kamath RS, Fraser AG, Dong Y, Poulin G, Durbin R, Gotta M, Kanapin A, Le BotN, Moreno S, Sohrmann M *et al.* (2003) Systematic functional analysis of the *Caenorhabditis elegans* genome using RNAi. *Nature* **421**, 231–237.
7 Espindola FS, Suter DM, Partata LB, Cao T, Wolenski JS, Cheney RE, King SM & Mooseker MS (2000) The light chain composition of chicken brain myosin-Va: calmodulin, myosin-II essential light chains, and 8-kDa dynein light chain/PIN. *Cell Motil Cytoskeleton* **47**, 269–281.
8 Fan J, Zhang Q, Tochio H, Li M & Zhang M (2001) Structural basis of diverse sequence-dependent target recognition by the 8 kDa dynein light chain. *J Mol Biol* **306**, 97–108.
9 Lee KH, Lee S, Kim B, Chang S, Kim SW, Paick JS & Rhee K (2006) Dazl can bind to dynein motor com-

plex and may play a role in transport of specific mRNAs. *EMBO J* **25**, 4263–4270.
10 Lo KW, Kan HM, Chan LN, Xu WG, Wang KP, Wu Z, Sheng M & Zhang M (2005) The 8-kDa dynein light chain binds to p53-binding protein 1 and mediates DNA damage-induced p53 nuclear accumulation. *J Biol Chem* **280**, 8172–8179.
11 Navarro C, Puthalakath H, Adams JM, Strasser A & Lehmann R (2004) Egalitarian binds dynein light chain to establish oocyte polarity and maintain oocyte fate. *Nat Cell Biol* **6**, 427–435.
12 Rodriguez-Crespo I, Yelamos B, Roncal F, Albar JP, Ortiz de Montellano PR & Gavilanes F (2001) Identification of novel cellular proteins that bind to the LC8 dynein light chain using a pepscan technique. *FEBS Lett* **503**, 135–141.
13 Puthalakath H, Huang DC, O'Reilly LA, King SM & Strasser A (1999) The proapoptotic activity of the Bcl-2 family member Bim is regulated by interaction with the dynein motor complex. *Mol Cell* **3**, 287–296.
14 Benison G, Karplus PA & Barbar E (2007) Structure and dynamics of LC8 complexes with KXTQT-motif peptides: swallow and dynein intermediate chain compete for a common site. *J Mol Biol* **371**, 457–468.
15 Williams JC, Roulhac PL, Roy AG, Vallee RB, Fitzgerald MC & Hendrickson WA (2007) Structural and thermodynamic characterization of a cytoplasmic dynein light chain–intermediate chain complex. *Proc Natl Acad Sci USA* **104**, 10028–10033.
16 Hall J, Hall A, Pursifull N & Barbar E (2008) Differences in dynamic structure of LC8 monomer, dimer, and dimer–peptide complexes. *Biochemistry* **47**, 11940–11952.
17 Wu H, Maciejewski MW, Takebe S & King SM (2005) Solution structure of the Tctex1 dimer reveals a mechanism for dynein–cargo interactions. *Structure* **13**, 213–223.
18 Barbar E (2008) Dynein light chain LC8 is a dimerization hub essential in diverse protein networks. *Biochemistry* **47**, 503–508.
19 Hodi Z, Rapali P, Radnai L, Molnár T, Szenes A, Kardos J, Buday L, Stafford WF & Nyitray L (2007) The LC8 family of dynein light chains: multifunctional chaperon-like proteins. *FEBS J* **274**, 106.
20 Benison G, Karplus PA & Barbar E (2008) The interplay of ligand binding and quaternary structure in the diverse interactions of dynein light chain LC8. *J Mol Biol* **384**, 954–966.
21 Day CL, Puthalakath H, Skea G, Strasser A, Barsukov I, Lian LY, Huang DC & Hinds MG (2004) Localization of dynein light chains 1 and 2 and their pro-apoptotic ligands. *Biochem J* **377**, 597–605.
22 Lightcap CM, Sun S, Lear JD, Rodeck U, Polenova T & Williams JC (2008) Biochemical and structural characterization of the Pak1–LC8 interaction. *J Biol Chem* **283**, 27314–27324.
23 Vedadi M, Lew J, Artz J, Amani M, Zhao Y, Dong A, Wasney GA, Gao M, Hills T, Brokx S *et al.* (2007) Genome-scale protein expression and structural biology of Plasmodium falciparum and related Apicomplexan organisms. *Mol Biochem Parasitol* **151**, 100–110.
24 Benison G, Chiodo M, Karplus PA & Barbar E (2009) Structural, thermodynamic, and kinetic effects of a phosphomimetic mutation in dynein light chain LC8. *Biochemistry* **48**, 11381–11389.
25 Liang J, Jaffrey SR, Guo W, Snyder SH & Clardy J (1999) Structure of the PIN/LC8 dimer with a bound peptide. *Nat Struct Biol* **6**, 735–740.
26 Rapali P, Radnai L, Suveges D, Harmat V, Tolgyesi F, Wahlgren WY, Katona G, Nyitray L & Pal G (2011) Directed evolution reveals the binding motif preference of the LC8/DYNLL hub protein and predicts large numbers of novel binders in the human proteome. *PLoS ONE* **6**, e18818.
27 Garcia-Mayoral MF, Martinez-Moreno M, Albar JP, Rodriguez-Crespo I & Bruix M (2010) Structural basis for the interaction between dynein light chain 1 and the glutamate channel homolog GRINL1A. *FEBS J* **277**, 2340–2350.
28 Garcia-Mayoral MF, Rodriguez-Crespo I & Bruix M (2010) Structural models of DYNLL1 with interacting partners: African swine fever virus protein p54 and postsynaptic scaffolding protein gephyrin. *FEBS Lett* **585**, 53–57.
29 Hall J, Karplus PA & Barbar E (2009) Multivalency in the assembly of intrinsically disordered dynein intermediate chain. *J Biol Chem* **284**, 33115–33121.
30 Williams JC, Xie H & Hendrickson WA (2005) Crystal structure of dynein light chain TcTex-1. *J Biol Chem* **280**, 21981–21986.
31 Lo KW, Naisbitt S, Fan JS, Sheng M & Zhang M (2001) The 8-kDa dynein light chain binds to its targets via a conserved (K/R)XTQT motif. *J Biol Chem* **276**, 14059–14066.
32 Hodi Z, Nemeth AL, Radnai L, Hetenyi C, Schlett K, Bodor A, Perczel A & Nyitray L (2006) Alternatively spliced exon B of myosin Va is essential for binding the tail-associated light chain shared by dynein. *Biochemistry* **45**, 12582–12595.
33 Wagner W, Fodor E, Ginsburg A & Hammer JA III (2006) The binding of DYNLL2 to myosin Va requires alternatively spliced exon B and stabilizes a portion of the myosin's coiled-coil domain. *Biochemistry* **45**, 11564–11577.
34 Benison G & Barbar E (2009) NMR analysis of dynein light chain dimerization and interactions with diverse ligands. *Methods Enzymol* **455**, 237–258.
35 Fan JS, Zhang Q, Tochio H & Zhang M (2002) Backbone dynamics of the 8 kDa dynein light chain dimer

reveals molecular basis of the protein's functional diversity. *J Biomol NMR* **23**, 103–114.
36 Radnai L, Rapali P, Hodi Z, Suveges D, Molnar T, Kiss B, Becsi B, Erdodi F, Buday L, Kardos J *et al.* (2010) Affinity, avidity, and kinetics of target sequence binding to LC8 dynein light chain isoforms. *J Biol Chem* **285**, 38649–38657.
37 Mammen M, Choi SK & Whitesides GM (1998) Polyvalent interactions in biological systems: implications for design and use of multivalent ligands and inhibitors. *Angew Chem Int Ed* **37**, 2754–2794.
38 Barbar E, Kleinman B, Imhoff D, Li M, Hays TS & Hare M (2001) Dimerization and folding of LC8, a highly conserved light chain of cytoplasmic dynein. *Biochemistry* **40**, 1596–1605.
39 Makokha M, Huang YJ, Montelione G, Edison AS & Barbar E (2004) The solution structure of the pH-induced monomer of dynein light-chain LC8 from Drosophila. *Protein Sci* **13**, 727–734.
40 Nyarko A, Cochrun L, Norwood S, Pursifull N, Voth A & Barbar E (2005) Ionization of His 55 at the dimer interface of dynein light-chain LC8 is coupled to dimer dissociation. *Biochemistry* **44**, 14248–14255.
41 Wang W, Lo KW, Kan HM, Fan JS & Zhang M (2003) Structure of the monomeric 8-kDa dynein light chain and mechanism of the domain-swapped dimer assembly. *J Biol Chem* **278**, 41491–41499.
42 Song C, Wen W, Rayala SK, Chen M, Ma J, Zhang M & Kumar R (2008) Serine 88 phosphorylation of the 8-kDa dynein light chain 1 is a molecular switch for its dimerization status and functions. *J Biol Chem* **283**, 4004–4013.
43 Song Y, Benison G, Nyarko A, Hays TS & Barbar E (2007) Potential role for phosphorylation in differential regulation of the assembly of dynein light chains. *J Biol Chem* **282**, 17272–17279.
44 Vadlamudi RK, Bagheri-Yarmand R, Yang Z, Balasenthil S, Nguyen D, Sahin AA, den Hollander P & Kumar R (2004) Dynein light chain 1, a p21-activated kinase 1-interacting substrate, promotes cancerous phenotypes. *Cancer Cell* **5**, 575–585.
45 Yang Z, Vadlamudi RK & Kumar R (2005) Dynein light chain 1 phosphorylation controls macropinocytosis. *J Biol Chem* **280**, 654–659.
46 Lightcap CM, Kari G, Arias-Romero LE, Chernoff J, Rodeck U & Williams JC (2009) Interaction with LC8 is required for Pak1 nuclear import and is indispensable for zebrafish development. *PLoS ONE* **4**, e6025.
47 Lei K & Davis RJ (2003) JNK phosphorylation of Bim-related members of the Bcl2 family induces Bax-dependent apoptosis. *PNAS* **100**, 2432–2437.
48 Guo A, Villen J, Kornhauser J, Lee KA, Stokes MP, Rikova K, Possemato A, Nardone J, Innocenti G, Wetzel R *et al.* (2008) Signaling networks assembled by oncogenic EGFR and c-Met. *Proc Natl Acad Sci USA* **105**, 692–697.
49 Chen YM, Gerwin C & Sheng ZH (2009) Dynein light chain LC8 regulates syntaphilin-mediated mitochondrial docking in axons. *J Neurosci* **29**, 9429–9438.
50 Makokha M, Hare M, Li M, Hays T & Barbar E (2002) Interactions of cytoplasmic dynein light chains Tctex-1 and LC8 with the intermediate chain IC74. *Biochemistry* **41**, 4302–4311.
51 Nyarko A, Hare M, Hays TS & Barbar E (2004) The intermediate chain of cytoplasmic dynein is partially disordered and gains structure upon binding to light-chain LC8. *Biochemistry* **43**, 15595–15603.
52 Tompa P (2005) The interplay between structure and function in intrinsically unstructured proteins. *FEBS Lett* **579**, 3346–3354.
53 Dunker AK, Silman I, Uversky VN & Sussman JL (2008) Function and structure of inherently disordered proteins. *Curr Opin Struct Biol* **18**, 756–764.
54 Fuxreiter M, Tompa P & Simon I (2007) Local structural disorder imparts plasticity on linear motifs. *Bioinformatics* **23**, 950–956.
55 Benison G, Nyarko A & Barbar E (2006) Heteronuclear NMR identifies a nascent helix in intrinsically disordered dynein intermediate chain: implications for folding and dimerization. *J Mol Biol* **362**, 1082–1093.
56 Hinds MG, Smits C, Fredericks-Short R, Risk JM, Bailey M, Huang DC & Day CL (2007) Bim, Bad and Bmf: intrinsically unstructured BH3-only proteins that undergo a localized conformational change upon binding to prosurvival Bcl-2 targets. *Cell Death Differ* **14**, 128–136.
57 Sickmeier M, Hamilton JA, LeGall T, Vacic V, Cortese MS, Tantos A, Szabo B, Tompa P, Chen J, Uversky VN *et al.* (2007) DisProt: the database of disordered proteins. *Nucleic Acids Res* **35**, D786–D793.
58 Lajoix AD, Gross R, Aknin C, Dietz S, Granier C & Laune D (2004) Cellulose membrane supported peptide arrays for deciphering protein–protein interaction sites: the case of PIN, a protein with multiple natural partners. *Mol Divers* **8**, 281–290.
59 Stein A & Aloy P (2008) Contextual specificity in peptide-mediated protein interactions. *PLoS ONE* **3**, e2524.
60 Aranda B, Achuthan P, Alam-Faruque Y, Armean I, Bridge A, Derow C, Feuermann M, Ghanbarian AT, Kerrien S, Khadake J *et al.* (2010) The IntAct molecular interaction database in 2010. *Nucleic Acids Res* **38**, D525–D531.
61 Hutchins JR, Toyoda Y, Hegemann B, Poser I, Heriche JK, Sykora MM, Augsburg M, Hudecz O, Buschhorn BA, Bulkescher J *et al.* (2010) Systematic analysis of human protein complexes identifies chromosome segregation proteins. *Science* **328**, 593–599.
62 Navarro-Lerida I, Martinez Moreno M, Roncal F, Gavilanes F, Albar JP & Rodriguez-Crespo I (2004)

Proteomic identification of brain proteins that interact with dynein light chain LC8. *Proteomics* **4**, 339–346.
63 Morshauser RC, Hu W, Wang H, Pang Y, Flynn GC & Zuiderweg ER (1999) High-resolution solution structure of the 18 kDa substrate-binding domain of the mammalian chaperone protein Hsc70. *J Mol Biol* **289**, 1387–1403.
64 Zhang W, Cheng GZ, Gong J, Hermanto U, Zong CS, Chan J, Cheng JQ & Wang LH (2008) RACK1 and CIS mediate the degradation of BimEL in cancer cells. *J Biol Chem* **283**, 16416–16426.
65 McCahill A, Warwicker J, Bolger GB, Houslay MD & Yarwood SJ (2002) The RACK1 scaffold protein: a dynamic cog in cell response mechanisms. *Mol Pharmacol* **62**, 1261–1273.
66 Jia D, Jurkowska RZ, Zhang X, Jeltsch A & Cheng X (2007) Structure of Dnmt3a bound to Dnmt3L suggests a model for de novo DNA methylation. *Nature* **449**, 248–251.
67 Schnorrer F, Bohmann K & Nusslein-Volhard C (2000) The molecular motor dynein is involved in targeting swallow and bicoid RNA to the anterior pole of Drosophila oocytes. *Nat Cell Biol* **2**, 185–190.
68 Wang L, Hare M, Hays TS & Barbar E (2004) Dynein light chain LC8 promotes assembly of the coiled-coil domain of swallow protein. *Biochemistry* **43**, 4611–4620.
69 Weil TT, Xanthakis D, Parton R, Dobbie I, Rabouille C, Gavis ER & Davis I (2010) Distinguishing direct from indirect roles for bicoid mRNA localization factors. *Development* **137**, 169–176.
70 Fejtova A, Davydova D, Bischof F, Lazarevic V, Altrock WD, Romorini S, Schone C, Zuschratter W, Kreutz MR, Garner CC *et al.* (2009) Dynein light chain regulates axonal trafficking and synaptic levels of Bassoon. *J Cell Biol* **185**, 341–355.
71 Stelter P, Kunze R, Flemming D, Hopfner D, Diepholz M, Philippsen P, Bottcher B & Hurt E (2007) Molecular basis for the functional interaction of dynein light chain with the nuclear-pore complex. *Nat Cell Biol* **9**, 788–796.
72 den Hollander P & Kumar R (2006) Dynein light chain 1 contributes to cell cycle progression by increasing cyclin-dependent kinase 2 activity in estrogen-stimulated cells. *Cancer Res* **66**, 5941–5949.
73 Dorsett M & Schedl T (2009) A role for dynein in the inhibition of germ cell proliferative fate. *Mol Cell Biol* **29**, 6128–6139.
74 O'Regan L, Blot J & Fry AM (2007) Mitotic regulation by NIMA-related kinases. *Cell Div* **2**, 25.
75 Schmidt JC, Kiyomitsu T, Hori T, Backer CB, Fukagawa T & Cheeseman IM (2010) Aurora B kinase controls the targeting of the Astrin–SKAP complex to bioriented kinetochores. *J Cell Biol* **191**, 269–280.
76 Tegha-Dunghu J, Neumann B, Reber S, Krause R, Erfle H, Walter T, Held M, Rogers P, Hupfeld K, Ruppert T *et al.* (2008) EML3 is a nuclear microtubule-binding protein required for the correct alignment of chromosomes in metaphase. *J Cell Sci* **121**, 1718–1726.
77 Nakano H, Funasaka T, Hashizume C & Wong RW (2010) Nucleoporin translocated promoter region (Tpr) associates with dynein complex, preventing chromosome lagging formation during mitosis. *J Biol Chem* **285**, 10841–10849.
78 Puthalakath H, Villunger A, O'Reilly LA, Beaumont JG, Coultas L, Cheney RE, Huang DC & Strasser A (2001) Bmf: a proapoptotic BH3-only protein regulated by interaction with the myosin V actin motor complex, activated by anoikis. *Science* **293**, 1829–1832.
79 Fimia GM, Stoykova A, Romagnoli A, Giunta L, Di Bartolomeo S, Nardacci R, Corazzari M, Fuoco C, Ucar A, Schwartz P *et al.* (2007) Ambra1 regulates autophagy and development of the nervous system. *Nature* **447**, 1121–1125.
80 Batlevi Y, Martin DN, Pandey UB, Simon CR, Powers CM, Taylor JP & Baehrecke EH (2010) Dynein light chain 1 is required for autophagy, protein clearance, and cell death in Drosophila. *Proc Natl Acad Sci USA* **107**, 742–747.
81 Crepieux P, Kwon H, Leclerc N, Spencer W, Richard S, Lin R & Hiscott J (1997) I kappaB alpha physically interacts with a cytoskeleton-associated protein through its signal response domain. *Mol Cell Biol* **17**, 7375–7385.
82 Jung Y, Kim H, Min SH, Rhee SG & Jeong W (2008) Dynein light chain LC8 negatively regulates NF-kappaB through the redox-dependent interaction with IkappaBalpha. *J Biol Chem* **283**, 23863–23871.
83 Jeong W, Chang TS, Boja ES, Fales HM & Rhee SG (2004) Roles of TRP14, a thioredoxin-related protein in tumor necrosis factor-alpha signaling pathways. *J Biol Chem* **279**, 3151–3159.
84 Tyagarajan SK & Fritschy JM (2010) GABA(A) receptors, gephyrin and homeostatic synaptic plasticity. *J Physiol* **588**, 101–106.
85 Fuhrmann JC, Kins S, Rostaing P, El Far O, Kirsch J, Sheng M, Triller A, Betz H & Kneussel M (2002) Gephyrin interacts with dynein light chains 1 and 2, components of motor protein complexes. *J Neurosci* **22**, 5393–5402.
86 Kim EY, Schrader N, Smolinsky B, Bedet C, Vannier C, Schwarz G & Schindelin H (2006) Deciphering the structural framework of glycine receptor anchoring by gephyrin. *EMBO J* **25**, 1385–1395.
87 Maas C, Tagnaouti N, Loebrich S, Behrend B, Lappe-Siefke C & Kneussel M (2006) Neuronal cotransport of glycine receptor and the scaffold protein gephyrin. *J Cell Biol* **172**, 441–451.

88 Haraguchi K, Satoh K, Yanai H, Hamada F, Kawabuchi M & Akiyama T (2000) The hDLG-associated protein DAP interacts with dynein light chain and neuronal nitric oxide synthase. *Genes Cells* **5**, 905–911.
89 Naisbitt S, Valtschanoff J, Allison DW, Sala C, Kim E, Craig AM, Weinberg RJ & Sheng M (2000) Interaction of the postsynaptic density-95/guanylate kinase domain-associated protein complex with a light chain of myosin-V and dynein. *J Neurosci* **20**, 4524–4534.
90 Boeckers TM (2006) The postsynaptic density. *Cell Tissue Res* **326**, 409–422.
91 Zhang W, Chan HM, Gao Y, Poon R & Wu Z (2007) BS69 is involved in cellular senescence through the p53–p21Cip1 pathway. *EMBO Rep* **8**, 952–958.
92 Kaiser FJ, Tavassoli K, Van den Bemd GJ, Chang GT, Horsthemke B, Moroy T & Ludecke HJ (2003) Nuclear interaction of the dynein light chain LC8a with the TRPS1 transcription factor suppresses the transcriptional repression activity of TRPS1. *Hum Mol Genet* **12**, 1349–1358.
93 Rayala SK, den Hollander P, Balasenthil S, Yang Z, Broaddus RR & Kumar R (2005) Functional regulation of oestrogen receptor pathway by the dynein light chain 1. *EMBO Rep* **6**, 538–544.
94 Rayala SK, den Hollander P, Manavathi B, Talukder AH, Song C, Peng S, Barnekow A, Kremerskothen J & Kumar R (2006) Essential role of KIBRA in co-activator function of dynein light chain 1 in mammalian cells. *J Biol Chem* **281**, 19092–19099.
95 Merino-Gracia J, García-Mayoral MF & Rodriguez-Crespo I (2011) The association of viral proteins with host cell dynein components during virus infection. *FEBS J* **278**, 2997–3011.
96 Nyarko A & Barbar E (2011) Light chain-dependent self-association of dynein intermediate chain. *J Biol Chem* **286**, 1556–1566.
97 King SJ, Brown CL, Maier KC, Quintyne NJ & Schroer TA (2003) Analysis of the dynein–dynactin interaction *in vitro* and *in vivo*. *Mol Biol Cell* **14**, 5089–5097.
98 Nyarko A & Barbar E (2010) Light chain-dependent self-association of dynein intermediate chain. *J Biol Chem* **286**, 1556–1566.
99 Varma D, Dawn A, Ghosh-Roy A, Weil SJ, Ori-McKenney KM, Zhao Y, Keen J, Vallee RB & Williams JC (2010) Development and application of *in vivo* molecular traps reveals that dynein light chain occupancy differentially affects dynein-mediated processes. *Proc Natl Acad Sci USA* **107**, 3493–3498.
100 Hoogenraad CC, Akhmanova A, Howell SA, Dortland BR, De Zeeuw CI, Willemsen R, Visser P, Grosveld F & Galjart N (2001) Mammalian Golgi-associated Bicaudal-D2 functions in the dynein–dynactin pathway by interacting with these complexes. *EMBO J* **20**, 4041–4054.
101 Dienstbier M, Boehl F, Li X & Bullock SL (2009) Egalitarian is a selective RNA-binding protein linking mRNA localization signals to the dynein motor. *Genes Dev* **23**, 1546–1558.
102 Rom I, Faicevici A, Almog O & Neuman-Silberberg FS (2007) Drosophila dynein light chain (DDLC1) binds to gurken mRNA and is required for its localization. *Biochim Biophys Acta* **1773**, 1526–1533.
103 Fridolfsson HN, Ly N, Meyerzon M & Starr DA (2010) UNC-83 coordinates kinesin-1 and dynein activities at the nuclear envelope during nuclear migration. *Dev Biol* **338**, 237–250.
104 Feng Y, Olson EC, Stukenberg PT, Flanagan LA, Kirschner MW & Walsh CA (2000) LIS1 regulates CNS lamination by interacting with mNudE, a central component of the centrosome. *Neuron* **28**, 665–679.
105 Stehman SA, Chen Y, McKenney RJ & Vallee RB (2007) NudE and NudEL are required for mitotic progression and are involved in dynein recruitment to kinetochores. *J Cell Biol* **178**, 583–594.
106 Kardon JR & Vale RD (2009) Regulators of the cytoplasmic dynein motor. *Nat Rev Mol Cell Biol* **10**, 854–865.
107 Galigniana MD, Harrell JM, O'Hagen HM, Ljungman M & Pratt WB (2004) Hsp90-binding immunophilins link p53 to dynein during p53 transport to the nucleus. *J Biol Chem* **279**, 22483–22489.
108 Buday L & Tompa P (2010) Functional classification of scaffold proteins and related molecules. *FEBS J* **277**, 4348–4355.
109 Han JD, Bertin N, Hao T, Goldberg DS, Berriz GF, Zhang LV, Dupuy D, Walhout AJ, Cusick ME, Roth FP *et al.* (2004) Evidence for dynamically organized modularity in the yeast protein–protein interaction network. *Nature* **430**, 88–93.
110 Martinez-Moreno M, Navarro-Lerida I, Roncal F, Albar JP, Alonso C, Gavilanes F & Rodriguez-Crespo I (2003) Recognition of novel viral sequences that associate with the dynein light chain LC8 identified through a pepscan technique. *FEBS Lett* **544**, 262–267.
111 Ewing RM, Chu P, Elisma F, Li H, Taylor P, Climie S, McBroom-Cerajewski L, Robinson MD, O'Connor L, Li M *et al.* (2007) Large-scale mapping of human protein-protein interactions by mass spectrometry. *Mol Syst Biol* **3**, 89.
112 Nomura M, Inaba K & Morisawa M (2000) Cyclic AMP- and calmodulin-dependent phosphorylation of 21 and 26 kDa proteins in axoneme is a prerequisite for SAAF-induced motile activation in ascidian spermatozoa. *Dev Growth Differ* **42**, 129–138.
113 Alonso C, Miskin J, Hernaez B, Fernandez-Zapatero P, Soto L, Canto C, Rodriguez-Crespo I, Dixon L & Escribano JM (2001) African swine fever virus protein p54 interacts with the microtubular motor complex

through direct binding to light-chain dynein. *J Virol* **75**, 9819–9827.
114 Puthalakath H, Strasser A & Huang DC (2001) Rapid selection against truncation mutants in yeast reverse two-hybrid screens. *BioTechniques* **30**, 984–988.
115 Regue L, Sdelci S, Bertran MT, Caelles C, Reverter D & Roig J (2011) DYNLL/LC8 controls signal transduction through the Nek9/Nek6 signaling module by regulating Nek6 binding to Nek9. *J Biol Chem* **286**, 18118–18129.
116 Fan JS, Zhang Q, Li M, Tochio H, Yamazaki T, Shimizu M & Zhang M (1998) Protein inhibitor of neuronal nitric-oxide synthase, PIN, binds to a 17-amino acid residue fragment of the enzyme. *J Biol Chem* **273**, 33472–33481.
117 Herzig RP, Andersson U & Scarpulla RC (2000) Dynein light chain interacts with NRF-1 and EWG, structurally and functionally related transcription factors from humans and drosophila. *J Cell Sci* **113** (Pt 23), 4263–4273.
118 Raux H, Flamand A & Blondel D (2000) Interaction of the rabies virus P protein with the LC8 dynein light chain. *J Virol* **74**, 10212–10216.
119 Jacob Y, Badrane H, Ceccaldi PE & Tordo N (2000) Cytoplasmic dynein LC8 interacts with lyssavirus phosphoprotein. *J Virol* **74**, 10217–10222.
120 Zhang J, Wang Q, Zhu N, Yu M, Shen B, Xiang J & Lin A (2008) Cyclic AMP inhibits JNK activation by CREB-mediated induction of c-FLIP(L) and MKP-1, thereby antagonizing UV-induced apoptosis. *Cell Death Differ* **15**, 1654–1662.
121 Okamura SM, Oki-Idouchi CE & Lorenzo PS (2006) The exchange factor and diacylglycerol receptor RasGRP3 interacts with dynein light chain 1 through its C-terminal domain. *J Biol Chem* **281**, 36132–36139.
122 Kubota T, Matsuoka M, Chang TH, Bray M, Jones S, Tashiro M, Kato A & Ozato K (2009) Ebolavirus VP35 interacts with the cytoplasmic dynein light chain 8. *J Virol* **83**, 6952–6956.
123 Su Y, Qiao W, Guo T, Tan J, Li Z, Chen Y, Li X, Li Y, Zhou J & Chen Q (2010) Microtubule-dependent retrograde transport of bovine immunodeficiency virus. *Cell Microbiol* **12**, 1098–1107.
124 Petit C, Giron ML, Tobaly-Tapiero J, Bittoun P, Real E, Jacob Y, Tordo N, De The H & Saib A (2003) Targeting of incoming retroviral Gag to the centrosome involves a direct interaction with the dynein light chain 8. *J Cell Sci* **116**, 3433–3442.
125 Havecker ER, Gao X & Voytas DF (2005) The Sireviruses, a plant-specific lineage of the Ty1/copia retrotransposons, interact with a family of proteins related to dynein light chain 8. *Plant Physiol* **139**, 857–868.
126 Emi T, Kinoshita T, Sakamoto K, Mineyuki Y & Shimazaki K (2005) Isolation of a protein interacting with Vfphot1a in guard cells of Vicia faba. *Plant Physiol* **138**, 1615–1626.
127 Yu J, Yu L, Chen Z, Zheng L, Chen X, Wang X, Ren D & Zhao S (2002) Protein inhibitor of neuronal nitric oxide synthase interacts with protein kinase A inhibitors. *Brain Res Mol Brain Res* **99**, 145–149.
128 Epstein E, Sela-Brown A, Ringel I, Kilav R, King SM, Benashski SE, Yisraeli JK, Silver J & Naveh-Many T (2000) Dynein light chain binding to a 3′-untranslated sequence mediates parathyroid hormone mRNA association with microtubules. *J Clin Invest* **105**, 505–512.
129 Yang P, Diener DR, Rosenbaum JL & Sale WS (2001) Localization of calmodulin and dynein light chain LC8 in flagellar radial spokes. *J Cell Biol* **153**, 1315–1326.
130 Abdu U, Bar D & Schupbach T (2006) spn-F encodes a novel protein that affects oocyte patterning and bristle morphology in Drosophila. *Development* **133**, 1477–1484.
131 Giot L, Bader JS, Brouwer C, Chaudhuri A, Kuang B, Li Y, Hao YL, Ooi CE, Godwin B, Vitols E *et al.* (2003) A protein interaction map of *Drosophila melanogaster*. *Science* **302**, 1727–1736.
132 Dosztanyi Z, Csizmok V, Tompa P & Simon I (2005) IUPred: web server for the prediction of intrinsically unstructured regions of proteins based on estimated energy content. *Bioinformatics* **21**, 3433–3434.
133 Obradovic Z, Peng K, Vucetic S, Radivojac P & Dunker AK (2005) Exploiting heterogeneous sequence properties improves prediction of protein disorder. *Proteins* **61**(Suppl 7), 176–182.
134 Lupas A (1996) Prediction and analysis of coiled-coil structures. *Methods Enzymol* **266**, 513–525.
135 Crooks GE, Hon G, Chandonia JM & Brenner SE (2004) WebLogo: a sequence logo generator. *Genome Res* **14**, 1188–1190.