Potential Role for Phosphorylation in Differential Regulation of the Assembly of Dynein Light Chains*

Received for publication, November 9, 2006, and in revised form, March 2, 2007 Published, JBC Papers in Press, April 11, 2007, DOI 10.1074/jbc.M610445200

Yujuan Song‡, Gregory Benison‡, Afua Nyarko‡, Thomas S. Hays§, and Elasar Barbar†1
From the ‡Department of Biochemistry and Biophysics, Oregon State University, Corvallis, Oregon 97331 and §Department of Genetics, Cell Biology, and Development, University of Minnesota, Minneapolis, Minnesota 55455

The homodimeric light chains LC8 and Tctex-1 are integral parts of the microtubule motor cytoplasmic dynein, as they directly associate with dynein intermediate chain IC and various cellular cargoes. These light chains appear to regulate assembly of the dynein complex by binding to and promoting dimerization of IC. In addition, both LC8 and Tctex-1 play roles in signaling, apoptosis, and neuronal development that are independent of their function in dynein, but it is unclear how these various activities are modulated. Both light chains undergo specific phosphorylation, and here we present biochemical and NMR analyses of phosphomimetic mutants that indicate how phosphorylation may regulate light chain function. For both LC8 and Tctex-1, phosphorylation promotes dissociation from IC while retaining their binding activity with other non-dynein proteins. Although LC8 and Tctex-1 are homologs having a common fold, their reduced affinity for IC upon phosphorylation arises by different mechanisms. In the case of Tctex-1, phosphorylation directly masks the IC binding site at the dimer interface, whereas for LC8, phosphorylation dissociates the dimer and indirectly eliminates the binding site. This modulation of the monomer-dimer equilibrium by phosphorylation provides a novel mechanism for discrimination among LC8 binding partners.

Cytoplasmic dynein is a principal cellular motor responsible for minus end directed traffic along microtubules. It is a large multisubunit protein complex involved in a variety of processes, including mitotic spindle assembly and orientation, chromosome segregation, intracellular trafficking of vesicles and mRNA, and the establishment of cell polarity (reviewed in Ref. (1)). The heavy chain subunits of dynein provide ATPase and microtubule binding functions, whereas the intermediate and light chain subunits are thought to mediate binding and transport of a wide array of cargo.

LC82 and Tctex-1, two of the three light chain subunits of cytoplasmic dynein, tightly associate with, and increase the structure of, disordered dynein intermediate chain (IC) (2, 3). The association with either dimeric light chain is postulated to be essential for dimerization of the intermediate chain (4), although recent work has challenged this hypothesis (5). In the cell, significant amounts of LC8 and Tctex-1 are not incorporated into the dynein complex, raising the possibility that these proteins have additional roles independent of dynein (6, 7). Consistent with this hypothesis, several proteins unrelated to dynein bind LC8 and Tctex-1 (8–20). Many of these interactions are interpreted to indicate that these molecules are cargoes transported by dynein, and except for the Swallow protein which is required for the proper localization of bicoid mRNA (14), there is little evidence for their active transport along microtubules by dynein. The question remains as to how these ubiquitously expressed highly conserved light chains are regulated for multiple functional roles both as part of dynein and as part of other complexes.

Recent investigations point to a role for phosphorylation of LC8 in tumorigenesis (18, 21) and in macropinocytosis (22). LC8 is phosphorylated at serine 88 by p21-activated kinase 1, resulting in abolished interaction with apoptotic protein Bim and reduced apoptosis. Phosphorylated LC8 is elevated specifically in human breast cancer tumors. Tctex-1 is phosphorylated upon interaction with the cytoplasmic domain of bone morphogenetic receptor type II, a member of the transforming growth factor β cell signaling superfamily. Disruption of this interaction is associated with primary pulmonary hypertension disease (10). Tctex-1 can also be phosphorylated by protein kinase C in vitro and functions independently of dynein in actin remodeling during neurite outgrowth (20).

Regulation by phosphorylation is observed for the other cytoplasmic dynein light chain, the LC7/roadblock subunit, which functions both as a dynein light chain and as a transforming growth factor-β signaling intermediate (23, 24). LC7 (also known as kmi23) can be serine-phosphorylated following transforming growth factor-β receptor activation. Similar to LC8 and Tctex-1, the LC7 light chain is a dimer and shows diverse binding properties but is nevertheless structurally distinct (25, 26). Interestingly, and reciprocal to the effect of phosphorylation...
Differential Regulation by Phosphorylation

The NMR structure of rat LC8 dimer bound to the Bim peptide with the backbone carbonyl of Ser-88 demonstrates that both Bim and nNOS bind in essentially the same groove created at the dimer interface, even though they do not share the consensus KXQQT recognition sequence for binding LC8 (31). For Tctex-1, high resolution structures are available for only the apo protein (32, 33). Thr-92 is solvent-exposed and close to a water channel at the dimer interface (Fig. 1).

Phosphorylation of Ser-88 in LC8 has been proposed as a potential regulatory mechanism that inhibits interactions with Bim (21). However, the NMR structure of the LC8 dimer bound to Bim peptide (31) shows that Ser-88 is not in close contact with Bim. In the present study, our analyses of phospho-mimetic LC8 mutants provide evidence that phosphorylation disrupts LC8 dimer stability. We conclude that loss of Bim binding is a secondary consequence of dimer dissociation. From association states of S88E and binding studies to IC and Swallow, we propose that phosphorylation promotes dissociation of LC8 and selects for tightly bound ligands that shift the equilibrium toward dimer. In contrast to LC8, the phosphomimetic mutant of Tctex-1 retains its association state in the native dimer but nonetheless exhibits reduced binding to IC. In this case, we attribute the loss of IC binding to the perturbation of a specific binding site by phosphorylation of Thr-92 and suggest that Thr-92 is part of the IC binding site in Tctex-1.

EXPERIMENTAL PROCEDURES

Mutagenesis, Protein Expression, and Purification—Mutations of single or double amino acid(s) of LC8 and Tctex-1 were prepared by QuikChange Multisite-Directed Mutagenesis kit (Invitrogen). Deletion mutant LC8-(1–87) was made by introducing a stop codon at Ser-88. The mutant DNAs were verified by automated sequencing before transformation into BL21 (DE3) cells for protein expression.

The cDNA (GenBank™ accession number AF263371) encoding the full-length Drosophila cytoplasmic dynein intermediate chain was used as the template in PCR reactions to generate the IC-(92–237) that includes the binding site of LC8 and Tctex-1. GST-fused IC-(92–237) was overexpressed and purified using glutathione-Sepharose 4B affinity columns (Amersham Biosciences) following the protocol provided by the manufacturer. Preparation of a construct of Swallow, Swa-(206–297), fused to GST was described earlier (34).

Protein expression and purification of LC8 and Tctex-1 mutants were performed as described elsewhere (2). All proteins were further purified on a Superdex 75 size exclusion pre-
paratory column, and purity of >95% was confirmed on a 15% SDS-PAGE. The molecular mass of each protein was determined by mass spectrometry. For LC8 WT with the His tag fusion peptide, the measured mass by MALDI-TOF was 12444 (calculated mass 12445), for H55K 12437 (calculated 12436), for S88E 12487 (calculated 12487), for H55A/S88E 12423 (calculated 12421), and for LC8-(1–87) 12301 (calculated 12301).

Peptides corresponding to the LC8 binding site on Swallow and IC were commercially synthesized, and their identity and purity verified by MALDI-TOF. The peptides were Swa-(281–297), (MYHIRSATSAKATQTDF) and IC-(123–138) (KETLVYTKQTQTTSTG).

Binding Studies—Binding of GST-IC-(92–237) to LC8 and Tctex-1 WT and mutants was assayed following previously published protocols (2, 3). Band intensities were measured by densitometry using the ImageQuant software (GE Healthcare). Intensities of mutant bands were normalized by dividing the WT band intensity by the GST-IC-(92–237) band intensity and followed by subtracting the background (control lane with no proteins). All densitometry figures represent at least three independent experiments.

NMR Spectroscopy—NMR spectra were collected at 25 °C on a 600-MHz Bruker DRX spectrometer and referenced with internal 2,2-dimethyl-2-silapentanesulfonic acid at zero ppm. Sample concentrations were 0.1–1 mM for LC8 WT and mutants in 50 mM sodium phosphate, pH 6.5, or 50 mM citrate phosphate, pH 5.5, with 50 mM NaCl, 2 mM benzamidine hydrochloride, 10% D2O, 1 mM sodium azide, and a protease inhibitor mixture (Roche Applied Science). 1H-15N heteronuclear single quantum correlation, HSQC, experiments were recorded and analyzed as reported earlier (4).

Peptide binding was quantified by NMR by recording a series of 1H-15N HSQC spectra on 15N-labeled S88E samples at increasing concentrations of either Swa or IC. The S88E concentration was 0.2 mM for Swa titrations and 0.4 mM for IC titrations.

Data Analysis—Peak intensities were obtained from fits to standard two-dimensional Lorentzian profiles. Mole fraction of the monomer was determined by the equation $f_m = I_m/(I_m + I_d + I_c)$, where $I_m$, $I_d$, and $I_c$ are the intensities of the peaks corresponding to the monomer, dimer, and complex, respectively. Similar equations were used to obtain $f_d$ and $f_c$. Curves were calculated from the law of mass action as shown in Reaction 1

$$2M + 2L \rightleftharpoons D + 2L \rightleftharpoons DL_2$$

where $K = [M]^2/[D]$, and $K_1 = [DL][L]/[DL_2] = [D][L]/[DL]$.

RESULTS AND DISCUSSION

Phosphomimetic Mutation S88E Promotes Dissociation of Dimeric LC8—Drosophila LC8 Ser-88 was replaced with glutamic acid as a phosphorylation mimic by site-directed mutagenesis. The S88E mutation as well as Ser-88 phosphorylation causes loss of binding to Bim (21). The hydroxyl group of
Ser-88 appears to be involved in dimer-stabilizing contacts because it forms a hydrogen bond with the backbone carbonyl of Ser-88\', and vice versa.

Size exclusion chromatography experiments were performed on several phosphomimetic LC8 variants (Fig. 2) and on dimeric WT and monomeric H55K LC8, whose association states were previously established (28). S88E elutes at 26.0 min, similar to the monomeric control H55K, strongly suggesting that phosphorylation of Ser-88 promotes dissociation of the dimer. The LC8 double mutant H55A/S88E also elutes as a monomer, although the single mutant H55A is a dimer (28). The deletion mutant LC8-(1–87), which is similar to S88E in showing no detectable interaction with Bim (21), elutes at 25.7 min. This is slightly earlier than monomeric LC8, and the peak is considerably broader, suggesting a mixture of monomer and dimer.

Phosphomimetic Mutants of LC8 Diminish Assembly with IC—Dynein intermediate chain IC shares the same consensus LC8 recognition motif K\(X\)TQT (35) with the non-dynein protein Bim. The Swallow protein, another LC8 binding partner, also requires the same recognition motif for binding LC8 (14). The NMR structure of LC8 bound to Bim shows that Bim binds in the grooves created at the dimer interface. We have obtained crystal structures demonstrating that IC and Swallow also bind in the same groove. Therefore, if monomerization eliminates the quaternary binding groove, no LC8 monomers, such as H55K, should bind Swallow, IC, and Bim.

Binding of WT LC8 to IC and the lack of binding of H55K to IC were verified by isothermal titration calorimetry (data not shown). A construct of IC spanning residues 92 to 237 fused to GST, GST-IC-(92–237), was used for binding measurements because it includes the binding sites of both LC8 (residues 123–138) and Tctex-1-(95–116) (4). GST fusion permits direct comparison with experiments below involving GST pulldown assays. A stoichiometry of 1:1 and dissociation constant of 1.6 \(\mu\)M indicate tight binding, consistent with fluorescence binding measurements using a similar IC construct without the GST tag (3). A change of enthalpy of \(-16.85\) kcal/mol and entropy of \(-29.9\) cal/mol\(^\circ\)C indicate that binding is enthalpically driven and entropically unfavorable. In contrast, no binding of GST-IC-(92–237) was detected with H55K.

The effect of phosphomimetic mutations of LC8 on assembly with IC and Swallow were probed with GST pulldown assays (Fig. 3). Binding of GST-IC-(92–237) is abolished for monomeric H55K and significantly reduced for monomeric mutants S88E and H55A/S88E but with varying binding efficiencies. The deletion mutant LC8-(1–87), which is a mixture of monomer and dimer, shows binding affinity similar to WT (Fig. 3, A and B). Binding studies of LC8 mutants using GST-Swa-(206–297), on the other hand, show that binding is also abolished with monomeric H55K, but the phosphomimetic mutants S88E, S88E/H55A and LC8-(1–87) show binding efficiency similar to WT (Fig. 3C). Binding of Swa-(206–297) to primarily monomeric phosphomimetic mutants is explained by a mass action effect on the monomer-dimer equilibrium as described below.

NMR Evidence for Linkage of Binding and Dimerization—Our working hypothesis is that monomeric LC8 does not bind Swallow, IC, and Bim and that regulation by phosphorylation at position 88 is primarily due to the effect of phosphorylation on shifting the monomer-dimer equilibrium toward monomer. A monomer-dimer equilibrium in S88E, H55A/S88E, and LC8-(1–87) but not H55K explains the presence of some IC binding to S88E and H55A/S88E and WT levels of binding to LC8-(1–87). H55K is a monomer and not in equilibrium with dimer,

---

3 G. Benison, P. A. Karplus, and E. Barbar, submitted for publication.
whereas in the other variants monomers and dimers are in equilibrium and monomers are favored. When IC is bound to dimers of S88E, H55A/S88E, or LC8-(1–87), the equilibrium is shifted as the bound dimer removes free dimer from the monomer-dimer pool and ultimately depletes the monomer. With Swallow, tight binding shifts the equilibrium considerably to dimer, and binding efficiency is similar to WT.

Evidence for this hypothesis is shown by NMR spectroscopy on S88E. $^1$H-$^{15}$N HSQC spectra of S88E have peaks indicative of both monomer and dimer LC8 (28) such as those corresponding to residues 9, 57, 59, 63, 76, 82, and 84 (Fig. 4). An overlay of spectra of monomeric H55K and dimeric WT LC8 (Fig. 4A) shows peaks with chemical shifts similar to those in the S88E spectrum (Fig. 4B). The populations of monomer and dimer in S88E are protein concentration-dependent. Spectra of 10-fold diluted samples have considerably lower intensity of dimer peaks (Fig. 5A). Relative populations obtained from peak intensities such as 63d and 63m at 1 mM concentration are 40% (dimer), 60% (monomer) (Fig. 4B), whereas at 0.1 mM, the populations are 10% (dimer), 90% (monomer) (Fig. 5A). When a 17-amino acid peptide of IC corresponding to the LC8 binding site is added to primarily monomeric S88E, no obvious change in the spectrum is observed (Fig. 5B). In contrast, when a peptide of the Swallow protein, Swa, corresponding to the LC8 binding site (34) is added, a significant chemical shift perturbation is observed.
similar to that of dimeric WT LC8 bound to Swa.\textsuperscript{4} For example, peaks corresponding to residues 59 and 63 have chemical shifts in S88E bound to Swa (Fig. 5C) similar to those of dimeric WT LC8 bound to Swa (Fig. 5D). Few peaks corresponding to a minor monomer population are still observed (Fig. 5C).

These data clearly show that S88E is in a monomer-dimer equilibrium at high protein concentration with a dissociation constant ~1 mM. In addition, binding and dimerization are linked; a tighter binding ligand, such as Swallow, shifts the equilibrium considerably toward dimer. In contrast, binding of IC at the same protein concentration does not alter the NMR spectral characteristics of monomer. The NMR binding data are consistent with GST pulldown studies showing S88E, S88E/H55A, and LC8-(1–87) with binding efficiency similar to WT for Swallow but diminished binding efficiency for IC (Fig. 3).

Quantitative Studies of Binding—Chemical shift titration experiments were recorded to estimate binding affinities of IC and Swa peptides to S88E. Fig. 6 shows changes in peak intensities corresponding to populations of monomer (M), dimer (D), and complex (C) in slow exchange, at increasing concentration of either Swa or IC. With Swa, ligand binding is tight enough to cause disappearance of monomer in favor of the bound complex, whereas at the same equivalence of IC, the major population of S88E still corresponds to monomer (Fig. 6, C and G). A $K_d$ for monomer-dimer equilibrium ($K_1$) of 1.4 mM was estimated from both titrations (Fig. 6, D and H). The ligand binding equilibrium ($K_f$) was estimated to be 2 $\mu$M for Swa and 340 $\mu$M for IC (Table 1). The >100-fold higher affinity of Swa suggests that Swa will compete for LC8 binding even in the presence of higher concentration of IC and that phosphorylated LC8 is unlikely to bind IC at physiological concentrations.

We conclude that in LC8 phosphorylation serves to modulate binding rather than eliminate it, by perturbing the monomer-dimer equilibrium. Upon phosphorylation, binding is possible at high protein concentrations, where the dimer is significantly populated, or at low protein concentrations but with tightly bound ligands that shift the equilibrium toward dimer. The variation in binding efficiency among LC8 mutants is due to differences in the monomer-dimer dissociation constants. The double mutant H55A/S88E is doubly perturbed, resulting in an equilibrium more shifted to the monomer and consequently reduced binding to IC. In contrast, the deletion mutant is primarily dimeric, resulting in increased binding to IC. The non-detectable binding with H55K is due to the complete absence of dimer (Fig. 3).

Phosphomimetic Mutant Tctex-1(T92E) Dissociates from the Dynein Complex but Remains a Homodimer—When mammalian Tctex-1 is phosphorylated \textit{in vitro} by protein kinase C at Thr-94 (Thr-92 in \textit{Drosophila}), or when the phosphomimetic replacement by Glu is made, it does not bind IC and therefore is presumed not to incorporate into the dynein complex (20). We have reproduced these experiments with \textit{Drosophila} Tctex-1 using GST pulldown experiments and the phosphomimetic mutant Tctex-1(T92E). Fig. 7, A and B, shows binding of GST-IC-(92–237) with Tctex-1 WT, T92A, and T92E mutants. The binding of T92A (lane 4) is significantly reduced whereas the binding of T92A (lane 3) is similar to WT (lane 2). Lane 1 shows GST-IC-(92–237) in the absence of lysates. The molecular weight marker is shown in the far left, B, quantification of binding from band intensities based on an average of three binding assays using proteins from crude lysates. C, size exclusion chromatograms of Tctex-1 WT and T92E mutant. Experiments were performed under the same conditions as in Fig. 2. Both proteins elute at 21.8 min, indicating that they are dimers. However, the phosphomimetic mutation does not affect the homodimeric association state of Tctex-1, unlike LC8(S88E). Fig. 7C shows an overlay of size exclusion chromatograms of dimeric Tctex-1 WT and T92E, both eluting at 21.8 min. Because the OH group on Thr-92 in \textit{Drosophila} is not completely solvent-accessible, it is not surprising that its perturbation does not detectably affect the quaternary structure. The negative charge introduced in T92E interferes with direct binding to residues 110–116 (LSVYNYQ), the IC segment correspond-

\begin{table}
\centering
\caption{Quantification of monomer-dimer ($K_d$) and ligand binding ($K_f$) equilibria.}
\begin{tabular}{lll}
\hline
Peptide & $K_d$ & $K_f$ \\
\hline
Swa & $1.4 \pm 0.2$ & $2 \pm 1$ \\
IC & $1.4 \pm 0.2$ & $340 \pm 40$ \\
\hline
\end{tabular}
\end{table}
Differential Regulation by Phosphorylation

FIGURE 8. A scheme for differential regulation of LC8 and Tctex-1 by phosphorylation. LC8 is phosphorylated by p21-activated kinase 1 and Tctex-1 by PKC. LC8 (red) can be a dimer or monomer, but Tctex-1 (green) is a dimer under our experimental conditions. Both are associated with the dynein complex (gray background) through direct binding to IC. Upon phosphorylation (yellow spheres) at a site that perturbs its protein-protein interface, but is nonoverlapping with the IC binding groove, LC8 dissociates from dynein and forms an equilibrating mixture of phosphorylated monomer and a minor population of phosphorylated dimer. Dissociation abolishes the dimer interface groove to which IC and other LC8 partners interact, and so their binding is not detectable in an equilibrating mixture favoring the monomer. However, binding of phosphorylated LC8 to a ligand with higher affinity than IC, such as Swallow (real green), is detectable because ligand binding and dimerization are linked. In contrast, phosphorylated Tctex-1 although the IC binding site is perturbed the dimer interface is not, and Tctex-1 dissociates as a phosphorylated dimer. Phosphorylation of Tctex-1 also disrupts binding to any ligand (blue) that shares its IC binding site.

In summary, this work suggests that phosphorylation of Thr-92 directly masks the IC binding site but does not affect dimer dissociation and that Thr-92, which is at the dimer interface, is part of the IC binding surface.

CONCLUSIONS

To the extent that phosphomimetic mutants behave like phosphorylated forms of LC8 and Tctex-1, this study provides evidence that binding of these light chains to IC and other ligands can be differentially regulated by phosphorylation. Moreover, the implied impact of phosphorylation on LC8 provides strong evidence that, as proposed earlier (29), the monomer-dimer equilibrium regulates LC8 function. LC8 phosphorylation occurs at a site distant from the IC binding groove but nonetheless inhibits IC binding. We show that the LC8(S88E) mutant promotes dissociation of the dimer to a folded monomer; the monomer-dimer equilibrium is retained but is shifted to favor monomer. Dissociation of the dimer eliminates the requisite groove created at the dimer interface and thereby results in abolishing binding to IC and other LC8 partners that interact in that groove. Thus, ligand binding and dimerization are linked processes. Whereas the LC8(S88E) mutant is predominantly monomer in the presence of IC, Swallow protein binds tightly to dimer and depletes the monomer by a mass action effect. In this way, phosphorylation of LC8 inhibits IC binding but does not necessarily prevent binding of other ligands outside the dynein system. Other proteins may bind phosphorylated LC8 either by tight assembly with the dimer like Swallow, or possibly by interaction with the folded monomer at a different binding site. In contrast, Tctex-1 phosphorylation at Thr-92 perturbs the IC binding site directly. This offers the interesting possibility that phosphorylation of Tctex-1 regulates IC binding without affecting interaction of non-dynein ligands at other sites. We speculate that phosphorylation is one mechanism for dissociating either light chain from dynein and targeting them to specific ligands and functions, for LC8 by shifting the monomer-dimer equilibrium and for Tctex-1 by directly disrupting the IC binding site, as illustrated in Fig. 8.

Acknowledgments—We thank the members of the Barbar laboratory for critical reading of the manuscript, Dr. Mike Hare for valuable suggestions, and Professor Mike Schimerlik for help with data fitting. We acknowledge the support of the nucleic acid and protein core and the mass spectrometry core in the Oregon State University Environmental Health Sciences Center (NIEHS, National Institutes of Health 00210).

REFERENCES

1. Vallee, R. B., Williams, J. C., Varma, D., and Barnhart, L. E. (2004) J. Neurosci. 24, 1363–1372
2. Makokha, M., Hare, M., Li, M., Hays, T., and Barbar, E. (2002) Biochemistry 41, 4302–4311
3. Nyarko, A., Hare, M., Hays, T. S., and Barbar, E. (2004) Biochemistry 43, 15595–15603
4. Benison, G., Nyarko, A., and Barbar, E. (2006) J. Mol. Biol. 362, 1082–1093
5. Lo, K. W., Kan, H. M., and Pfister, K. K. (2006) J. Biol. Chem. 281, 9552–9559
6. King, S. M., Barbaresi, E., Dillman, J. F., Patelking, R. S., Carson, J. H., and Pfister, K. K. (1996) J. Biol. Chem. 271, 19358–19366
7. Li, M. G., Serr, M., Newman, E. A., and Hays, T. S. (2004) Mol. Biol. Cell 15, 3005–3014
8. Campbell, K. S., Cooper, S., Dessing, M., Yates, S., and Buder, A. (1998) J. Immunol. 161, 1728–1737
9. Mueller, S., Cao, X. M., Welker, R., and Wimmer, E. (2002) J. Biol. Chem. 277, 7897–7904
10. Machado, R. D., Rudarakanchana, N., Atkinsion, C., Flanagan, J. A., Harrison, R., Morrell, N. W., and Trembath, R. C. (2003) Hum. Mol. Genet. 12, 3277–3286
11. Nagano, F., Orita, S., Sasaki, T., Naito, A., Sakaguchi, G., Maeda, M., Watanabe, T., Kominami, E., Uchiyama, Y., and Takai, Y. (1998) J. Biol. Chem. 273, 30065–30068
12. Puthalakath, H., Huang, D. C., O’Reilly, L. A., King, S. M., and Strasser, A. (1999) Mol. Cell 3, 287–296
13. Raux, H., Flaman, A., and Blondel, D. (2000) J. Virol. 74, 10212–10216
14. Schnorrer, F., Bohmann, K., and Nusslein-Volhard, C. (2000) Nat. Cell Biol. 2, 185–190
15. Jaffrey, S. R., and Snyder, S. H. (1996) Science 274, 774–777
16. Lo, K. W., Kan, H. M., Chan, L. N., Xu, W. G., Wang, K. P., Wu, Z., Sheng, M., and Zhang, M. (2005) J. Biol. Chem. 280, 8172–8179
17. Kaiser, F. J., Tavassoli, K., Van den Bernd, G. J., Chang, G. T., Horstemke, B., Moroy, T., and Ludecke, H. J. (2003) Hum. Mol. Genet. 12, 1349–1358
18. Rayala, S. K., den Hollander, P., Balasenthil, S., Yang, Z., Broaddus, R. R., and Kumar, R. (2005) EMBO Rep. 6, 538–544
19. Tai, A. W., Chuang, J. Z., Bode, C., Wolfrum, U., and Sung, C. H. (1999) Cell 97, 877–887
20. Chuang, J. Z., Yeh, T. Y., Bollati, F., Conde, C., Canavosio, F., Caceres, A., and Sung, C. H. (2005) Dev. Cell 9, 75–86
21. Vadlamudi, R. K., Bagheri-Yarmand, R., Yang, Z., Balasenthil, S., Nguyen, D., Sahin, A. A., den Hollander, P., and Kumar, R. (2004) Cancer Cell 5, 575–585
22. Yang, Z., Vadlamudi, R. K., and Kumar, R. (2005) J. Biol. Chem. 280, 654–659
23. Tang, Q., Staub, C. M., Gao, G., Jin, Q., Wang, Z., Ding, W., Aurigemma, R. E., and Mulder, K. M. (2002) Mol. Biol. Cell 13, 4484–4496
24. Jin, Q., Ding, W., Staub, C. M., Gao, G., Tang, Q., and Mulder, K. M. (2005) Cell. Signal. 17, 1363–1372
25. Ilango, U., Ding, W., Zhong, Y., Wilson, C. L., Gropp, J. C., Trbovich, J. T., Zuniga, J., Demeler, B., Tang, Q., Gao, G., Mulder, K. M., and Hinck, A. P. (2005) J. Mol. Biol. 352, 338–354
26. Song, J., Tyler, R. C., Lee, M. S., Tyler, E. M., and Markley, J. L. (2005) J. Mol. Biol. 354, 1043–1051
27. Talbott, M., Hare, M., Nyarko, A., Hays, T. S., and Barbar, E. (2006) Biochemistry 45, 6793–6800
28. Nyarko, A., Cochrun, L., Norwood, S., Pursifull, N., Voth, A., and Barbar, E. (2005) Biochemistry 44, 14248–14255
29. Barbar, E., Kleinman, B., Imhoff, D., Li, M., Hays, T. S., and Hare, M. (2001) Biochemistry 40, 1596–1605
30. Liang, J., Jaffrey, S. R., Guo, W., Snyder, S. H., and Clardy, J. (1999) Nat. Struct. Biol. 6, 735–740
31. Fan, J., Zhang, Q., Tochio, H., Li, M., and Zhang, M. (2001) J. Mol. Biol. 306, 97–108
32. Williams, J. C., Xie, H., and Hendrickson, W. A. (2005) J. Biol. Chem. 280, 21981–21986
33. Wu, H., Maciejewski, M. W., Takebe, S., and King, S. M. (2005) Structure (Camb.) 13, 213–223
34. Wang, L., Hare, M., Hays, T. S., and Barbar, E. (2004) Biochemistry 43, 4611–4620
35. Lo, K. W., Naisbitt, S., Fan, J. S., Sheng, M., and Zhang, M. (2001) J. Biol. Chem. 276, 14059–14066
36. DeLano, W. L. (2002) The PyMOL Molecular Graphics System, DeLano Scientific, San Carlos, CA