Synergy of Silent and Hot Spot Mutations in Importin β Reveals a Dynamic Mechanism for Recognition of a Nuclear Localization Signal*

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Molecular recognition of the importin β-binding (IBB) domain of importin α by importin β is critical for the nuclear import of protein cargoes containing a classical nuclear localization signal. We have studied the function of four conserved tryptophans of importin β (Trp-342, Trp-430, Trp-472, and Trp-864) located at the binding interface with the IBB domain by systematic alanine substitution mutagenesis. We found that Trp-864 is a mutational hot spot that significantly affects IBB-binding and import activity, whereas residues Trp-342, Trp-430, and Trp-472 are mutationally silent when analyzed individually. Interestingly, the combination of the hot spot at residue Trp-864 with mutations in the other three tryptophans gives rise to a striking synergy that diminishes IBB domain binding by up to ~1000-fold and, in turn, abolishes import activity. We propose that importin β uses the tryptophans to select and stabilize a helical conformation of the IBB domain, which, in turn, conveys specific, high affinity binding.

Importin β (also known as karyopherin β-1) represents the prototype of the importin β-superfamily of nuclear transport receptors (1–3). The interaction of importin β with import cargoes involves recognition of the nuclear localization signal (NLS), either directly, as in the case of cargoes like parathyroid hormone-related protein (PTHrP) (4), or via an adaptor. The adaptors importin α and snurportin, which are involved in the import of classical NLS (cNLS) (1–3) containing cargoes and trimethylguanosine (m3G)-capped U small nuclear ribonucleoprotein (snRNP) particles (5), respectively, interact with importin β via an N-terminal basic importin β-binding (IBB) domain (1–3). The IBB domain represents the minimal structural region of importin α that binds to importin β with high affinity and can itself serve as a functional NLS (1–3). For example, large cargoes such as β-galactosidase fused to the IBB domain are specifically imported into the nucleus of permeabilized cells by importin β (6, 7). Translocation of the import complex through the nuclear pore complex (NPC) involves multiple rounds of interaction of importin β with nucleoporins (8). An essential cofactor is the small GTPase Ran, which, in its GTP-bound form, dissociates importin β from nucleoporins and from the adaptor-cargo complex (1–3).

The crystal structure of importin β complexed with the IBB domain of importin α (residues 11–54) was solved by x-ray crystallography to a resolution of 2.3Å (9). Importin β contains a modular structure built of 19 tandem HEAT repeats arranged to form a superhelix. Each HEAT repeat represents a secondary structure motif formed by two helices connected by a loop. The HEAT repeat array contains two structurally and functionally distinct surfaces. The convex outer surface has nucleoporin-binding sites (10), and the concave internal face binds Ran (HEAT repeats 1–8) (11) and cargoes. Whereas the IBB domain binds mostly to the C terminus of importin β (HEAT repeats 7–19) (9), PTHrP interacts with the N-terminal HEAT repeats 2–11 in a region of the protein that overlaps with the Ran-binding domain (4).

As seen in the crystal structure of importin β complexed with the IBB domain of importin α, residues 22–51 of the IBB domain form a straight helix, whereas the N-terminal moiety (residues 11–21) adopts a more extended conformation, interrupted between residues 13–15 by a 3₁₀ helix. The importin β-IBB domain-binding interface involves an extended network of interactions, which accounts for over 40 specific bonds including electrostatic, hydrophobic, and Van der Waals contacts (9). Likewise, the binding cavity of importin β is highly enriched in acidic residues and affords an ideal environment for the folding of a basic peptide. This has led to the idea that importin β may serve both as an import receptor and a chaperone to keep small basic proteins from aggregating (12).

Using the three-dimensional structure of the importin β-IBB domain complex as a guide, we have analyzed the molecular basis for the recognition of the IBB domain of importin α. Through systematic alanine substitution mutagenesis, we have targeted four tryptophans of importin β located at the binding interface. We have identified a single tryptophan that is essential for efficient binding to the IBB domain and severely diminishes the binding affinity after mutation to alanine. Moreover, we have demonstrated that this hot spot can synergize with mutations in other tryptophans at the binding interface, which only marginally affect the interaction when analyzed alone.

Based on these data, we propose a model for recognition of the IBB domain by importin β that involves the selection of a population of distinct helical IBB conformers by the receptor.

**EXPERIMENTAL PROCEDURES**

Analysis of the Importin βIBB Domain-binding Interface—The crystal structure of the importin βIBB domain complex (9) (Protein Data Bank accession number 1QGK) was extensively analyzed using the

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The abbreviations used are: NLS, nuclear localization signal; cNLS, classical nuclear localization signal; PTHrP, parathyroid hormone-related protein; IBB, importin β binding; NPC, nuclear pore complex; GST, glutathione S-transferase; BSA, bovine serum albumin.
RESULTS

Conserved Tryptophans of Importin β at the Binding Interface with the IBB Domain—One of the intriguing features of the importin β-IBB-binding interface is the presence of four conserved tryptophans in importin β, Trp-342, Trp-430, Trp-472, and Trp-864 (Fig. 1a), which are located in close proximity (3.8–4.2 Å) to four highly conserved IBB residues (9), namely Lys-18/22 (Fig. 1, b and c) and Arg-13/51 (Fig. 1, d and e). Whereas the indole rings of Trp-430, Trp-472, and Trp-864 point directly toward the ε-amino-terminal groups of IBB-Lys18/22 and the guanidinium group of IBB-Arg-51, respectively, W342 is oriented more toward the backbone of IBB-Arg-13. The extended conformation adopted by IBB-residues Lys-18/22 and Arg-13/51 exposes the polar guanidinium and ε-amino-terminal groups to engage in bidentate and monodentate interactions with at least other six residues of importin β and four residues from the IBB domain (Fig. 1, b–e).

To understand the importance of the four conserved tryptophans in the recognition of the IBB domain, we generated libraries of importin β point mutants (Table I) in which each tryptophan was replaced by an alanine, a residue that is compatible with α-helical folding. A first generation library consisted of the four Trp → Ala single point mutants. Additional tryptophans in these single point mutants were then mutated to produce six double and four triple Trp → Ala mutants, representing the second and third generation mutant libraries, respectively. In total, fourteen Trp → Ala importin β mutants were expressed in E. coli and purified to homogeneity. Similar to wild type importin β, all the Trp → Ala mutants displayed high solubility under physiological salt conditions (see “Experimental Procedures”) and were eluted as a monomer by gel filtration chromatography (data not shown). In addition, circular dichroism analysis revealed that the Trp → Ala mutations did not alter the secondary structure content of the mutants (data not shown). Together, these data indicate that the point mutations did not disrupt the overall folding of importin β.

Binding of Importin β (Trp → Ala) Mutants to the IBB Domain—We used a solid phase binding assay to study the effect of the Trp → Ala mutations on the interaction of importin β with the IBB domain of importin α. The IBB domain fused to GST was adsorbed to microtiter plates, and the binding of either wild type importin β or the Trp → Ala mutants was analyzed in a concentration range of 0.01–100 nM (Fig. 2, a–c). For wild type importin β we reproducibly measured an apparent dissociation constant (K_d) for the IBB domain of about 1 nM (Fig. 2a and Table I). Whereas the first generation importin β (Trp → Ala) mutants in residues Trp-342, Trp-430, and Trp-472 did not show a significant decrease in their binding affinity for the IBB domain, the mutation of Trp-864 reduced the dissociation constant for the IBB domain by ∼35-fold (K_d = 35.1 nM; Fig. 2a and Table I). This suggested that Trp-864 is a mutational hot spot (21). Second generation Trp → Ala mutants in which the Trp-864 was not mutated were still able to bind the IBB domain with nearly wild type affinity (Fig. 2b and Table I). In contrast, all second generation Trp → Ala mutants...
containing a combination of a single silent mutation (Trp342, Trp430, and Trp472) and the hot spot Trp864 showed a dramatic decrease in their binding affinity (~360–450-fold; see Table I). An even more pronounced synergy of silent and hot spot mutations was observed for the third generation Trp→Ala importin β mutants (Fig. 2c). In this case, the only triple mutant that showed sufficiently strong binding to accurately measure a $K_d$ in the 0.01–100 nM importin β concentration was the triple mutant W342A/W430A/W864A. We therefore repeated the binding assay using up to a 10-fold higher concentration of importin β (0.01–1 μM range) for both second and third generation mutants (Fig. 2d). From these measurements the apparent dissociation constant of second and third generation mutants containing the hot spot W864A was reduced to ~400 and 950 nM, respectively (Table I). However, because the binding isotherms did not reach saturation in these cases, these values should be viewed as approximations.

**Functional Characterization of Importin β Trp→Ala Mutants in Nuclear Import**—To investigate whether the decreased binding affinity of the importin β mutants for the IBB domain of importin α resulted in a reduced level of nuclear import of cNLS-cargo, we assayed the Trp→Ala mutants in a nuclear import assay involving permeabilized HeLa cells reconstituted with recombinant transport factors (18–19) (Fig. 3). Bovine serum albumin (BSA) coupled to a cNLS peptide was used as the cargo for import. In the presence of ATP, wild type importin β stimulated import of BSA-cNLS by ~4-fold (designated as 100% in Fig. 3) with respect to a control reaction wherein no recombinant importin β was supplied to the cells. Whereas the single point mutants targeting residues Trp342, Trp430, and Trp472 supported nuclear import of BSA-cNLS cargo at nearly wild type levels, the W864A mutant yielded only ~60% of the level of import (Fig. 3). In contrast, the second generation mutants yielded a significantly lower level of import, which was most pronounced for constructs containing the hot spot W864A mutation. For instance, the import levels with the W342A/W430A and W342A/W472A double mutants were about 50% of wild type importin β, and the levels with double mutants containing the T864A substitution were only ~25–40% of the wild type (Fig. 3). Finally, the third generation mutants bearing the hot spot W864A mutation were almost completely inactive in the nuclear import of NLS-BSA, whereas the triple mutant carrying the three weaker mutations (W342A/W430A/W472A) was only 50% reduced in import.

**Translocation of Importin β Trp→Ala Mutants Through the NPC**—We next examined whether the importin β triple mutants that were strongly deficient in supporting the import of cNLS-containing cargoes also were impaired in their ability to translocate through the NPC. Wild type importin β or the triple mutants containing the W864A mutation were microinjected in the cytoplasm of NRK cells, and at various times the cells were fixed and importin β localized by immunofluorescence staining with antibodies to the S-epitope tag (Fig. 4a and b). As shown for the triple mutant W342A/W430A/W864A, the injected importin β mutant was strongly concentrated in the nucleus after 1 min (Fig. 4) as well as after longer times (data not shown). Similar results were obtained for the other two triple mutants that were highly deficient in nuclear import (W342A/W472A/W864A and W430A/W472A/W864A, data not shown). In related studies, it has been reported that the C-terminally deleted importin β mutants, which were deficient for binding to the IBB domain, also efficiently accumulated in the nucleus permeabilized HeLa cell in the absence of exogenous transport factors (22). We conclude that the binding of importin β to the importin αcNLS-cargo complex and the ability of the receptor to translocate through the NPC are not coupled obligatorily. It is unlikely that the efficient nuclear translocation of the importin β Trp→Ala triple mutants observed in the microinjection experiments occurs as a consequence of their binding to importin α-independent cargoes like the PTHrP (4), because the interaction between the non-classical-NLS of the PTHrP and importin β critically involves tryptophans Trp342, Trp430, and Trp472 (4).

**DISCUSSION**

**Cation-π Interactions at the Importin βIBB-binding Interface**—The mutagenesis of conserved tryptophans at the importin β IBB domain-binding interface described above has revealed a key role of the tryptophans in the molecular recognition of the IBB domain. We propose that, at least in the case of residues Trp340, Trp472, and Trp864, the tryptophans engage in cation-π interactions with residues of the IBB domain. The cation-π interaction is a type of long-range, non-covalent intermolecular force between cationic residues and aromatic side chains. The planar ring of the tryptophans provides π electrons, whereas the cationic guanidinium and ε-amino-terminal groups of arginines and lysines, respectively, provide the cationic groups. The importance of cation-π interactions in protein structure is becoming increasingly recognized (23–24). In contrast to hydrophobic interactions, cation-π contacts are electrostatic interactions between dipoles and are completely abolished by alanine mutagenesis, as alanine lacks the delocalized nucleophilic charge of an aromatic ring.

To further investigate our predictions, we used the program CaPTURE (Cation-π Trends Using Realistic Electrostatics) (24), which predicts and quantifies cation-π interactions based on ab initio calculations from three-dimensional structures. Interestingly, CaPTURE predicts that three of the four importin β tryptophans at the binding interface that we studied engage in energetically significant intermolecular cation-π interactions with IBB residues. The energetic contributions were estimated to be approximately ~5.41 kcal/mole and ~4.61 kcal/mole for the Trp864IBB-Arg51 and Trp430IBB-Lys18 pairs and ~2.67 kcal/mole for Trp472IBB-Lys22 pair, respectively (Table II). Moreover, as predicted by visual examination of the three-dimensional structure, the potential energetic contribution due to a cation-π interaction for the Trp342IBB-Arg13 pair was not significant (~2 kcal/mol), as the ring of the tryptophan points more toward the main chain of the IBB-Arg13

**TABLE I**

| First generation | Second generation | Third generation |
|------------------|-------------------|-----------------|
| Mutant           | $K_d$ (nM)        | $K_d$ (nM)      | $K_d$ (nM)      |
| Wild type        | 0.65 ± 0.02       | 1.41 ± 0.18     | 0.78 ± 0.10     |
| W342A            | 1.01 ± 0.11       | 35.85 ± 5.99    | 1.05 ± 0.08     |
| W430A/W342A      | 3.97 ± 0.29       | 11.91 ± 0.62    | 11.91 ± 0.62    |
| W430A/W472A      | 426.9 ± 14.0      | 2.39 ± 0.15     | 364.5 ± 35.4    |
| W430A/W864A      | 405.0 ± 49.5      | 3.97 ± 0.15     | 405.0 ± 49.5    |
| W472A/W864A      | 364.5 ± 35.4      | 364.5 ± 35.4    | 364.5 ± 35.4    |
| W342A/W430A      | 5.99 ± 0.62       | 5.99 ± 0.62     | 5.99 ± 0.62     |
| W342A/W472A      | 14.0 ± 0.62       | 14.0 ± 0.62     | 14.0 ± 0.62     |
| W342A/W430A/W864A| 859.2 ± 45.5      | 859.2 ± 45.5    | 859.2 ± 45.5    |
| Wild type        | 1.12 ± 0.15       | 1.12 ± 0.15     | 1.12 ± 0.15     |
| W342A/W340A      | 68.1 ± 0.62       | 68.1 ± 0.62     | 68.1 ± 0.62     |
| W342A/W430A      | 59.5 ± 0.95       | 59.5 ± 0.95     | 59.5 ± 0.95     |
| W342A/W472A      | 114.0 ± 0.62      | 114.0 ± 0.62    | 114.0 ± 0.62    |
| W342A/W430A/W472A| 18.45 ± 7.30      | 18.45 ± 7.30    | 18.45 ± 7.30    |
| W342A/W430A/W864A| 810.4 ± 56.9      | 810.4 ± 56.9    | 810.4 ± 56.9    |
| W342A/W472A/W864A| 899.2 ± 56.5      | 899.2 ± 56.5    | 899.2 ± 56.5    |

$^a$ $K_d$ apparent (± S.D.). Data derived from plots of Fig. 2.
than to the cationic guanidinium group. These values reinforce the concept that Trp-864 is a hot spot mutation but do not explain the synergy of hot and silent point mutations observed in second and third generations (see below).

Based on our reexamination of the structure of the import adaptor importin α (25–26), we note that potential cation-π interactions also are found at the binding interface between importin α and the conserved arginines/lysines of both classical monopartite (25) and bipartite (26) NLS-peptides. Because both importin α and importin β may have evolved from a common precursor (4, 27), it is possible that cation-π interactions represent an evolutionary conserved feature of nucleoplasmic transport factors governing NLS recognition.

Population Selection and Binding—The dynamics of the importin β-IBB domain binding interface cannot be understood without considering the structural plasticity of the ligand, the IBB domain. Previous studies have shown that the IBB domain adopts different conformations in different structural contexts. Whereas it is mostly α-helical when bound to importin β (9), it is essentially unstructured in solution (28). Furthermore, a portion of the domain adopts an extended conformation when bound to the eNLS binding groove of importin α, suggesting an auto-inhibitory mechanism for binding to the NLS cargo (29).

Such conformational flexibility suggests that, in solution, the nuclear import reactions in digitonin-permeabilized cells were conducted with wild type (w.t.) importin β and 14 Trp → Ala mutants. Each import reaction was repeated twice, the two values were averaged, and the mean value was plotted in a histogram. Under optimal condition an ∼4-fold stimulation of nuclear import of BSA-NLS was observed with added wild type importin β (which corresponds to 100% on the y-axis) as compared with a negative control lacking exogenous importin β. The level of nuclear import obtained with the Trp → Ala importin β mutants is plotted as a percentage of the wild type import. The level of BSA-NLS accumulation observed under conditions of ATP depletion was subtracted from each import reaction (∼5–10% of the total).

FIG. 2. Isotherms depicting the binding of wild type and importin β Trp → Ala mutants to the IBB domain of importin α. First generation (a), second generation (b), and third generation (c) importin β Trp → Ala mutants were analyzed in the concentration range 0.01–1 nM. In panel d, the second and third generation mutants were re-assayed in a concentration range of 0.1–1 μM. The curves obtained in each panel were obtained by averaging three independent measurements carried out under identical conditions. The apparent dissociation constants (Kd) calculated using the GraphPad Prism program are presented in Table I. wt, wild type.

FIG. 3. Functional characterization of Trp → Ala mutants of importin β. Nuclear import reactions in digitonin-permeabilized cells were conducted with wild type (w.t.) importin β and 14 Trp → Ala mutants. Each import reaction was repeated twice, the two values were averaged, and the mean value was plotted in a histogram. Under optimal condition an ∼4-fold stimulation of nuclear import of BSA-NLS was observed with added wild type importin β (which corresponds to 100% on the y-axis) as compared with a negative control lacking exogenous importin β. The level of nuclear import obtained with the Trp → Ala importin β mutants is plotted as a percentage of the wild type import. The level of BSA-NLS accumulation observed under conditions of ATP depletion was subtracted from each import reaction (∼5–10% of the total).
Molecular Recognition of the IBB Domain by Importin β

IBB domain exists in a range of conformations with low energy barriers between them. Similar structural plasticity was reported previously for human immunodeficiency virus type 1 (HIV-1) Rev (30), another small, basic importin β-binding protein (1–3), and probably reflects an intrinsic propensity of arginine-rich polypeptides (30).

The view of the IBB domain as a population of dynamically interconverting conformers (31, 32) suggests that the recognition of the IBB domain by importin β may follow a population selection model (31, 32). Accordingly, the receptor would select the helical conformation of the IBB domain, which complements most favorably the importin β-binding cavity (Fig. 5), because of the electrostatic complementarity of basic IBB side chains projecting along the helix peptide and acidic residues of importin β. The binding of the helical IBB-conformer would be energetically driven by the large enthalpic nature of the binding,2 which is accompanied by the formation of over 40 specific contacts between the protein and the peptide (9). Moreover, the high affinity binding of a distinct helical IBB conformer must be dependent on the degree of structural stabilization of the helix imposed by importin β. We propose that the tryptophans of importin β control this step of the binding reaction by stabilizing the helical conformation of the IBB domain primarily via cation–π interactions with IBB-Arg-51 and Lys-18/22.

In this regard, the position of the tryptophans in importin β provides a rational explanation for the existence of weak and hot spots mutations. Whereas a single residue Trp-864 would stabilize the C terminus of the IBB helix, three separated residues near the N-terminal end of the helix could play overlapping roles. Residues Trp-430 and Trp-472 are located in the immediate proximity to the N terminus of the IBB helix (Fig. 5), and Trp-342 is next to the 310 helix (IBB-residues 15–18) (Fig. 1a). The hot spot can be interpreted as a destabilization of the C terminus of the IBB helix due to the loss of the single tryptophan located in that area (33). Conversely, it is possible that mutations W342A, W430A, and W472A do not individually play a critical role, as they can complement each other. The synergy of hot spot and silent mutants would derive from the fact that each of the three silent mutations on the N terminus of the IBB helix is enhanced by a mutation destabilizing the C terminus of the IBB helix, which leads to the inability of importin β to select the helical form of the IBB domain.

Conclusion—Our data strongly suggest that the importin β-IBB domain binding interface is not a static structural state wherein a protein and the ligand fit together as lock and key, as originally proposed by Chothia and Janin (36). Rather the molecular recognition of the IBB domain by importin β may be dissected into the following two distinct events: (i) the selection of a helical IBB domain sub-population inside the acidic groove of importin β, which stabilizes the IBB-helix (residues 22–51) via tryptophan residues; and (ii) the stable binding of the IBB-side chains arranged on the surface of the IBB-helix to the acidic binding cavity of importin β. The concept that the specificity of a NLS is encoded in its primary as well as secondary structure may be crucial for understanding the flexible yet highly specific recognition of a NLS by nuclear transport receptors.

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**FIG. 4.** Subcellular localization of microinjected importin β. Wild type (w.t.) importin β (a, right panel) and the triple mutant W342A/W430A/W864A (342×430×864; b, right panel) were microinjected in the cytoplasm of NRK cells together with a microinjection marker (a and b, left panels) to verify the integrity of the plasma membrane and cell nucleus.

**FIG. 5.** Model for involvement of tryptophans in selection of the helical IBB domain by importin β. Tryptophans 430, 472, and 864 (represented as space-filled objects) located at the N and C termini of the IBB-helix, respectively, are suggested to stabilize the interaction of the C-terminal domain of importin β with the IBB domain helical form. The IBB-helix is mostly unfolded when not bound to importin β, but is in equilibrium with a population of the helically folded form.

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**TABLE II**

| Pair         | Contribution (kcal/mol) |
|--------------|-------------------------|
| Trp-864 : Arg-51 | ~-5.41                 |
| Trp-472 : Lys-22  | ~-2.67                 |
| Trp-430 : Lys-18  | ~-4.61                 |
| Trp-342 : Arg-13  | ~-2.00                 |
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REFERENCES
1. Macara, I. G. (2001) *Microbiol. Mol. Biol. Rev.* 65, 570–594
2. Gorlich, D., and Kutay, U. (1999) *Annu. Rev. Cell Dev. Biol.* 15, 607–660
3. Mattaj, I. W., and Englmeier, L. (1998) *Annu. Rev. Biochem.* 67, 265–306
4. Cingolani, G., Bednenko, J., Gillespie, M. T., and Gerace, L. (2002) *Mol. Cell* 10, 1345–1353
5. Huber, J., Cronshagen, U., Kadokura, M., Marshallsay, C., Wada, T., Sekine, M., and Luhrmann, R. (1998) *EMBO J.* 17, 4114–4126
6. Nachury, M. V., and Weis, K. (1999) *Proc. Natl. Acad. Sci. U. S. A.* 96, 9459–9464
7. Lyman, S. K., Guan, T., Wodrich, H., and Gerace, L. (2002) *J. Cell Biol.* 159, 55–67
8. Mittwoch, U., and Arndt, A. (1999) *FEBS Lett.* 450, 145–149
9. Cingolani, G., Petosa, C., Weis, K., and Mueller, C. W. (1999) *Nature* 399, 221–229
10. Baylies, R., Lotsowy, T., and Stewart, D. (2000) *Cell* 102, 99–108
11. Vetter, I. R., Arndt, A., Kutay, U., Gorlich, D., and Wittinghofer, A. (1999) *Cell* 97, 635–646
12. Jakel, S., Mingot, J. M., Schwarzmaier, P., Hartmann, E., and Gorlich, D. (2002) *EMBO J.* 21, 377–386
13. Jones, T. A., Zou, J. Y., Cowan, S. W., and Kjeldgaard, M. (1991) *Acta Crystallogr. Sect. A* 47, 110–115
14. Esnouf, R. M. (1997) *Acta Crystallogr. Sect. D Biol. Crystallogr.* 55, 938–940
15. Merritt, E. A., and Bacon, D. J. (1997) *Methods Enzymol.* 277, 505–524
16. Ben-Efraim, I., and Gerace, L. (2001) *J. Cell Biol.* 152, 411–417
17. Delphin, C., Guan, T., Melchior, F., and Gerace, L. (1997) *Mol. Biol. Cell* 8, 2379–2390
18. Adam, S. A., Sterne-Marr, R., and Gerace, L. (1992) *Methods Enzymol.* 219, 97–110
19. Melchior, F. (1998) *Methods Mol. Biol.* 88, 265–273
20. Frutet, F., Guan, T., Subauste, C., Hahn, K., and Gerace, L. (2002) *J. Cell Biol.* 156, 617–633
21. Clackson, T., and Well, J. A. (1995) *Science* 267, 383–386
22. Rose, S., Imamoto, N., Tachibana, T., Shimamoto, T., and Yoneda, Y. (1997) *J. Cell Biol.* 139, 841–849
23. Dougherty, D. A. (1996) *Science* 271, 163–166
24. Gallivan, J. P., and Dougherty, D. A. (1999) *Proc. Natl. Acad. Sci. U. S. A.* 96, 9459–9464
25. Conti, E., Uy, M., Leighton, L., Bleher, G., and Kuriyan, J. (1998) *Cell* 94, 193–204
26. Conti, E., and Kuriyan, J. (2000) *Structure Fold. Des.* 8, 329–338
27. Malik, H. S., Eckbath, T. H., and Goldfarb, D. S. (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 13738–13742
28. Cingolani, G., Lashuel, H. A., Gerace, L., and Muller, C. W. (2000) *FEBS Lett.* 484, 291–296
29. Kube, B. (1999) *Nat. Struct. Biol.* 6, 388–397
30. Ye, X., Gorm, A., Frederick, R., Hu, W., Majumdar, A., Xu, W., McLendon, G., Ellington, A., and Patel, D. J. (1999) *Chem. Biol.* 6, 657–669
31. Kumar, S., Ma, B., Tsai, C. J., Sinha, N., and Nussinov, R. (2000) *Protein Sci.* 9, 10–19
32. Ma, B., Shatsky, M., Wolfson, H. J., and Nussinov, R. (2002) *Protein Sci.* 11, 184–197
33. DeLano, W. L. (2002) *Curr. Opin. Struct. Biol.* 12, 14–20
34. Burley, S. K., and Petsko, G. A. (1985) *Science* 229, 23–28
35. Burley, S. K., and Petsko, G. A. (1986) *FEBS Lett.* 203, 139–143
36. Chothia, C., and Janin, J. (1975) *Nature* 256, 705–758
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