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A Model of the Complex between Cyclin-Dependent Kinase 5 and the Activation Domain of Neuronal Cdk5 Activator

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Tau protein kinase II (TPKII) is a heterodimer comprising a catalytic cyclin-dependent kinase subunit (Cdk5) and a regulatory protein called neuronal Cdk5 activator (Nck5a). TPKII is somewhat reminiscent, therefore, of the Cdk2-cyclin complex important in cell cycle regulation. In fact, although the amino acid sequence of Nck5a has little similarity to those of cyclins, recent experimental results obtained by site-directed mutagenesis studies have indicated that its activation domain, Nck5a*, may adopt a conformation of the cyclin-fold structure. Based on this structural inference, a 3-dimensional model of the Cdk5-Nck5a*-ATP complex was derived from the X-ray structure of Cdk2-cyclinA-ATP complex. The computed structure for TPKII is fully compatible with experimental data derived from studies of the Cdk5-Nck5a system, and also predicts which amino acid residues might be involved in formation of the Cdk5-Nck5a* interface and ATP binding pocket in TPKII. The computational structure also shows the interactive region of Nck5a* and the T-loop of Cdk5, a critical region in TPKII which functions as a gate-control-lever of the catalytic cleft. Furthermore, a physical mechanism is put forth to explain why the activation of TPKII is not dependent upon phosphorylation of the Cdk5 subunit, a puzzle long-standing in this area. These findings provide a model with which to consider design of compounds which might serve as inhibitors of TPKII. **© 1999 Academic Press**

Key Words: **cyclin-dependent kinase 5; cyclin-box; neuronal Cdk5 activator; ATP binding pocket; T-loop.**

Ser/Thr protein kinases play an important role in regulation of the eukaryotic cell cycle [1, 2]. These regulatory kinases are referred to as cyclin-dependent kinases (Cdk) because the catalytic kinase subunit becomes active only in complexation with a regulatory cyclin subunit [3]. In the case of Cdk2, binding of the cyclin subunit lends basal kinase activity to the complex, but full activity is produced only after phosphorylation of a threonine residue in the T-loop region of Cdk2; this phosphorylation is catalyzed by Cdk-activating kinase (CAK). Therefore, the activation of Cdk2 is a two step process: (1) binding with cyclin to produce low level catalytic activity, and (2) subsequent phosphorylation by CAK to enhance the activity.

Cyclin-dependent kinase 5 (Cdk5) shows a high degree of sequence identity to Cdk2, but is not activated by cyclins. Rather, Cdk5 is activated by the neuronal-specific activator protein, Nck5a, a molecule which shows minimal sequence similarity to cyclins. Nck5a has also been referred to in the literature as p35, and p25, an N-terminally truncated form of p35 [4]. Although Cdk5 is distributed in tissues throughout the body, Nck5a is found solely in brain. Accordingly, Cdk5-Nck5a is thought to play a central role in the coordination of the balance between kinases and phosphatases involved in neurite outgrowth in postmitotic neurons [5]. The complex of Cdk5 and Nck5a has been termed tau protein kinase II or TPKII [6], and its putative involvement in tau hyper-phosphorylation leading to formation of neurofibrillary tangles (NFT) has suggested that TPKII might be a therapeutic target in Alzheimer's Disease (AD).

In contrast to the dual activation scheme for Cdk2 by cyclin subunits and phosphorylation, the Cdk5-Nck5a complex is fully active and neither requires, nor undergoes phosphorylation by CAK to enhance its catalytic activity [4]. Based upon studies with truncation mutants, it has recently been determined that the activation domain of Nck5a consists of the region spanning residues 150 to 291 [7], where residues 150-200 were sufficient for binding to Cdk5, and residues 279-291

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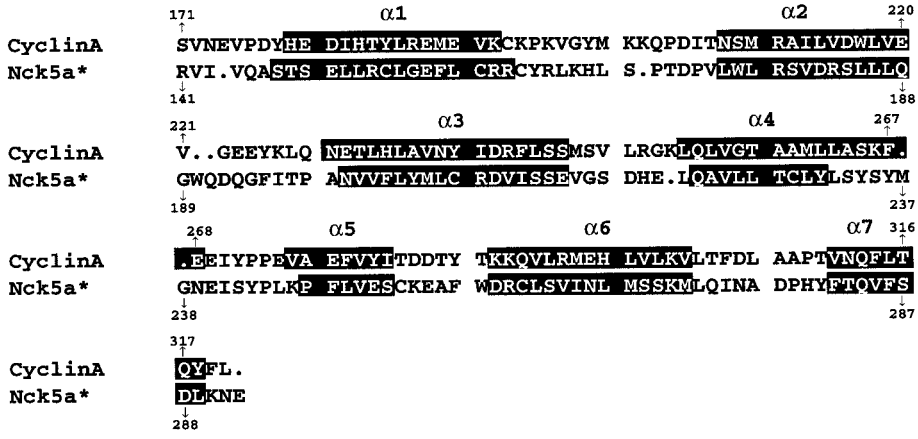

FIG. 1. Sequence alignment between human cyclinA and Nck5a* according to Tang et al. [7]. The sequence segments with white character codes inscribed in a black box represent the α-helix regions. There are seven helices, marked by α1, α2, α3, α4, α5, α6, and α7. They are respectively His179-Lys192, Asn208-Glu224, Asn229-Ser245, Leu253-Glu268, Val275-Ile281, Lys288-Val301, Val311-Tyr318 for the X-ray structure of cyclinA, and Ser147-Arg162, Leu-176-Gln188, Asn200-Glu215, Gln223-Tyr231, Pro247-Ser252. Asp259-Met272, and Phe282-Leu289 for the computed structure of Nck5a* (cf. Fig. 4).

were needed for activation of Cdk5 *in vitro* [4]. In fact, it has been known that the amino terminal segment of Nck5a is not required for activation of Cdk5 and that N-terminally truncated forms of the protein, such as p25 and p20, which contain the binding and activation regions defined above, are fully competent to activate the kinase [4].

In this report, we present a 3-dimensional model for the activation domain of Nck5a, in complexation with Cdk5 and ATP. We define the activation domain as Nck5a*. Modeling was done relative to the published X-ray structure of the Cdk2-cyclinA-ATP complex [8]. Since the human Cdk5-Nck5a complex is of potential significance in AD, we would hope that this model

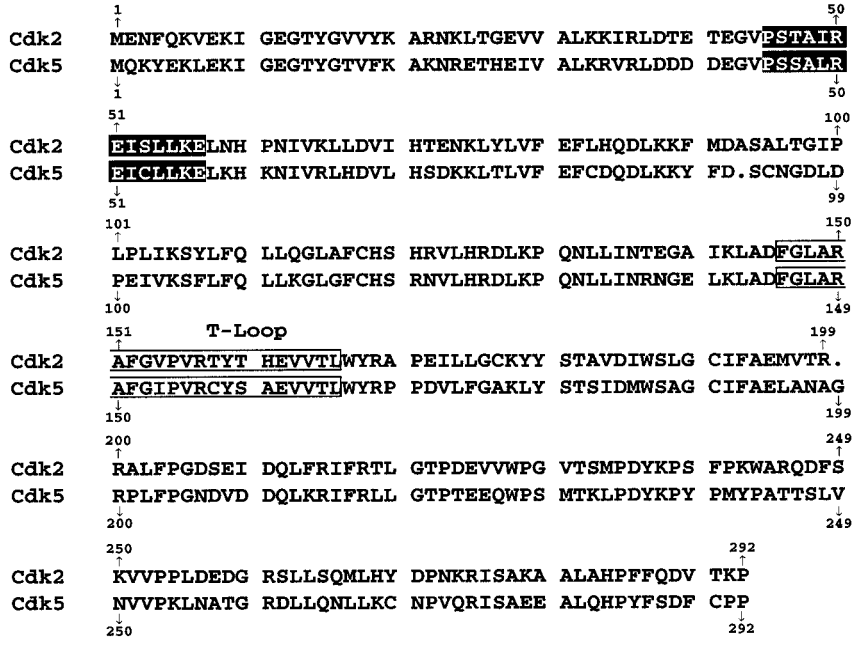

FIG. 2. Sequence alignment between human Cdk2 and Cdk5 obtained by using the BESTFIT program in the GCG package [10]. The two sequence segments with white character codes inscribed in a black box represent the PSTAIRE helix region of Cdk2 and the PSSALRE helix region of Cdk5, respectively. The sequence enclosed in an open box is the T-loop region. It consists of 21 residues: Phe146-Leu166 for Cdk2 and Phe145-Leu165 for Cdk5. With the exception of four residues, the sequence of the T-loop in Cdk5 is identical to that in Cdk2. Note that the sequence of Cdk5 as originally reported by Meyerson et al. [5] and deposited in SwissProt data bank contained two minor errors: (1) the residue at sequence position 89 should be K rather than N, and (2) the residue Y at position 90 was missed. The correct sequence of Cdk5 can be obtained from EMBL data bank with the Accession Number of X66364 (personal communication with the corresponding author of ref. 5).

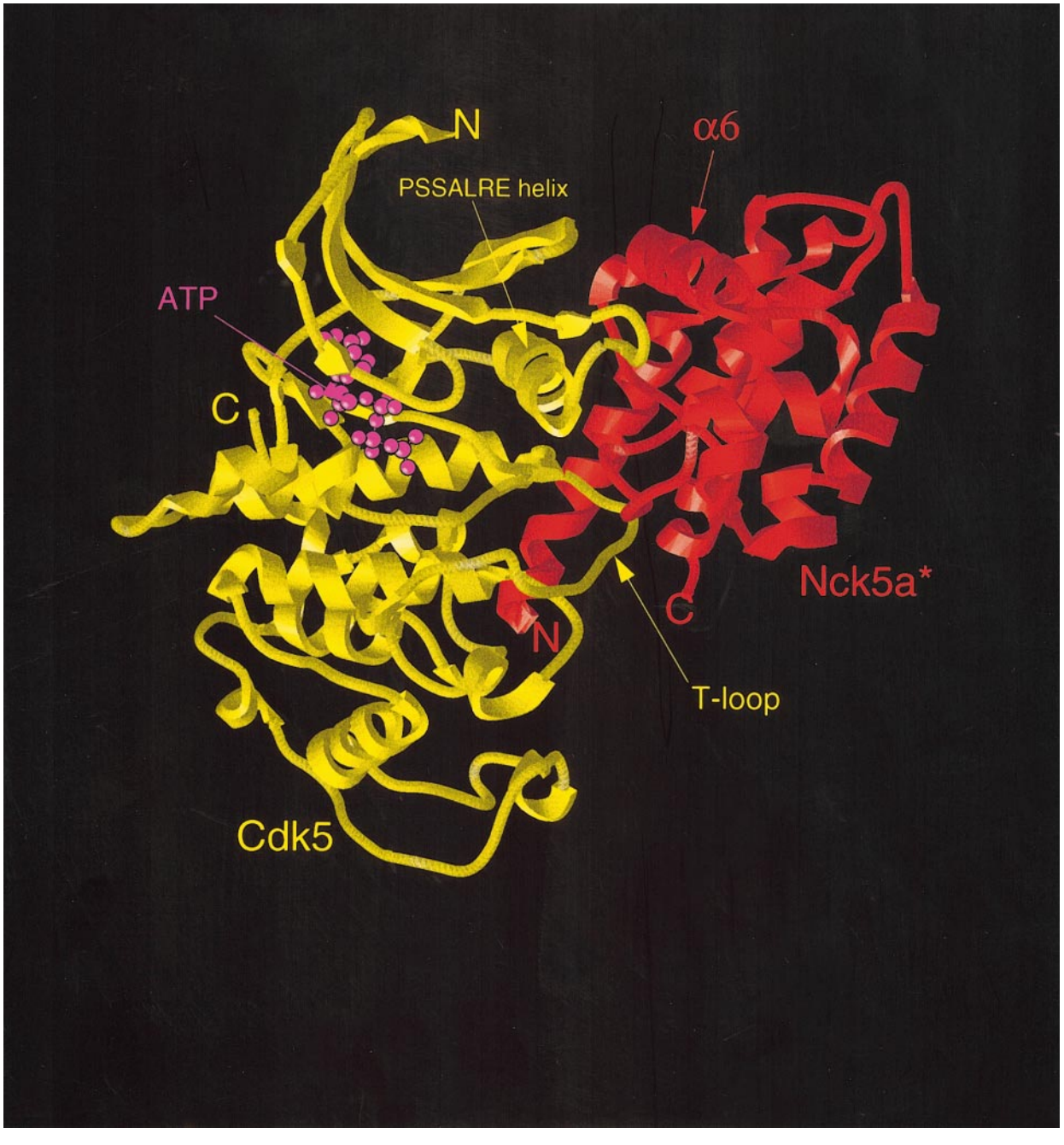

FIG. 3. A schematic drawing of the Cdk5-Nck5a*-ATP complex. Cdk5 is colored yellow, Nck5a* red, and ATP purple (shown as a ball-and-stick representation). The characters N and C colored with yellow and red indicate the N- and C-terminals of Cdk5 and Nck5a*, respectively. The Cdk5-Nck5a* interface is formed by an interlocking array of Cdk5 and Nck5a* elements, involving the PSSALRE helix, the T-loop, portions of the N- and C-terminal lobes from Cdk5 (cf. Fig. 2), and the 1st, 4th, 5th, 6th, and 7th helices from Nck5a* (cf. Figs. 1 and 4). The residues forming the Cdk5-Nck5a* interface are given in Table 1.

might provide a structural basis for the study of this important enzyme and of molecules which block its activity. Moreover, the model may help explain how a non-cyclin protein subunit is able to activate a cyclin-dependent kinase with no requirement for phosphorylation.

STRATEGY AND MODELING METHODOLOGY

Nck5a differs from other activators of cyclin-dependent kinases in that its amino acid sequence is only marginally similar to the cyclin consensus sequence. However, recent mutagenesis studies [7] have provided strong support to the suggestion that Nck5a may, in part, assume a conformation similar to that of cyclinA [9]. In Fig. 1, the

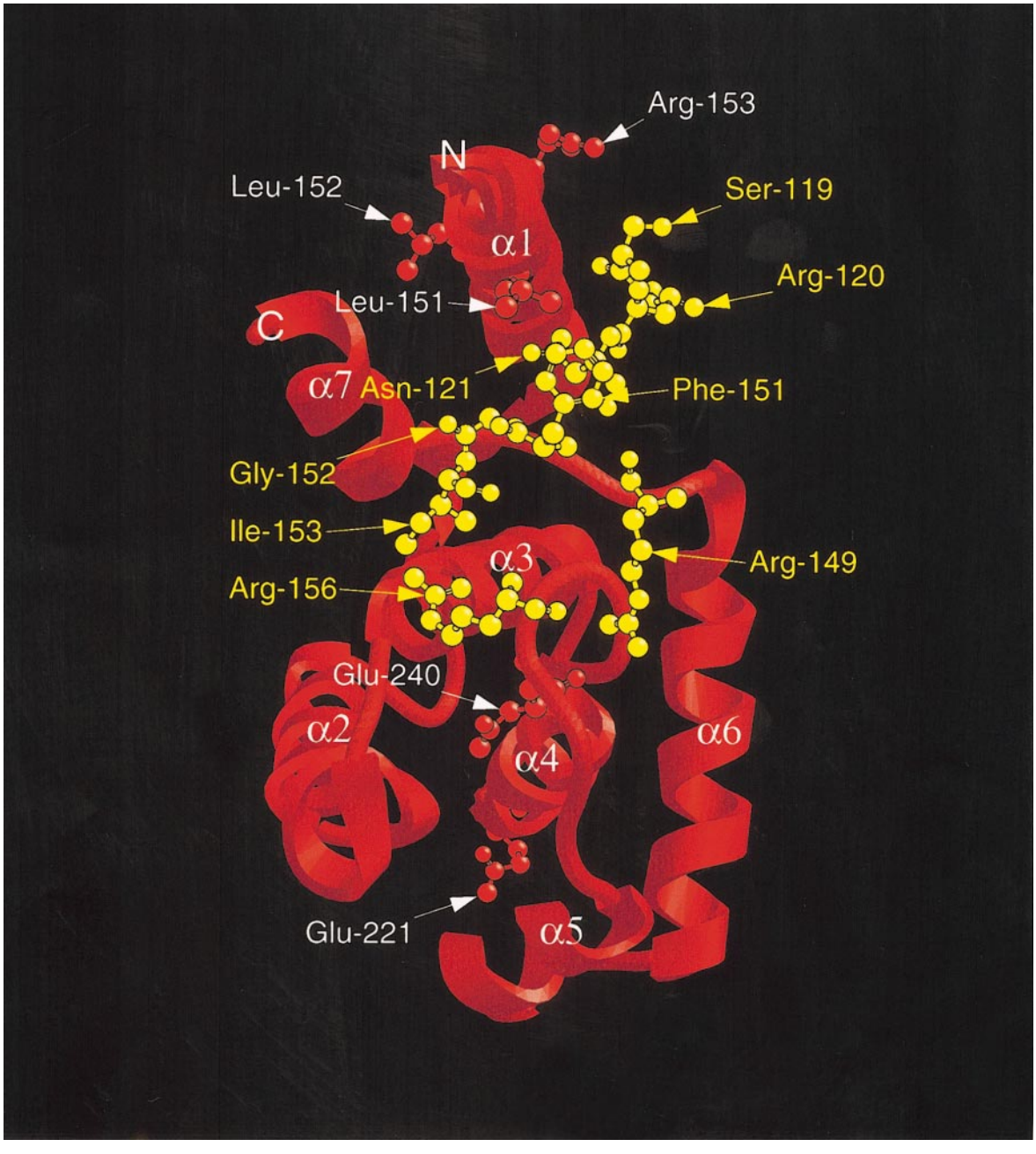

FIG. 4. A ribbon and ball-stick drawing to mark the seven helices, α1-α7, of Nck5a* and highlight some relevant residues discussed in the text. The residues of Cdk5 are colored yellow, and those of Nch5a* colored red (cf. Fig. 3).

sequence of the conserved core region of Nck5a (corresponding to the activation domain as termed Nck5a*) is aligned with that of cyclinA, based upon some key amino acid substitution mutants of Nck5a studied by Tang et al. [7]. They found that there are common structural features between Nck5a* and cyclinA, with respect to both protein folding and Cdk activation. This information provided a feasible avenue toward modeling of Nck5a* based upon the sequence alignment in Fig. 1, and the X-ray structure of cyclinA as the template.

As for Cdk5, it shares a high degree of sequence similarity with other Cdks [5]. The sequence alignment of Cdk2 and Cdk5 shown in Fig. 2 was obtained by the BESTFIT program in the GCG package [10]. Since the similarity and identity between Cdk5 and Cdk2 are 70% and 60%, respectively, the 3-dimensional structure of Cdk5 can be quite reliably modeled based on the X-ray structure of Cdk2. Moreover, given the arguments for the common mode of structural interactions in the Cdk2-cyclinA and Cdk5-Nck5a* complexes [7], coupled with the X-ray crystallographic structure of the Cdk2-cyclinA-ATP complex [8], it was possible to model structures for both Nck5a* and the Cdk5-Nck5a*-ATP complex.

In the present study, the segment match modeling method [11] was adopted during computation. This method uses a database of

known protein structures to build an unknown target structure from the amino acid sequence. The target structure is first broken into a set of short segments. The database is then searched for matching segments on the basis of amino acid sequence similarity and compatibility with the target structure. The process is repeated 10 times and an average model is generated, followed by energy minimization to create the final model. This procedure was originally shown to be highly accurate for eight test proteins ranging in size from 46 to 323 residues, where the all-atom root-mean-square deviation of the modeled structures is between 0.93 Å and 1.73 Å [11]. Recently, this method was successfully applied to model the structure of the protease domain of caspase-8 based on the X-ray structure of ICE [12], and to model the CARDs (caspase recruitment domains) of Apaf-1, caspase-9, Ced-4 and Ced-4 based on the NMR structure of RAIDD CARD [13]. The structure thus obtained for the Cdk5-Nck5a*-ATP complex was further refined by energy minimization with respect to all the side chains using AMBER (**A**ssisted **M**odel **B**uilding with **E**nergy **R**efinement) force field [14]. The final structure is given in Fig. 3. The examination by ProCheck [15] for the current model has indicated its rationality from the structural point of view.

RESULTS AND DISCUSSION

The features of the Cdk5 (yellow)-Nck5a* (red)-ATP (purple) complex shown in Fig. 3 are described as follows.

Overall structure. The structure of Cdk5 consists of two lobes: the N-terminal lobe (residues 1-85) is rich in β-strands; the C-terminal lobe (residues 86-291) is formed mostly by α-helices. ATP binds in a deep cleft between the two lobes which contains catalytic residues as will be discussed later. Nck5a* contains seven helices, α1, α2, α3, α4, α5, α6, and α7 (Figs. 1 and 4). It binds to one side of the catalytic cleft, interacting with both the N- and C-terminal lobes of Cdk5 to form a large, continuous protein-protein interface.

Structure of Nck5a.* The hydrophobic core of the Nck5a* subunit is its 4th helix (α4: Gln223-Tyr231), which plays a pivotal role in maintaining the cyclin-like fold. The rationale for the computed Nck5a* structure (Fig. 4) can be addressed by the following points**. (1)** According to the observation of Tang et al. [7], Leu151 and Leu152 of Nck5a are the two residues that contribute to Cdk5 activation by participating in an essential hydrophobic interaction. It was found from the computed structure that Leu151 and Leu152 are very close to Ser119, Arg120, and Asn121 of Cdk5, as well as its Phe151, Gly152, and Ile153. The last three residues are all within the sensitive activation-controlling T-loop region (Fig. 2). In particular, Phe151, which corresponds to Phe152 of Cdk2, is thought to be a key residue in interacting with Leu151 and Leu152 for activating Cdk5 [7]. **(2)** Although α1, the 1st helix of Nck5a*, is a part of the Cdk5-Nck5a* interface, it was observed that Arg153 of the helix had little effect on the activation of Cdk5 [7], suggesting that Arg153 has no direct contact with any residue in Cdk5. This is supported by the current structure, from which it can be seen that the side chain of Arg153 points away from the interface without any direct interaction with the residues of Cdk5, and its distance to the T-loop is even farther away. **(3)** Two glutamate residues in Nck5a*, Glu221 and Glu240, were substituted individually by alanine [7]. It was observed that while substitution of Glu221 had only little effect, substitution of Glu240 markedly decreased the kinase activation by Nck5a*. These workers assumed that like Glu269 in cyclinA, Glu240 of Nck5a* participates in the Cdk5 activation by interacting with Arg149 of the T-loop. This assumption is also fully supported by our computed structure, from which it is observed that the distance from Arg149 to Glu221 is greater than 26 Å. Accordingly, there is no direct contact whatsoever between Arg149 and Glu221 (Fig. 4). In contrast, the distance between Glu240 of Nck5a* and Arg149 of the T-loop is much closer. Actually, our model does predict that there is a hydrogen bond between Arg149 and Glu240 that helps to hold the T-loop of Cdk5 with Nck5a*, as will be further elaborated below.

Binding pocket of ATP. There is a deep cleft between the two lobes of Cdk5 that forms a pocket for ATP (Fig. 5). The constituents of the pocket are defined by those residues that have at least one heavy atom, i.e., an atom other than hydrogen, with a distance $\leq$5 Å from a heavy atom of the ATP. The pocket thus defined consists of 21 residues. They are Ile10, Gly11, Glu12, Gly13, Thr14, Val18, Ala31, Val64, Phe80, Glu81, Phe82, Cys83, Asp84, Asp86, Lys89, Lys128, Gln130, Asn131, Leu133, Ala143, and Asp144. It would be intriguing to probe the binding pocket by site-directed mutagenesis of single amino acids as an avenue to find out which of the 21 residues are the most sensitive for the catalytic activity.

Structure of the Cdk5-Nck5a interface.* The Cdk5-Nck5a* interface is formed by an interlocking array of Cdk5 and Nck5a* elements (Fig. 3), including the PSSALRE helix and T-loop (cf. Fig. 2), portions of the N- and C-terminal lobes from Cdk5, and the 1st, 4th, 5th, 6th, and 7th helices (cf. Figs. 1 and 4) from Nck5a*. A large surface area of 3,461 Å^2 is buried upon the binding of Cdk5 and Nck5a*. The residues involved in the Cdk5-Nck5a* interface are given in Table 1. The interface consists of 88 residues, of which 46 are from Cdk5 and 42 from Nck5a*. Note that Cdk5 was identified as a Cdk-related protein due to the sequence segment that has the conserved PSSALRE motif of cdc2 used for the kinase nomenclature [5]. The PSSALRE helix, which corresponds to the PSTAIRE helix of Cdk2 (Fig. 2), lies parallel to the 6th helix (α6) of Nck5a* (Fig. 3) and is surrounded by the C-terminal of the 4th helix, N-terminal of the 5th helix, as well as part of the loop between these two helices. The PSSALRE helix is thus bound by an extended patch of

TABLE 1

The Residues[a] Involved in the Cdk5-Nck5a* Interface

Cdk5			Nck5a*		
Asp-39	Asp-40	Asp-41	Arg-141	Val-142	Ile-143
Glu-42	Gly-43	Val-44	Val-144	Ala-146	Ser-147
Pro-45	Ser-46	Ser-47	Glu-150	Leu-151	Cys-154
Leu-49	Arg-50	Ile-52	Leu-155	Glu-157	Phe-158
Cys-53	Leu-54	Lys-56	Arg-161	Asn-200	Tyr-231
Glu-57	Val-69	His-71	Leu-232	Ser-235	Tyr-236
Ser-72	Asp-73	Lys-74	Met-237	Gly-238	Asn-239
His-118	Ser-119	Arg-120	Glu-240	Ile-241	Ser-242
Asn-121	Arg-125	Ala-148	Tyr-243	Lys-246	Pro-247
Arg-149	Ala-150	Phe-151	Asp-259	Arg-260	Ser-263
Gly-152	Ile-153	Pro-154	Asn-266	Leu-267	Ser-270
Val-155	Arg-156	Tyr-158	Gln-274	Ile-275	Asn-276
Glu-161	Thr-180	Ser-181	Ala-277	Asp-278	Thr-283
Val-272	Gln-273	Arg-274	Gln-284	Ser-287	Asp-288
Ile-275	Ser-276	Glu-278			
Glu-279					

[a] Residues listed under Cdk5 have at least one heavy atom with a distance ≤5 Å from a heavy atom of Nck5a*, and residues under Nck5a* have at least one heavy atom with a distance ≤5 Å from a heavy atom of Cdk5.

TABLE 2

List of Hydrogen Bonds between Cdk5 and Nck5a*

Cdk5	Nck5a*	Number of hydrogen bonds
Asp-39	Arg-260	2
Asp-39	Ser-263	1
Asp-40	Lys-246	1
Asp-40	Arg-260	2
Asp-41	Lys-246	1
Glu-42	Tyr-243	1
Val-44	Tyr-231	1
Ser-46	Ser-235	1
Gly-57	Arg-161	1
Ala-120	Glu-157	1
Arg-149	Glu-240	1
Ala-150	Met-237	1
Tyr-158	Ser-242	1
Val-272	Arg-141	1
Ser-276	Ile-143	1
Glu-278	Ser-147	1

hydrophobic interactions. The T-loop region consists of 21 residues (residues 145-165). The sequence of the T-loop in Cdk5 is almost the same as that in Cdk2, except for four residues (Fig. 2). In studying the binding of Cdk2 and cyclinA, the T-loop region of Cdk2 has been a major focus. This is because it was observed that the T-loop blocked the entrance to the catalytic cleft in the free kinase [8, 16], and binding of cyclinA induced large conformational and positional changes in the T-loop, significantly relieving the blockade of the catalytic cleft observed in free Cdk2.

*Hydrogen bonds between Cdk5 and Nck5a**. There are 469 hydrogen bonds that form a hydrogen bond network in the Cdk5-Nck5a*-ATP complex. Of the 469 hydrogen bonds, 296 are intra-molecular hydrogen bonds within Cdk5, 150 intra-molecular hydrogen bonds within Nck5a*, one intra-molecular hydrogen bond within ATP, 18 inter-molecular hydrogen bonds between Cdk5 and Nck5a* (Table 2), and four inter-molecular bonds between Cdk5 and ATP. The residues in Cdk5 that are involved in forming hydrogen bonds with ATP are Glu-81, Cys-83, Gln-130, and Asp-144. The hydrogen bonds between the T-loop and the other part of the complex are listed in Table 3, and the hydrogen bonds between the PSSALRE helix and the other part of the complex are listed in Table 4.

Why is phosphorylation not required for the activation of Cdk5-Nck5a?* A major difference in the mode of activation of Cdk2-cyclinA as compared with that for Cdk5-Nck5a is that the former complex involves obligate phosphorylation of a threonine residue in the T-loop by CAK [3]. Cdk5-Nck5a neither requires, nor undergoes phosphorylation in the T-loop. So far there is no explanation for this difference [7]. However, a comparison of our model with the unphosphorylated Cdk2-cyclinA-ATP complex [8] in the following three aspects may shed some light on this question. **(1) Energy barrier.** As reported by Russo et al. [16], the binding of the phosphate group in the Cdk2 pocket moves the phosphorylated Thr-160 side chain by 6.1 Å, and the backbones of residues 154-162 in the T-loop are displaced by 5.3-7.1 Å. Meanwhile, a dra-

TABLE 3

List of Hydrogen Bonds[a] between the T-loop[a] and the Other Part of Cdk5-Nck5a*

T-loop	Cdk5-Nck5a*	Number of hydrogen bonds
Phe-151	Asn-121 of Cdk5	1
Arg-149	Leu-123 of Cdk5	1
Thr-164	Asp-126 of Cdk5	1
Gly-146	Arg-50 of Cdk5	1
Leu-147	Arg-125 of Cdk5	1
Arg-149	Leu-123 of Cdk5	1
Val-155	Tyr-179 of Cdk5	1
Tyr-158	Tyr-179 of Cdk5	1
Glu-161	Arg-125 of Cdk5	2
Glu-161	Arg-50 of Cdk5	2
Glu-161	Arg-125 of Cdk5	1
Val-163	Arg-168 of Cdk5	2
Thr-164	Tyr-167 of Cdk5	1
Leu-165	Arg-168 of Cdk5	1
Leu-165	Gln-211 of Cdk5	1
Cys-157	Lys-177 of Cdk5	1
Ala-150	Met-237 of Nck5a*	1
Arg-149	Glu-240 of Nck5a*	1
Tyr-158	Ser-242 of Nck5a*	1

[a] The T-loop of Cdk5 is in the region of residues 145-165 (see Fig. 2).

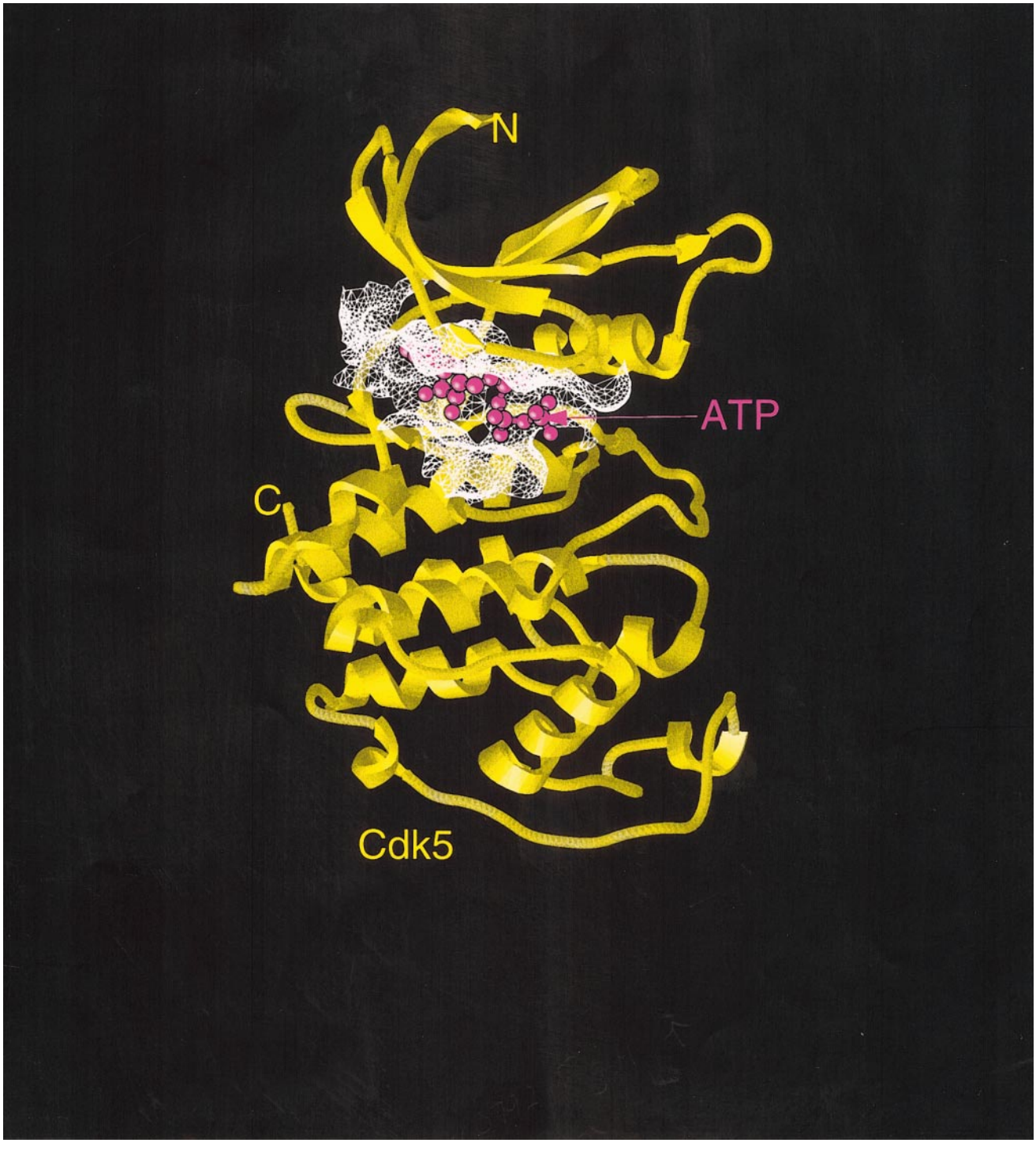

FIG. 5. The binding pocket of ATP, consisting of the following 21 residues: Ile10, Gly11, Glu12, Gly13, Thr14, Gly16, Val18, Ala31, Val64, Phe80, Glu81, Phe82, Cys83, Asp84, Asp86, Lys127, Gln129, Asn130, Leu132, Ala142, and Asp143. The yellow mesh represents the van der Waals surfaces of those heavy atoms that are within 5 Å from the ATP and form its pocket as defined in the text.

matic conformational change also occurs for the PSTAIRE helix with a rotation of roughly 90 degrees about its helical axis, so that the backbone atoms in the helix are as much as 8.5 Å away from their counterparts in free Cdk2. But for the Cdk5-Nck5a* complex it is very difficult to have the T-loop make such a dramatic internal movement because it is tightly tethered by 22 hydrogen bonds to the other part of the complex (Table 3), as

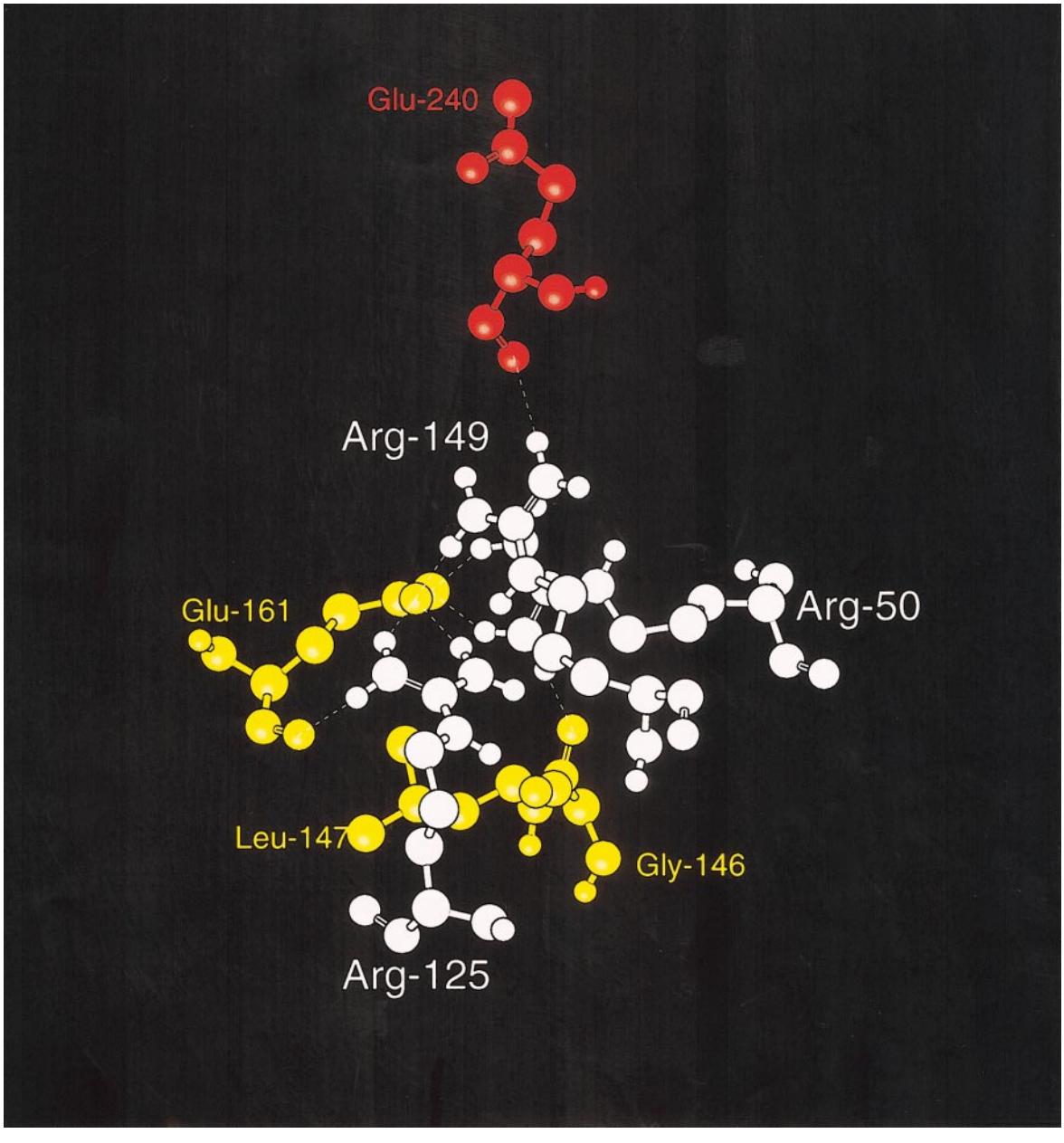

FIG. 6. The nine hydrogen bonds that neutralize the side chains of Arg-50, Arg-124, and Arg-148. The relevant residues of Cdk5 are printed in yellow, and those of Nck5a* in red. The hydrogen bonds are represented by white dotted lines.

compared with 14 hydrogen bonds for the case of the unphosphorylated Cdk2-cyclinA complex. Also, according to our model, it is hard to make the corresponding PSSALRE helix in Cdk5-Nck5a* undergo the kind of dramatic movement seen in the PSTAIRE helix of Cdk2 because the PSSALRE helix is tightly held by 13 hydrogen bonds to the other part of the complex (Table 4), compared with only four hydrogen bonds in the case of unphosphorylated Cdk2-cyclinA. In other words, a very high energy barrier must be overcome in order to allow a similar type of phosphorylation occurring in the Cdk5-Nck5a complex. **(2) Hydrogen bond network transduction.** The residue to be phosphorylated in Cdk2-cyclinA-ATP complex is Thr-160, whose side chain forms a hydrogen bond with the side chain of His-161. The corresponding residue in Cdk5 that might undergo phosphorylation is Ser-159 (Fig. 2). However, Ser-159 does not form any hydrogen bond with other residues in the Cdk2-Nck5a* complex, implying that Ser-159 might not be able to trigger off the aforementioned conformational changes in Cdk5 through the hydrogen bond network as Thr-160 does in Cdk2 even after being phosphorylated. This is

TABLE 4

List of Hydrogen Bonds[a] between the PSSALRE Helix[a] and the Other Part of Cdk5-Nck5a* Complex

PSSALRE helix	Cdk5-Nck5a*	Number of hydrogen bonds
Glu-51	Tyr-15 of Cdk5	1
Glu-51	Lys-33 of Cdk5	1
Leu-54	Leu-58 of Cdk5	1
Lys-56	Lys-59 of Cdk5	1
Glu-57	Lys-59 of Cdk5	2
Glu-57	Arg-120 of Cdk5	2
Arg-50	Gly-146 of Cdk5	1
Arg-50	Glu-161 of Cdk5	2
Glu-57	Arg-161 of Nck5a*	1
Ser-46	Ser-235 of Nck5a*	1

[a] The PSSALRE helix of Cdk5 is in the region of residues 45-57 (see Fig. 2).

fully consistent with the fact that mutation of Ser-159 to an alanine residue has no effect on enzyme activity [4]. **(3) Charge neutralization.** As pointed out by Russo et al. [16], the physical basis for the phosphorylation of Cdk2-cyclinA is that the cationic side chains of Arg-50, Arg-126, and Arg-150 in Cdk2 need to be neutralized by charge-stabilized hydrogen bonds with the three oxygens of the phosphate. However, for the current Cdk5-Nck5a*-ATP model, the situation is completely different. According to the sequence alignment (Fig. 2), the corresponding three arginines in Cdk5 are Arg-50, Arg-125, and Arg-149. As shown in Fig. 6, their side chains in the Cdk5-Nck5a* complex have already been neutralized by forming nine hydrogen bonds with the side chain of Glu-161 and the backbone carbonyl groups of Gly-146 and Leu-147 in Cdk5 as well as the side chain of Glu-240 in Nck5a*, and hence need not be neutralized by phosphorylation.

CONCLUSION

The Cdk5-Nck5a*-ATP complex was modeled based on the X-ray structure of Cdk2-CyclinA-ATP complex. Although many of the amino acid residues in cyclinA that are in direct contact with Cdk2 are not conserved in Nck5a*, the results thus obtained are fully compatible with previous theoretical hypotheses [9, 17] and experimental deductions [7]. The computed structure of the Cdk5-Nck5a*-ATP complex offers a 3-dimensional model for studying the activation domain of neuronal Cdk5 activator, and its interaction with Cdk5. Particularly, the structure presented here can be used to address the long-standing question concerning why the activation of Cdk5-Nck5a is not dependent upon phosphorylation.

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