Solution NMR Study of the Monomeric Form of p13\textsuperscript{suc1} Protein Sheds Light on the Hinge Region Determining the Affinity for a Phosphorylated Substrate*

Received for publication, December 10, 2001, and in revised form, January 18, 2002
Published, JBC Papers in Press, January 25, 2002, DOI 10.1074/jbc.M111741200

Benoît Odaert‡‡, Isabelle Landrieu‡, Klaas Dijkstra§, Gea Schuurman-Wolters§, Peter Casteels§, Jean-Michel Wieruszkeski‡, Dirk Inzé‡, Ruud Scheek‡, and Guy Lippens‡‡

From the ‡CNRS-Université de Lille 2 UMR 8525, Institut Pasteur de Lille-Institut de Biologie de Lille, 59019 Lille Cedex, France, §The Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, 9747 Groningen, The Netherlands, and the §Laboratorium voor Genetica, Department of Plant Genetics, Flanders Interuniversity Institute for Biotechnology (VIB), Universiteit Gent, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium

Cyclin-dependent kinase subunit (CKS) proteins bind to cyclin-dependent kinases and target various proteins to phosphorylation and proteolysis during cell division. Crystal structures showed that CKS can exist both in a closed monomeric conformation when bound to the kinase and in an inactive C-terminal β-strand-exchanged conformation. With the exception of the hinge loop, however, both crystal structures are identical, and no new protein interface is formed in the dimer. Protein engineering studies have pinpointed the crucial role of the proline 90 residue of the p13\textsuperscript{suc1} CKS protein from \textit{Schizosaccharomyces pombe} in the monomer-dimer equilibrium and have led to the concept of a loaded molecular spring of the β-hinge motif. Mutation of this hinge proline into an alanine stabilizes the protein and prevents the occurrence of swapping. However, other mutations further away from the hinge as well as ligand binding can equally shift the equilibrium between monomer and dimer. To address the question of differential affinity through relief of the strain, here we compare the ligand binding of the monomeric form of wild-type \textit{S. pombe} p13\textsuperscript{suc1} and its hinge mutant P90A in solution by NMR spectroscopy. We indeed observed a 5-fold difference in affinity with the wild-type protein being the most strongly binding. Our structural study further indicates that both wild-type and the P90A mutant proteins adopt in solution the closed conformation but display different dynamic properties in the C-terminal β-sheet involved in domain swapping and protein interactions.

Progression through the cell cycle is tightly regulated at all levels with a central role for the CDK\textsuperscript{2} complex. Activation of the kinase requires binding of a cyclin protein (1) as well as phosphorylations and dephosphorylations of specific residues (2). Additionally a small subunit called cyclin-dependent kinase subunit (CKS) is essential for cell cycle progression (3), and the cell is unable to enter or exit mitosis depending on the time at which the CKS is removed (4). Although this small protein does not modulate directly the catalytic function of the CDK complex, it is involved in the interaction with regulators of the kinase complex (5). CKS enhances the Cdc25 phosphatase phosphorylation by the CDK complex (5), binds to the phosphorylated anaphase-promoting complex responsible for the cyclin degradation that is necessary to exit mitosis (6), and is required for SCFSkp2-mediated ubiquitinylation of the phosphorylated p27kip1 (7). We have recently shown that the \textit{Schizosaccharomyces pombe} p13\textsuperscript{suc1} protein binds via its anion-binding site to a Cdc25 phosphatase peptide in a phosphorylation-dependent manner (8).

A potential binding site for the phosphorylated substrate was suggested by the presence of a sulfate anion in the crystal structure of CKSHs2 (9). Several other structures have been solved, including the human CKSHs1 (10), the \textit{S. pombe} p13\textsuperscript{suc1} (11–13), and the \textit{Saccharomyces cerevisiae} CKS1 (14), and all contained the conserved cluster of basic residues that form the potential anion-binding site. A recent NMR study has confirmed that those residues map to the interaction site with the phospho-(Ser/Thr)-Pro motif on the ligand (8). The conserved CKS fold further contains a four-stranded β-sheet flanked by two α-helices where the four-stranded β-sheet has on one side a patch of conserved hydrophobic residues that bind tightly to the C-terminal domain of the kinase as revealed by the crystal structure of the human CKSHs1-CDK2 complex (15).

Several crystal structures have revealed that the CKS monomers can form a dimer by exchange of their respective C-terminal β-strands (β4) (9, 12–14). The mechanism of this domain swapping remains unknown, and no consensus model has yet been achieved. Mutation studies (16, 17) and molecular dynamics simulations of protein unfolding (18) of the \textit{S. pombe} p13\textsuperscript{suc1} CKS monomeric protein suggest that dimerization occurs in the early events of the folding process via an intermediate state or even imply \textit{in vivo} chaperone molecules not yet characterized. However, it is clear that this domain swapping requires only a minimal conformational change in the hinge region preceding the β4-strand. Moreover, the conservation of this HXPEPH motif among the different members of the CKS family argues for a biologically relevant role that might be related to a conformational control of the CKS association with...
the CDK complexes (19, 20). Indeed, as this hinge region switches from a turn (in the closed globular form) to an extended conformation (in the open exchanged form), the protein in its dimeric form can no longer interact with the kinase (15). Very recently it was shown that single point mutations of residues as far as 20 Å from the hinge motif or, alternatively, ligand binding can equally shift the equilibrium between both forms toward the monomeric form (21).

Whereas these last results suggest that the strain accumulated throughout the protein is actively used to enhance ligand binding, many questions remain, one of the most important being the uncertainty on the solution conformation of this β4-strand. Small angle x-ray scattering studies on the native S. pombe p13mut CKS monomeric protein in solution yielded an unexpected large gyration radius, suggesting that CKS even in its monomeric form is at least partially in an open conformation with this C-terminal β-strand protruding in solution (22). In the same S. pombe p13mut CKS protein, mutation of the first proline residue of the hinge region to an alanine (p13P90A) has been reported to prevent the swapping process, to increase the protein stability, and to simplify the folding kinetics (23).

To address the question of differential affinity through relief of the strain, here we compare the ligand binding of the monomeric form of wild-type S. pombe p13mut and its hinge mutant P90A in solution by NMR spectroscopy. We indeed observed a 5-fold difference in affinity with the wild-type protein being the most strongly binding. We therefore continued our NMR analysis to characterize structurally the CKS protein in solution and to investigate the role of the proline residue Pro-90 (bold underlined) in the hinge region (motif HXPEPH). We found that both wild-type and the P90A mutant proteins adopt in solution the closed conformation but display different dynamic properties in the C-terminal β-sheet involved in domain swapping and protein interactions. The flexibility imposed by the loaded molecular spring that is the hinge region can provide for the diversity in binding partners that the CKS protein has to recognize during the cell cycle.

MATERIALS AND METHODS

15N,13C-Labeled Protein Production and Purification—The p13mut gene was cloned at the NdeI and EcoRI sites of the T7 promoter-based vector pRK172 (3). The proline mutation into alanine at residue position Pro-90 was obtained by PCR using primers containing a single point mutation converting the proline GCA codon into an alanine CCA codon.

For production of uniformly 15N,13C-labeled p13mut proteins, BL21 cells hosting the plasmid were grown in M9 minimal medium with 15NH4Cl (1 g liter−1) and 15Cigluco (2 g liter−1) (Cambridge Isotope Laboratories, Cambridge, MA) to A600 = 0.6–1.0 and induced with 0.4 mM isopropyl-1-thio-β-D-galactopyranoside for 2 h. After centrifugation for 10 min at 5000 × g, the cells were resuspended in Tris-HCl buffer (50 mM, pH 8) containing 1 mM phenylmethylsulfonyl fluoride, 1 mM MgCl2, and traces of DNase and RNase and disrupted with a French press. After centrifugation of the solution for 2 h at 4 °C at 17,000 × g, the supernatant was dialyzed overnight at 4 °C against the Tris-HCl buffer. Proteins were purified with a Q anion chromatography-Sepharose column equilibrated in the Tris-HCl buffer with a NaCl elution gradient (0–0.5 m) followed by a gel filtration step with a Superdex 75 column equilibrated with 50 mM Tris-HCl, NaCl buffer. At each step, fractions containing the pure monomeric form of p13mut proteins as checked by native and SDS-polyacrylamide gels were pooled together and concentrated. The dimeric fraction represented less than 5% of the wild-type protein and was separated from the monomeric fraction during the purification process. NMR samples were prepared by dialysis of the protein solution against 100 mM NaCl, 50 mM NaHPO4/Na2HPO4, solution (pH 6.8) in 5%–95% D2O/H2O and concentrated by centrifugation to a final protein concentration of 2 mM (Vivaspin 5 kDa).

NMR Experiments—NMR data were recorded at 20 °C on a Bruker DMX600 (Pasteur Lille) and on a Varian Inova 600 (Groningen) spectrometer equipped with a triple resonance 5-mm probe with z gradient coil. Sequential backbone resonance assignments of p13mut and p13P90A protein were achieved using the following pairs of triple resonance experiments: HNCA/HN(CO)CA, HNCO/HN(CA)CO, CB-CA(CA)/CO, CBCA(CO)NH, or HNCACB and combination of sensitivity enhancement versions of 15N-edited HSQC-NOESY/HSQC-TOCSY (24). For the 1H/15N exchange studies, the 1H NMR sample was diluted by a factor of 2 in a deuterated phosphate buffer at 20 °C. A series of 15N HSQC spectra was acquired every 0.5 μm (four times), 10 (five times), 20, 80 (six times), and 160 (nine times) min. To identify the slowest exchangeable protons, a similar study was performed after a quick dialysis (1 h) against deuterated buffer. Three classes of exchange were defined: highly exchangeable protons (within 20 min), moderately protected (time of exchange <1 day), and highly protected residues (time of exchange >1 day). For the thermal studies, a series of 15N HSQC spectra was acquired at 50 μm protein concentration at different temperatures ranging from 5 °C to 50 °C in steps of 5 °C. All data were processed on a Silicon Graphics O2 work station with the program SNARF, version 0.8.9 (Frans van Hoesel, University of Groningen).

For the titration experiments, a sample of the wild-type p13mut was prepared in a 50 mM Tris buffer as described previously (8). Increasing amounts of unlabeled synthetic peptide of sequence EQPLpTPVTDL (where pT is phosphothreonine) (25) were added to a 0.5 mM 15N-labeled p13mut sample. Final concentrations were successively 0.5/0.125 mM, 0.5/0.25 mM, 0.5/0.50 mM, 0.5/1.0 mM, 0.5/2.5 mM, 0.5/5.0 mM, and 0.5/10 mM. 1H-15N HSQC spectra were measured at each titration point. The binding constant was calculated as described previously (8).

RESULTS

Assignment of the p13P90A Mutant Protein—The good dispersion of the 15N HSQC spectrum indicates that the protein adopts a folded conformation in solution, justifying further efforts to the determination of the solution conformation (Fig. 1). We therefore attempted to assign the backbone resonances by heteronuclear NMR experiments. A complete backbone assignment, with the exception of the single residue Arg-39 in the
tide to determine an affinity constant, we found a value of $180 \pm 20 \mu M$, indicative of a 5-fold stronger binding of the phosphopeptide to the wild-type protein than to the hinge mutant (8). Even at the highest concentration of peptide, however, no additional peaks corresponding to those only present in the HSQC spectrum of the mutant protein could be observed.

Secondary Structure in Solution of the CKS Protein—As the mutation allowed a complete assignment of the spectrum, we decided to first characterize the structure of the CKS protein in solution on the basis of the mutant NMR data. The secondary structure derived on the basis of the $^{13}C\alpha$, $^{13}C\beta$, $^{13}CO$, and $^1H\alpha$ chemical shift index CSI (27) was confirmed by characteristic sequential NOE contacts. NN(I, I + 1) and $\alpha$N(I, I + 1) NOEs of, respectively, strong and weak intensity coincide with upfield shifts of the $^1H\alpha/^{13}C\alpha/^{13}Co$ profile and downfield shifts of the $^{13}C\beta$ frequencies in the segments 12–19, 45–49, 67–71, and 103–107. The three first segments correspond in the crystal structure, respectively, to the $\alpha$-1-, $\alpha$-2-, and $\alpha$4-helices. The fourth segment, unsolved in the crystal structures due to dynamic disorder, adopts a helical conformation as observed for the CKS1 protein (14). Despite the lack of helical CSI pattern, the segment 53–56 probably does adopt the short $\alpha$3-helix observed in the crystal structure as suggested by strong sequential NN(I, I + 1), $\alpha$N(52, 54), and $\alpha$N(53, 55) NOE contacts. Strong $\alpha$N(I, I + 1) NOEs, associated with a significant downfield shift of the $^1H\alpha/^{13}C\alpha/^{13}Co$ profile and upfield $^{13}C\beta$ values defined a $\beta$-extended conformation for the strands $\beta$1 (residues 26–28), $\beta$2 (residues 36–43), and $\beta$4 (residues 94–99) of the four-stranded $\beta$-sheet. The segments (8–11 and 78–80) are equally extended but not involved in the formation of $\beta$-sheet, and the absence of any secondary CSI pattern between residues 82 and 85 confirmed that the strand $\beta$3 is not in a regular conformation in solution in agreement with the crystal structure. The C-terminal elongation of the $\alpha$1-helix and the $\beta$1-strand is interrupted by the presence of the proline residues Pro-20 and Pro-29 as supported by the disruption of the CSI pattern in the vicinity of these residues.

The presence of nonsequential backbone $\alpha\alpha$, $\alpha$N, and NN connectivities supports the presence of a four-stranded $\beta$-sheet in the solution structure (Fig. 3). The numerous nonsequential NOEs between the $\beta$2- and $\beta$4-strands indicate that they participate in the formation of a regular antiparallel $\beta$-sheet. Finally the reversal of the protein chain in the $\beta$-hinge region of the C-terminal $\beta$-sheet is supported by strong sequential NN(89, 90), NN(90, 91), and NN(93, 94) NOEs and weak intensity $\alpha$N(89, 91), $\alpha$B(89, 92), and $\beta$N(89, 92) NOEs. All these results support the view that the protein adopts in solution the overall global fold of the closed form of the zinc-mediated dimer described by x-ray studies (11).

Hydrogen/Deuterium Exchange Experiments on Both Wild-type and Mutant Proteins—$^1H/\label{H}^2D$ exchange experiments were used to detail the protection of the amide protons against the solvent in terms of accessibility and hydrogen bonding. As our working pH for the NMR experiments was relatively high ($>6.3$), we observed that most of the amide protons in the mutant protein exchanged within the first 20 min. The remaining 36 residues showed intermediate exchange rates with a time constant around 24 h or very slow exchange rates with a protection extending over 2 days. Mapping of the three exchange rate classes of the P90A mutant protein on the crystal structure (Fig. 2c) shows that most of the highly protected residues are located in the $\beta$2-strand (residues 37, 38, 41, 42, and 43) and $\beta$4-strand (residues 94–99). In contrast to the central $\beta$-strands of the $\beta$-sheet, moderate protection was observed for the external $\beta$1-strand (residue 26) and $\beta$3-strand (residues 82 and 83). A similar trend was observed for the

---

2 B. Odaert, I. Landrieu, K. Dijkstra, G. Schuurman-Wolters, P. Casteels, J.-M. Wierzuszek, D. Inze, R. Scheek, and G. Lippens, manuscript submitted.
residues of the α1- (residues 15, 18, and 22), α2- (residues 47, 51), and α4-helices (residues 70, 71, 72) and hydrophobic residues 55, 66, 74, and 76 implied in the formation of the core of the protein.

An exchange experiment on the wild-type protein gave very similar results, indicating that both proteins are protected to the same extent and in the same regions. Especially protection of amide protons of residues 37, 41, 43, 98, and 99 showed that...
the β4-strand is in a regular conformation in the wild-type protein as well (Fig. 2c).

**Thermal Effect on the 15N HSQC Spectrum of Both Proteins**—For a better characterization of the line broadening observed for the resonances of the four-stranded β-sheet in the wild-type and mutant proteins, we studied the effect of temperature on the NMR parameters by acquiring a series of 15N HSQC spectra for both proteins between 5 °C and 50 °C (Fig. 4). To prevent aggregation, the thermal study was performed at low protein concentration (50 µM) and at temperatures well below the unfolding temperature (65 °C).

Raising the temperature led to a line narrowing for the resonances of most of the residues, probably due to an accelerated overall tumbling of the molecules. In contrast, resonances of the residues at the N-terminal and C-terminal extremities disappeared at higher temperatures due to an increase of their exchange rates with water.

The major difference between the two proteins resides in the behavior of those residues whose resonances were broadened or missing from the spectrum of the wild-type protein. An increase of temperature made those resonances reappear at the same chemical shift values as the corresponding resonances in the mutant protein at the same temperature. In particular, residues in the β4-strand (from 93 to 97) and in the neighboring region of the β3-strand (from 65 to 67) showed this behavior (Fig. 4). Residues in the β-hinge region (from Tyr-85 to Glu-91) could not be identified in the wild-type protein spectrum even at high temperatures.

These thermal studies also allowed us to determine the amide thermal coefficients. High values in disordered or accessible parts of the protein are usually indicative of a significant degree of flexibility. We observed that some of the residues in the β-hinge region of the mutant protein, such as His-88, Val-89, and His-93, displayed even higher coefficients than those found for the flexible N- and C-terminal extremities, suggesting a high degree of internal flexibility for the β-hinge region. Such results are in accordance with the conformational heterogeneity observed in the crystal structures for this region. A similar trend is observed for the residue Tyr-27 interacting with the proline Pro-29, which introduces a bulge in the β1-strand. For the rest of the protein, no significant difference in thermal coefficients between the wild-type and the mutant proteins was observed.

**DISCUSSION**

The small CKS protein is both functionally and structurally a remarkable protein module. A ubiquitous subunit of the CDKcyclin complexes, it is essential for a correct progression of the cell cycle (3, 4, 28), but its precise role as a targeting unit with a clear definition of its multiple binding partners is still being investigated. As for its structure, x-ray crystallography has identified two distinct conformations, each associated with a different assembly state: the monomer, in which the β4-strand is inserted in the central β-strand of the globular protein (11), and the β-strand exchanged dimer, in which the same β4-strand inserts into the β-sheet of the other protein partner (13). The structural change between both forms is limited to the β-hinge region that is very conserved across the species for which a CKS member was identified. Folding studies demonstrated that the hinge, and more particularly the proline at position 90 in p13 suc1, is a source of kinetic heterogeneity in the β-sheet (23). Nevertheless, despite the module being the prototype of a system that can undergo proline-dependent oligomerization with arm exchange (29), a number of questions relating to its solution structure and dynamics remain unanswered.

The crystal structure of the CKS-CDK2 complex (15) indicated without any ambiguity that the CDK kinase recruits the globular monomeric form, and further modeling even excluded binding of the dimeric form of CKS to the kinase. Very recently elegant protein engineering studies have shed some further light on the connection between folding and function by showing an enhanced binding of a phosphorylated peptide from Cdc25 to the strained monomer rather than to the dimer (21). This confirms previous observations that the thermodynamics of protein-protein interactions are not incompatible with a high degree of flexibility of the unbound partners despite the entropic cost of binding (30, 31). However, how the changes in the β-hinge translate into a signal for altered ligand binding cannot directly be inferred from the various crystal structures as the domain swapping does not lead to any obvious structural difference, and no new protein-protein interface is formed. Both structures seemingly differ in their thermodynamic properties, however, with the β-hinge acting as a loaded “molecular spring” that is relieved in the dimer. Most importantly this
hinge confers strain not only at the local level but to the whole protein body. A different approach to obtain the relieved state is the mutation of the key residue Pro-90 to an alanine, which results in a major population of the monomeric form for the mutant protein and the disappearance of two populations with different unfolding kinetics (17, 21).

The aim of our present study was to use solution NMR techniques to clarify in atomic detail the role of the β-hinge in the structure and function of p13suc1. A very strong indication that the overall architecture of both the wild-type and mutant proteins are identical is given in Fig. 1. Indeed, the chemical shift being a very sensitive probe of three-dimensional environment, the near identity of both HSQC spectra confirms that both proteins share the same structure. The assignment of the mutant protein gave the necessary basis for further structural investigations. Both CSI and backbone NOE data agreed with the presence of the major secondary structure elements as observed in the crystal structure. Both structural elements as observed in the crystal structure. More specifically the presence of a central β-sheet formed by the β2- and β4-strands was confirmed by a high density of interstrand NOE contacts (Fig. 3). However, these data are still not conclusive as a strand in fast exchange between the core of the protein and the solvent, spending a reasonable fraction of time in the direct neighborhood of β2, still could give similar NOE contacts, the NOE effect being heavily biased toward folded conformations (32). We therefore performed 1H/D exchange experiments that should be very sensitive to exposure, be it partial, of the amide functions to the solvent. The finding of the highest protection factors for the central residues of the β2- and β4-strands confirmed unambiguously that both strands form a stable β-sheet affected by some degree of internal dynamics but without partial disruption. We thus conclude that the mutant protein forms a stable globular monomer in solution, be it characterized by some dynamics in its central β2-β4 folding core.

Upon studying the wild-type protein, the fact that 12 correlation peaks in the HSQC spectrum were missing when compared with that of the P90A mutant came as a surprise. The full assignment of the mutant protein allowed the assignment of these missing peaks mainly to residues in the hinge and on the β2- and β2-strands. Spectra at higher temperature indicated that the correlation peaks are present at exactly the same position as in the mutant protein but retain a broader character (Fig. 4). We can therefore extrapolate that at room temperature these missing peaks are present but are broadened beyond detection because of an exchange term with unfavorable characteristic time (33). Significantly line broadening associated with motions in the 100-μs-ms time range have been frequently observed for protein regions involved in protein-protein interactions (30, 31). Similarly as in the mutant protein, however, the dynamics cannot be associated with even a partial unfolding of the β4-strand. Indeed those residues of the wild-type p13suc1 whose correlation peak was visible in the HSQC spectrum proved equally protected against rapid 1H/D exchange. An example of those residues are Arg-99, which forms a salt bridge with the side chain of Glu-37 and packs against the side chains of Trp-82 (β3) and Arg-30 (β1). Because it is not very close to the hinge region, its dynamics do not preclude observation of its amide proton, which was not replaced by deuterium even after a week (Fig. 2c). We hence conclude that the mutation of Pro-90 into an alanine residue does not significantly change the overall structure but changes the dynamics of the hinge region and β4-strand. The exchange data on both proteins indicate a good protection against hydrogen/deuterium exchange for residues at the C terminus of this β4-strand, strongly arguing against even a transient swapping in solution of the whole strand. The mapping of the binding site of a synthetic phosphorylated Cdc25 peptide on both proteins furthermore confirms that the same residues are involved in substrate recognition (Fig. 2b). However, those residues implicated in ligand binding are not directly associated with the enhanced dynamics: their correlation peaks are visible in the HSQC and are not significantly broadened compared with the same correlations in the HSQC spectrum of the mutant. The 5-fold increase in affinity as measured in a titration experiment therefore can be interpreted as a form of signal transduction as was recently concluded on the basis of a differential affinity of the same phosphopeptide for the monomeric form of p13suc1 compared with its dimeric form (21). Our spectral data show moreover that substrate binding does not remove the kinetic heterogeneity observed around the hinge region as even at the highest concentration of peptide (with almost all of the p13 molecules in the bound conformation) no additional peaks could be observed in the HSQC spectrum.

Functionally the CKS protein was previously shown to stimulate strongly the regulatory phosphorylations of Cdc25, Myt1, and Wee1 by the Cdc2/Cyclin B complex (5). In the same study, it was concluded that CKS and the WW domain of Pin1, a prolyl cis/trans isomerase that was found to be essential for correct cell cycle progression (34, 35), may normally play antagonistic roles in controlling the G2/M transition. Whereas we had previously observed this molecular competition in vitro (8), the weak affinity of the mutant CKS protein (900 μM) compared with that of the WW domain (150 μM) suggested that CKS was a rather poor competitor, which did not fit well with the observations with the Xenopus cell extracts. Our data on the wild-type CKS protein presented here, however, show that the relative affinities of CKS and the Pin1 WW domain are very comparable and confirm therefore their potential role as molecular competitors for the same phosphorylated substrates.

The CKS proteins through their role as binding modules are not involved in a single complex with unique molecular partners but in multiple CDK complexes with even more substrate proteins. Flexibility to adapt to partners on both sides might therefore be a crucial feature of its structure and would be necessary for both interacting sides of the protein. As both binding sites have in common residues of the central β4-strand (8, 15), destabilizing this structure element to some extent while maintaining it in a regular structural context might be the solution that nature has adopted to allow simultaneous binding to the CKD complex and to its many phosphorylated substrates. The hinge with its two proline residues confers the required dynamics and as such is an essential structural feature of the CKS family, explaining its strict sequence conservation. To evaluate the flexibility of this central strand in the full complex, we are currently extending our NMR study to the complex of CKS with CKD2 and hope to elucidate the role of the central β-strand dynamics in the near future.

REFERENCES
1. Jeffrey, P. D., Russo, A. A., Polyaek, K., Gibbes, E., Hurwitz, J., Massague, J. & Pavletich, N. P. (1995) Nature 376, 313–320
2. Lew, D. J. & Kornbluth, S. (1996) Curr. Opin. Cell Biol. 8, 795–804
3. Brinzuela, L., Dravetta, G. & Beach, D. (1997) EMBO J. 6, 3537–3541
4. Patra, D. & Dunphy, W. G. (1996) Genes Dev. 10, 1503–1515
5. Patra, D., Wang, S. X., Kumagai, A. & Dunphy, W. G. (1999) J. Biol. Chem. 274, 36839–36842
6. Patra, D. & Dunphy, W. G. (1998) Genes Dev. 12, 2549–2559
7. Ganoth, D., Bornstein, G., Ko, T. K., Larsen, B., Tyers, M., Pagano, M. & Hershko, A. (2001) Nat. Cell Biol. 3, 321–324
8. Landrieu, I., Odaert, B., Wieruszewski, J.-M., Droboeq, H., Inzé, D. & Lippens, G. (2001) J. Biol. Chem. 276, 1434–1438
9. Parge, H. E., Arvai, A. S., Murtari, D., Reed, S. I. & Tainer, J. A. (1993) Science 262, 387–395
10. Arvai, A. S., Bourne, Y., Hickey, M. J. & Tainer, J. A. (1995) J. Mol. Biol. 249, 835–842
11. Endicott, J. A., Noble, M. E., Garman, E. F., Brown, N., Rasmussen, B., Nurse, P. & Johnson, L. N. (1995) EMBO J. 14, 1004–1014
Solution NMR Study of the Monomeric Form of p13^suc1 Protein

12. Bourne, Y., Arvai, A. S., Bernstein, S. L., Watson, M. H., Reed, S. I., Endicott, J. E., Noble, M. E., Johnson, L. N. & Tainer, J. A. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 10232–10236
13. Khazanovich, N., Bateman, K. S., Chernaia, M., Michalak, M. & James, M. N. G. (1996) Structure 4, 299–309
14. Bourne, Y., Watson, M. H., Arvai, A. S., Bernstein, S. L., Reed, S. I. & Tainer, J. A. (2000) Structure Fold. Des. 8, 841–850
15. Bourne, Y., Watson, M. H., Hickey, M. J., Holmes, W., Rocque, W., Reed, S. I. & Tainer, J. A. (1996) Cell 84, 863–874
16. Schymkowitz, J. W. H., Rousseau, F., Irvine, L. R. & Itzhaki, L. S. (2000) Structure 8, 89–100
17. Schymkowitz, J. W. H., Rousseau, F. & Itzhaki, L. S. (2000) J. Mol. Biol. 301, 199–204
18. Alonso, D. O. V., Alm, E. & Daggett, V. (2000) Structure 8, 101–110
19. Endicott, J. A. & Nurse, P. (1995) Structure 3, 321–323
20. Pines, J. (1996) Curr. Biol. 6, 1399–1402
21. Schymkowitz, J. W. H., Rousseau, F., Wilkinson, H. R., Friedler, A. & Itzhaki, L. S. (2001) Nat. Struct. Biol. 8, 888–892
22. Birck, C., Vachette, P., Welch, M., Swaren, P. & Samama, J. P. (1996) Biochemistry 35, 5577–5585
23. Rousseau, F., Schymkowitz, J. W. H., Sánchez del Pino, M. & Itzhaki, L. S. (1998) J. Mol. Biol. 284, 503–519
24. Grzesiek, S., Bax, A., Hu, J. S., Kaufman, J., Palmer, I., Stahl, S. J., Tjandra, N. & Wingfield, P. T. (1997) Protein Sci. 6, 1248–1263
25. Korchuganov, D. S., Nolde, S. B., Reihartkh, M. Y., Grekhov, V. Y., Schulga, A. A., Ermolyuk, Y. S., Kirpichenkov, M. P. & Arseniev, A. S. (2001) J. Am. Chem. Soc. 123, 2068–2069
26. Pühl, M., Chen, H. A., Kristensen, S. M. & Driscoll, P. C. (1999) J. Biomol. NMR 14, 307–320
27. Wishart, D. S. & Sykes, B. D. (1994) J. Biomol. NMR 4, 171–180
28. Moreno, S., Hayles, J. & Nurse, P. (1989) Cell 58, 361–372
29. Bergdoll, M., Remy, M.-H., Cagnon, C., Masson, J.-M. & Dumas, P. (1997) Structure 5, 391–401
30. Feher, V. A. & Cavanagh, J. (1999) Nature 400, 289–293
31. Akke, M., Liu, J, Cavanagh, J., Erickson, H. P. & Palmer, A. G., III. (1998) Nat. Struct. Biol. 5, 55–59
32. Burgi, R., Pitera, J. & Van Gusteren, W. F. (2001) J. Biomol. NMR 19, 305–320
33. Clore, G. M., Szabo, A., Bax, A., Kay, L. E., Driscoll, P. C. & Gronenborn, A. M. (1990) J. Am. Chem. Soc. 112, 4989–4991
34. Lu, K. P., Hanes, S. D. & Hunter, T. (1996) Nature 380, 544–547
35. Yaffe, M. B., Schultkowski, M., Shen, M., Zhou, X. Z., Stukenberg, P. T., Rahfeld, J. U., Xu, J., Kuang, J., Kirschner, M. W., Fischer, G., Cantley, L. C. & Lu, K. P. (1997) Science 278, 1957–1966