Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 10618–10622, November 1993
Biochemistry

A conserved helix motif complements the protein kinase core

MICHEL VERON*, ELZBIETA RADZIO-ANDZELM†, IGOR TSIGELNY†, LYNN F. TEN EYCK†‡, AND SUSAN S. TAYLOR†§

*Unité de Biochimie Cellulaire, Centre National de la Recherche Scientifique: URA1129, Institut Pasteur, 75724, Paris, Cedex 15, France; †Department of Chemistry, 0654, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0654; and ‡San Diego Supercomputer Center, P.O. Box 85608, San Diego, CA 92186-9784

Communicated by Helen M. Ranney, July 9, 1993 (received for review May 19, 1993)

ABSTRACT **Residues 40–300 of the mammalian catalytic (C) subunit of cAMP-dependent protein kinase define a conserved bilobal catalytic core shared by all eukaryotic protein kinases. Contiguous to the core is an extended amphipathic α-helix (A helix). Trp^{30}, a prominent feature of this helix, fills a deep hydrophobic pocket between the two lobes on the surface opposite to the active site. The C subunit in *Dictyostelium discoideum* shows sequence conservation of residues 40–350 with the mouse enzyme but contains an N-terminal extension of 332 residues. A sequence corresponding to the A helix contiguous to the core is absent. However, we have now identified a remote A-helix motif (residues 77–98). When the core of the *Dictyostelium* C subunit was modeled, based on the mouse C subunit, complementarity between this putative A helix and the surface of the core was found to be conserved. Analysis of other protein kinases reveals that the A-helix motif is not restricted to cAMP-dependent protein kinase. In the Src-related family of protein kinases, for example, an A helix is very likely contiguous to the core, thus serving as a linker between the conserved catalytic core and the Src homology 2 domain. We predict that an A-helix motif complementary to the core will be a conserved feature of most eukaryotic protein kinases.**

cAMP-dependent protein kinase (cAPK), one of the first protein kinases to be purified (1), is one of the simplest members of a large family of enzymes that play key regulatory roles in all eukaryotic cells. The sequence similarities between the catalytic (C) subunit of cAPK and $pp60^{src}$ (2) first established that these diverse enzymes share a conserved catalytic core (3). The C subunit thus serves as a prototype for all protein kinases (4). Being the first crystallized protein kinase, the C subunit also provides a template for the proposed folding of the polypeptide chain in this family and defines where the highly conserved residues are located (5–7).

The conserved core includes residues 40–300 in the mammalian C subunit (m-C) (8). Residue 43 initiates an antiparallel β-sheet, the prominent feature of the small lobe. The large lobe, beginning at residue 128, is dominated by α-helices. The single strand, Glu^{121} to Glu^{127}, linking the two domains is a key feature of the adenine binding pocket (6).

A dominant feature of the first 40 residues that lie outside the core is the A helix, an α-helix that spans the surface of both lobes. A myristoyl moiety at the N terminus stabilizes the enzyme (9, 10). The C-terminal 50 residues wrap as an extended chain around the surface of both lobes (5, 6).

m-C, one of the smallest protein kinases, is highly conserved in size and sequence throughout the vertebrates, as well as in *Aplysia* (11), *Caenorhabditis elegans* (12), and *Drosophila* (13). Only in more primitive phyla does its size vary. The yeast C subunits, for example, TPK1–3, all contain additional sequence at the N terminus (14). A putative C subunit (Dd-C) also was recently identified in *Dictyostelium discoideum*. Although this enzyme contains an additional 332 residues at its N terminus, subsequent expression of the kinase core (15) and characterization of the full-length protein in *Dictyostelium* (16) established that this full-length protein is the *in vivo* C subunit of cAPK. The sequence similarities between Dd-C and m-C begin with residue 40 in m-C and extend through the C terminus (Fig. 1). However, in considering this structure of a diversified C subunit that still reassociates with regulatory (R) subunits and is activated by cAMP (15, 16) an immediate question arises about the exposed surface occupied by the A helix in m-C. The conserved nature of this surface in the *Dictyostelium* enzyme led us to search for a complementary sequence in the large N-terminal extension. Although much of this proline-rich sequence consists of glutamine, threonine, and asparagine repeats, between residues 77 and 98 lies a sequence that shares similarities with the A-helix motif of m-C (Fig. 1). We propose that this segment corresponds to the missing A helix and complements the surface of the core of the *Dictyostelium* enzyme. Analysis of the overall protein kinase family suggests that the A-helix motif is a conserved feature of many protein kinases.

METHODS

Modeling. To test our hypothesis, two structures, Dd-C and $pp60^{c\text{-}src}$, were modeled based on the coordinates of the mouse C subunit as described (17, 18). The sequences were first aligned manually and then fit into the HOMOLOGY program (version 2.0; Biosym Technologies, San Diego). After the side chains were replaced, the model was subjected to energy minimization with DISCOVER (version 2.8; Biosym Technologies) until the maximum gradient of any atom was <0.005 kcal/(mol·Å). The backbone atoms in the region of interest were not altered during the replacement procedure and were kept fixed during energy minimizations.

RESULTS AND DISCUSSION

The A-Helix Motif and Its Complementarity to the Core. Prior to considering other protein kinases, the sites of interaction between the N-terminal segment and the conserved catalytic core in the mouse C subunit were mapped. Residues 1–14, encoded by exon I (19), define a myristoylation motif (20), a conserved feature of the mammalian, *Drosophila*, and *Aplysia* C subunits. The recombinant enzyme is not myristoylated, and residues 1–14 cannot be visualized (5). In the crystal structure of the intact mammalian enzyme, the myristoyl moiety occupies a hydrophobic pocket and anchors the

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: cAPK, cAMP-dependent protein kinase; C subunit, catalytic subunit; m-C, mammalian C subunit; Dd-C, *Dictyostelium discoideum* C subunit; PTK, protein-tyrosine kinase; SH2, Src homology 2.

§To whom reprint requests should be addressed.

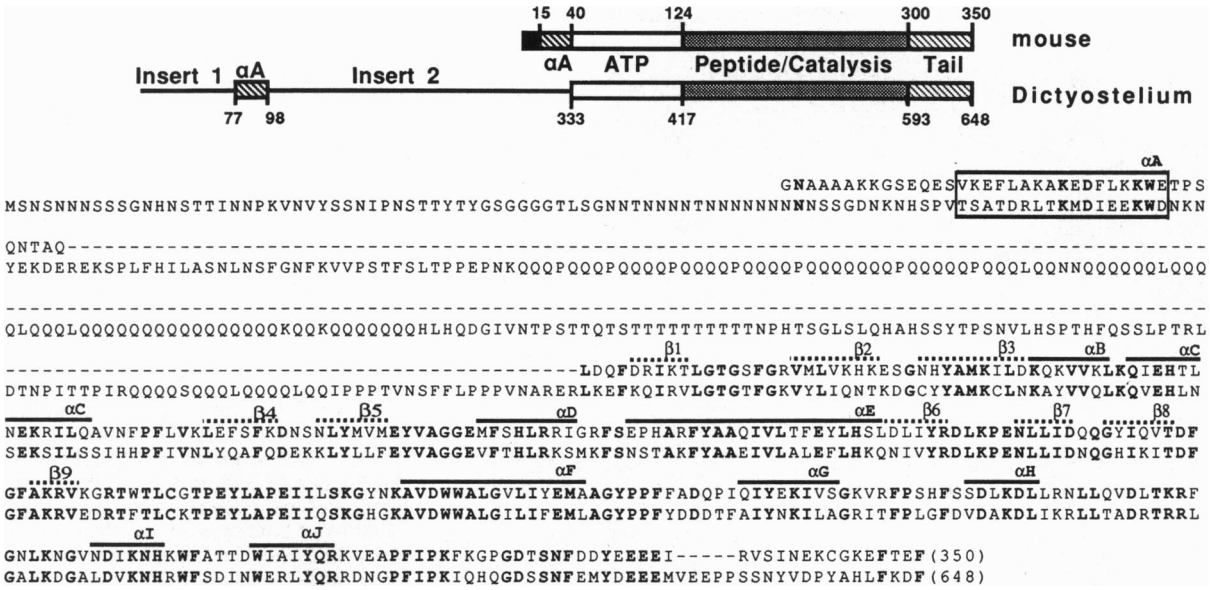

FIG. 1. Sequence alignment and modular structure of the mammalian and *Dictyostelium* C subunits. (*Upper*) Structural motifs associated with both C subunits. (*Lower*) Sequence alignment correlated with features of secondary structure (α-helices A–J, solid overlines; β-strands 1–9, dotted overlines) based on the crystal structure of the mouse C subunit. The position of the A helix (αA) (boxed) is based on the coordinates of the recombinant C-subunit ternary complex (6), where residues 1–14 were not visualized.

N terminus firmly to the core (10). Residues 15–35, defined here as the A-helix motif, are encoded by exon II (19). The characteristic feature of this segment is an amphipathic helix that binds to the surface of both lobes. The space-filling model in Fig. 2 shows the general topology of this surface on the core and its interactions with the A-helix motif, whereas specific interactions are shown in Fig. 3*A*.

A prominent feature of the A helix is Trp^{30}, which fills a deep hydrophobic pocket between the two lobes on the surface opposite to the active site (Fig. 4*A*). Phe^{26} also fills this pocket. The residues comprising this pocket originate from both lobes. The indole ring of Trp^{30} is stabilized, in particular, by stacking between Arg^{93} from the small lobe and Arg^{190} from the large lobe. The close proximity of Thr^{197}, an essential phosphorylation site in m-C (22), is also shown in Figs. 2 and 3*A*.

Dd-C Contains an A-Helix Motif. After recognizing that a potential A-helix motif might lie in the region that is noncontiguous to the core, we first modeled the core (residues 333–593) of Dd-C. The overall sequence identity in this core is 54% (Fig. 1). The C-terminal region, residues 594–648, shows 40% identity, but since this segment does not interact directly with the A-helix motif, it was not included in the model. Residues 77–98 were then modeled into an α-helix and an extended chain according to the alignment shown in Fig. 1. After removal of poor contacts and strains by energy minimization of all side chains, this modeled A-helix motif was superimposed onto the exposed surface of the core and again subjected to energy minimization.

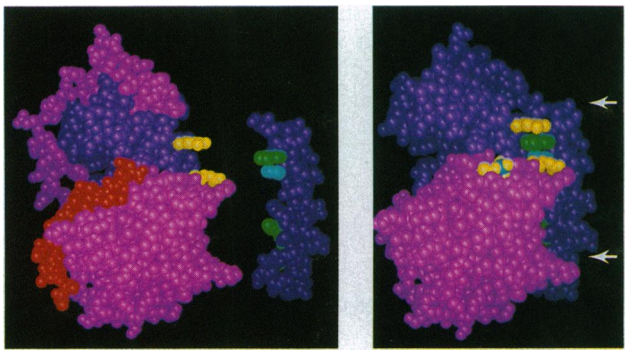

FIG. 2. Space-filling model of the binary complex of the mammalian C subunit showing interactions of the N-terminal helix and acylation site with the core. Residues 1–127 are purple, residues 128–350 are pink, and the peptide is red. Arrows show the A-helix boundaries. Trp^{30} and the myristate are green, Phe^{26} is turquoise, and Arg^{190} and Arg^{93} are yellow. (*Left*) Entire C subunit with residues 1–34 displaced to the right. (*Right*) Core plus residues 1–34. Thr^{197} is shown in yellow with the phosphorous in blue. Figs. 1, 3, and 4 were generated with INSIGHT II (Biosym Technologies, San Diego).

Like m-C, a dominant conserved feature of this model of Dd-C, shown in Fig. 3*B*, is a tryptophan residue (Trp^{91}) that protrudes from the A-helix and fills a hydrophobic pocket precisely between the two lobes (Figs. 3*B* and 4*B*). This tryptophan in m-C is anchored in part by hydrophobic interactions with two arginines. Arg^{190}, at the end of β-strand 9 in the large lobe and beneath the tryptophan ring, is conserved as Arg^{483} in Dd-C. Unlike m-C, where this arginine is hydrogen-bonded to the α-carbonyl of Trp^{30}, Arg^{483} can ion pair with the side chain of Glu^{88} in the proposed A helix, and this could contribute further stability. The other arginine in m-C, Arg^{93}, is above the tryptophan ring and lies at the end of the C helix in the small lobe. Arg^{93} follows the conserved glutamate, Glu^{91}, an invariant feature of the ATP binding site in all protein kinases. In Dd-C, Arg^{93} is replaced with serine (Ser^{386}). Ser^{386} can hydrogen bond with the side chain of Lys^{94}, and Lys^{94}, in turn, is fixed by interactions with the α-carbonyl of Trp^{91} in the A helix and Ser^{390}. The methylene carbons of Lys^{94} also provide hydrophobic contacts to the tryptophan ring. Phe^{26} in m-C is replaced with Ile^{87}, thus conserving the hydrophobic nature of this site. The side chain of Lys^{90} is also within hydrogen-bonding distance of the α-carbonyl of Ser^{389}.

From modeling of the core and the potential complementary A-helix motif, it is clear that Dd-C does, in fact, resemble the intact mammalian enzyme in its entirety except for the myristoylation motif. We predict, specifically, that residues 99–333 constitute a large insert whereas residues 77–98 comprise the missing A-helix motif and complement the exposed surface of the core (see Fig. 7). Residues 1–76 constitute an additional insert. The function of the two large inserts remains to be established.

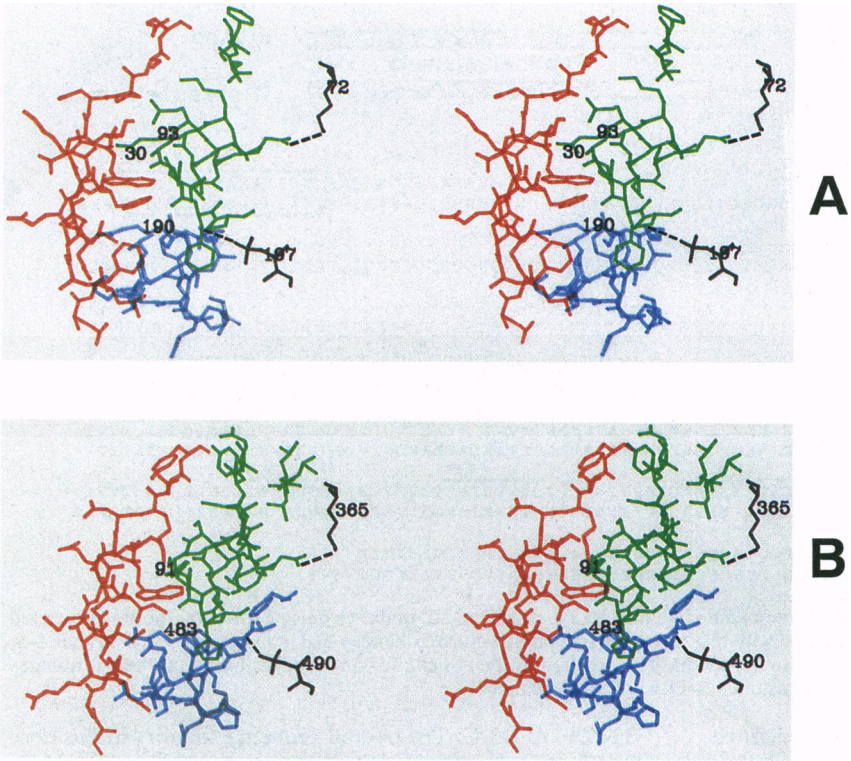

FIG. 3. Stereoview of residues in the A helix interacting with residues in the core. (*A*) Structure of the recombinant mouse C subunit (21). The A-helix motif is red, the small lobe green, and the large lobe blue. Lys[72] and Glu[91] (conserved features of the ATP binding site) and their salt bridge are black. Phospho-Thr[197] in the large lobe is shown in black with its salt bridge to Lys[189] shown as a dashed line. (*B*) Model of the corresponding region in Dd-C. The proposed A-helix motif is red, the small lobe green, and the large lobe blue. Lys[375], equivalent to Lys[72] in the mouse enzyme, is shown in black, with its salt bridge to Glu[394] indicated as a dashed line. Phospho-Thr[490] is shown in black with its salt bridge to Arg[482] shown as a dashed line.

General Features of A-Helix/Core Interactions. The modeled interactions of the A-helix motif with the core in Dd-C share many common features with m-C. These contacts, mapped diagrammatically in Fig. 5, constitute an extended network of interactions between the A-helix motif and many elements of secondary structure in both lobes. Although the precise nature of each interaction site is not conserved with respect to hydrophobic contacts and hydrogen bonding, complementarity is conserved. The general features of the A-helix motif and its complementarity to the core can be summarized as follows. (*a*) The general topography of the core surface with a deep hydrophobic cleft between the lobes is conserved. (*b*) The hydrophobic surface of the A-helix complements this surface. While the A helix might vary in length, a tryptophan near the end of the A helix is critical to fill the hydrophobic pocket. (*c*) The hydrophobic pocket consists of conserved motifs from both lobes. In the small lobe, the segment extending from the end of the C helix to the apex of the αC–$\beta 4$ loop provides the major contact sites for the A-helix motif. This region has a sequence motif, EXXhhXXhZ, where E is the invariant glutamate that ion pairs with Lys[72] and the h residues are hydrophobic. The first two hydrophobic residues contribute to the pocket that binds the tryptophan. Residue Z ring stacks with a conserved aromatic residue in the E helix. This αC–$\beta 4$ loop extends over the surface of the large lobe and is sandwiched between the large lobe and the A helix. (*d*) Two residues in β-strand 9, Lys[189] and Arg[190], bridge the A helix with the active site in m-C and provide an important link that traverses the large lobe and links the hydrophobic pocket with the essential phosphorylation site Thr[197]. The proximity of the hydrophobic pocket to the phosphorylated Thr[197] is shown in Figs. 2 and 3. Both Lys[189] and Arg[165] (which precedes the catalytic loop) bind this phosphate and render it phosphatase-resistant (21). Arg[165] is conserved in most protein kinases, and many protein kinases are also phosphorylated at a serine, threonine, or tyrosine that is analogous to Thr[197] (9, 23).

Implications for the Protein Kinase Family. Is this observation unique to the C subunits of cAPK or is it a more general motif that is an important feature of all protein

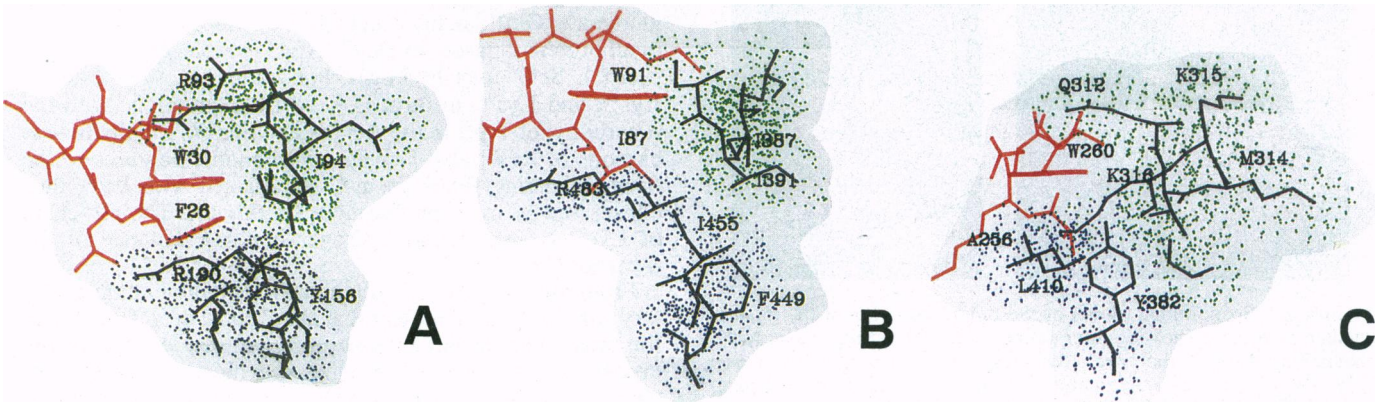

FIG. 4. The hydrophobic pocket filled by a tryptophan from the A helix. The major hydrophobic residues lining the pocket are indicated with their corresponding electron shell. Residues in the small lobe are green and those in the large lobe are blue. The A-helix segment filling this pocket is red. (*A*) m-C. (*B*) Dd-C model. (*C*) pp60[src] model. The van der Waals radii used for C, N, O, and S atoms were 1.55, 1.40, 1.35, and 1.81 Å, respectively, as specified in INSIGHT II.

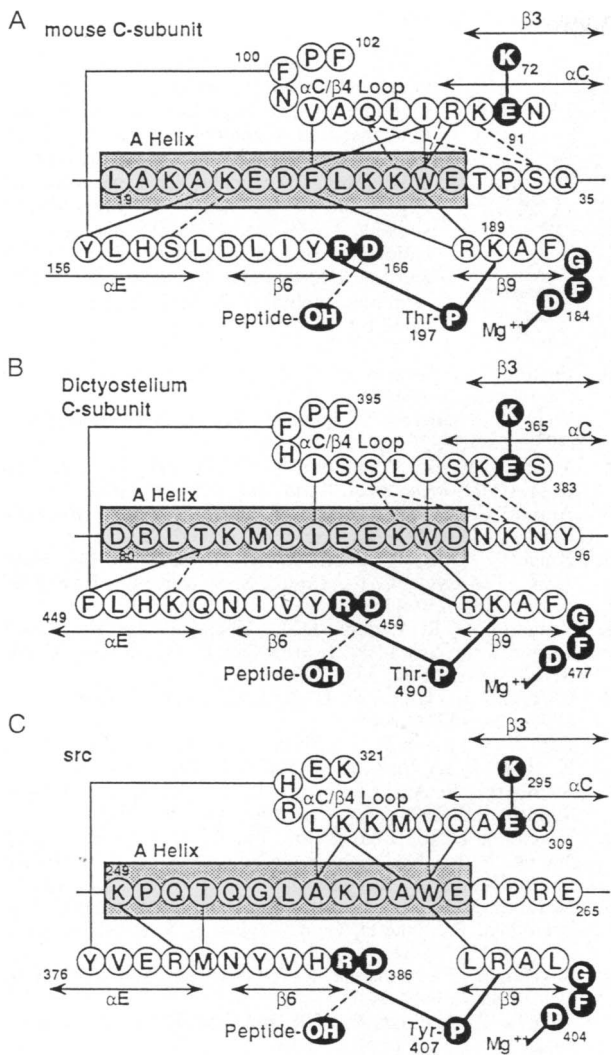

FIG. 5. Mapping of interactions between the A-helix motif and the core. Distances of <3.5 Å were counted as sufficient for electrostatic (thick line) or hydrogen (broken line) bonding, whereas distances of <4.0 Å were considered sufficient for hydrophobic (thin line) contacts. (*A*) m-C. (*B*) Dd-C. (*C*) pp60^{src}.

kinases? In searching for the A-helix motif, there are at least four possibilities. (*i*) Like m-C, it can be contiguous to the core. (*ii*) Like Dd-C, it can lie N-terminal but not contiguous to the core. (*iii*) It could lie C-terminal to the core. (*iv*) It could be part of another subunit. In manually searching for A-helix motifs, we focused, in particular, on a conserved tryptophan to align with Trp^{30} in the m-C A helix.

A general survey of the protein kinase family reveals that the A-helix motif is by no means unique to cAPKs. Several, in fact, very likely have amphipathic α-helices that lie contiguous to the core (Fig. 6). These helices may not be as long as the A helix in m-C, but all have a conserved Trp near what is predicted to be the C terminus of a helix. The most striking examples are the PTKs, where at least 50% seem to have an A-helix motif. The Src-related PTKs, in particular, all appear to have a contiguous A-helix that links the Src homology 2 (SH2) domain and the core. Some of the receptor PTKs, such as the insulin receptor (29) and the platelet-derived growth factor receptor (30), appear to have a contiguous A helix whereas others, such as the epidermal growth factor receptor, do not or at least do not have tryptophan as part of the motif.

Possible examples of protein kinases that, like Dd-C, contain a noncontiguous A helix N-terminal to the core are the Ca^{2+}/phospholipid-dependent protein kinases (protein kinases C) and the myosin light-chain kinases. These enzymes do not appear to have amphipathic helices contiguous to the core but have several conserved tryptophans in the large N-terminal segment preceding the core. The yeast C subunits of cAPK (14) have no conserved tryptophans in the extended sequences preceding the core but do have several conserved aromatic residues that are potential candidates for an A-helix motif. We have not yet clearly identified cases where the helix might be provided by an extension C-terminal to the core.

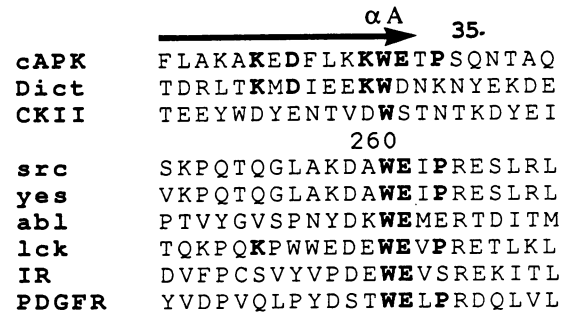

FIG. 6. Sequence alignment of the A-helix motifs. Sequences are shown for protein-serine/threonine kinases [cAPK, m-C; Dict, Dd-C; CKII, casein kinase II (24)] and protein-tyrosine kinases (PTKs) [Src (25); Yes (26); Abl (27); Lck (28); IR, insulin receptor (29), and PDGFR, platelet-derived growth factor (30)].

Some small protein kinases, such as cdc2 (31) and the mitogen-activated protein kinases (32), do not contain extensions at either end of the core. Therefore, if they have a missing A helix, it must be provided by another protein. Suc1 or the cyclins are potential candidates for cdc2, and recent modeling of cdc2, based on the crystallographic coordinates of m-C and charged-to-alanine mutagenesis of cdc2, predicts that Suc1 is the most plausible (23). The structure of cdk2, a homolog of cdc2, was solved recently and confirms that the surface that complements the A-helix motif in m-C is conserved (33).

The A-Helix Motif in the Src Family of PTKs. To test our prediction that the Src family has an A-helix motif, the core of pp60$^{c\text{-}src}$ was modeled. While there are some important inserts near the active site for the PTKs (18), these are not important for consideration here and can be ignored. Modeling reveals immediately a deep hydrophobic pocket between the lobes (Fig. 4*C*), as in m-C. The segment that immediately precedes the core can be modeled readily into an amphipathic helix with the conserved tryptophan, Trp^{260}, aligned with Trp^{30} in the C subunit. The complementarity of this helix with the core (Fig. 5*C*) is consistent with the general rules summarized above. This alignment requires a deletion of six residues between the end of the A helix and the beginning of β-strand 1 in the small lobe, but this does not significantly disturb the folding of the small lobe. The general location of the A helix relative to the core and the SH2 domain is shown in Fig. 7. Although the exact role of this amphipathic helix for kinase function remains to be established, the model makes a strong argument for conserved interactions that mimic in part those seen between the core and the A helix in the C subunit of cAPK. This motif in pp60^{src} would provide a direct link between the core and the SH2 domain that terminates with residue 249. It also brings the SH2 domain in close proximity to Tyr^{527}, the inhibitory tyrosine phosphorylation site located in the C-terminal tail (34).

Future Aims. So far, most of the attention devoted to the protein kinase structure has focused on the active site; however, the surface that lies distal to the active site could also be important for kinase function. Since the core of the *Dictyostelium* (15) and yeast (35) C subunits can be expressed as active proteins, the A helix is obviously not essential for activity. However, it could be important for stability. The

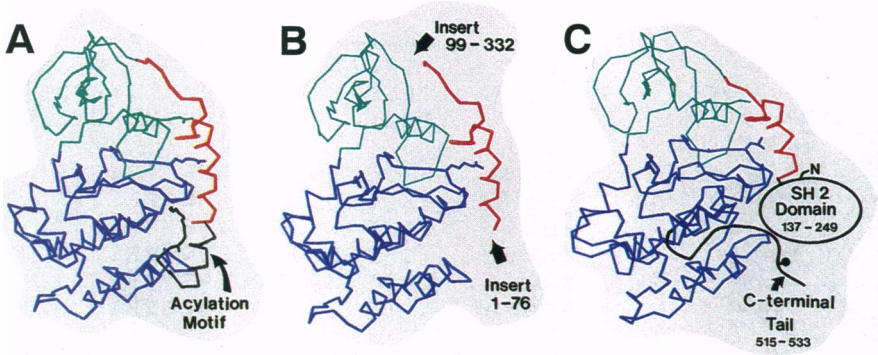

FIG. 7. Various A-helix motifs. α-Carbon backbone showing A-helix motif (red), small lobe (green), and large lobe (blue). Additional nonconserved segments are black. (*A*) m-C showing Trp^{30}, Arg^{93}, Arg^{190}, and the myristoylation motif. (*B*) Model of Dd-C showing Trp^{91}, Arg^{483}, and the two inserts. (*C*) Model of $pp60^{src}$ showing Trp^{260}, Leu^{410}, Gln^{312}, the general location of the C-terminal tail including Tyr^{527} (●), and the SH2 domain. The remainder of the N terminus, including the SH3 domain, is indicated by N.

structure of an open conformation of m-C also emphasizes the stability of the A-helix/core interface in contrast to the rotation of the antiparallel β-sheet in the small lobe (10). Potential A-helix motifs also should be considered before expression of any protein kinase in foreign cells such as *Escherichia coli*, since expression of a stable enzyme may require more than the conserved core. Since the A-helix motif can also be linked indirectly to the active site, it could also be a docking site for other proteins, thus serving as an effector site. To determine exactly how communication between the A helix and the core takes place and to determine the structural and functional role of the helix will require experimentation with different protein kinases. We propose, however, that the A-helix motif is a conserved and functionally important feature of most protein kinases.

This research was supported by the American Cancer Society (Grant BE-48 to S.S.T.), Association de la Recherche contre le Cancer (ARC 6438 to M.V.), and Institute National de la Santé et de la Recherche Médicale (CRE 920113 to M.V.). E.R.-A. is supported by Public Health Service Training Grant 5T32 DK07233.

1. Walsh, D. A., Perkins, J. P. & Krebs, E. G. (1968) *J. Biol. Chem.* **243,** 3763–3765.
2. Barker, W. C. & Dayhoff, M. O. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 2836–2839.
3. Hanks, S. K., Quinn, A. M. & Hunter, T. (1988) *Science* **241,** 42–52.
4. Taylor, S. S., Knighton, D. R., Zheng, J., Ten Eyck, L. F. & Sowadski, J. M. (1992) *Annu. Rev. Cell Biol.* **8,** 429–462.
5. Knighton, D. R., Zheng, J., Ten Eyck, L. F., Ashford, V. A., Xuong, N.-h., Taylor, S. S. & Sowadski, J. M. (1991) *Science* **253,** 407–414.
6. Zheng, J., Knighton, D. R., Ten Eyck, L. F., Karlsson, R., Xuong, N.-h., Taylor, S. S. & Sowadski, J. M. (1993) *Biochemistry* **32,** 2154–2161.
7. Taylor, S. S., Knighton, D. R., Zheng, J., Sowadski, J. M., Gibbs, C. S. & Zoller, M. J. (1993) *Trends Biochem. Sci.* **18,** 84–89.
8. Knighton, D. R., Zheng, J., Ten Eyck, L. F., Xuong, N.-h., Taylor, S. S. & Sowadski, J. M. (1991) *Science* **253,** 414–420.
9. Yonemoto, W., McGlone, M. L. & Taylor, S. S. (1993) *J. Biol. Chem.* **268,** 2348–2352.
10. Zheng, J., Knighton, D. R., Xuong, N.-h., Taylor, S. S., Sowadski, J. M. & Ten Eyck, L. F. (1993) *Prot. Sci.* **2,** 1559–1573.
11. Bueshausen, S., Bergold, P., Sturner, S., Elste, A., Roytenberg, V., Schwartz, J. H. & Bayley, H. (1988) *Neuron* **1,** 853–864.
12. Gross, R. W., Bagchi, S., Lu, X. & Rubin, C. S. (1990) *J. Biol. Chem.* **265,** 6896–6907.
13. Foster, J. L., Higgins, G. C. & Jackson, F. R. (1988) *J. Biol. Chem.* **263,** 1676–1681.
14. Toda, T., Cameron, S., Sass, P., Zoller, M. J. & Wigler, M. (1987) *Cell* **50,** 277–287.
15. Mann, S. K. O., Yonemoto, W. M., Taylor, S. S. & Firtel, R. A. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 10701–10705.
16. Anjard, C., Etchebehere, L., Pinaud, S., Veron, M. & Reymond, C. D. (1993) *Biochemistry* **32,** 9532–9538.
17. Knighton, D. R., Pearson, R. B., Sowadski, J. M., Means, A. R., Ten Eyck, L. F., Taylor, S. S. & Kemp, B. E. (1992) *Science* **258,** 130–135.
18. Knighton, D. R., Cadena, D. L., Zheng, J., Ten Eyck, L. F., Taylor, S. S., Sowadski, J. M. & Gill, G. N. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 5001–5005.
19. Chrivia, J. C., Uhler, M. D. & McKnight, G. S. (1988) *J. Biol. Chem.* **263,** 5739–5744.
20. Carr, S. A., Biemann, K., Shoji, S., Parmalee, D. C. & Titani, K. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6128–6131.
21. Steinberg, R. A., Cauthron, R. D., Symcox, M. M. & Shuntoh, H. (1993) *Mol. Cell. Biol.* **13,** 2332–2341.
22. Knighton, D. R., Bell, S. M., Zheng, J., Ten Eyck, L. F., Xuong, N.-h., Taylor, S. S. & Sowadski, J. M. (1993) *Acta Crystallogr. D* **49,** 357–361.
23. Marcote, M. J., Knighton, D. R., Basi, G., Sowadski, J. M., Brambilla, P., Draetta, G. & Taylor, S. S. (1993) *Mol. Cell. Biol.* **13,** 5122–5131.
24. Saxena, A., Padmanabha, R. & Glover, C. V. C. (1987) *Mol. Cell. Biol.* **7,** 3409–3417.
25. Takeya, T., Feldman, R. A. & Hanafusa, H. (1982) *J. Virol.* **44,** 1–11.
26. Sukegawa, J., Semba, K., Yamanashi, Y., Nishizawa, M., Miyajima, N., Yamamoto, T. & Toyoshima, K. (1987) *Mol. Cell. Biol.* **7,** 41–47.
27. Shtivelman, E., Lifshitz, B., Gale, P. R., Roe, B. A. & Canaani, E. (1986) *Cell* **47,** 277–284.
28. Koga, Y., Caccia, N., Toyonaga, B., Spolski, R., Yanagi, Y., Yoshikai, Y. & Mak, T. W. (1986) *Eur. J. Immunol.* **16,** 1643–1646.
29. Ebina, Y., Ellis, L., Jarnagin, K., Edery, M., Graf, L., Clauser, E., Ou, J.-H., Masiarz, F., Kan, Y. W., Goldfine, I. D., Roth, R. A. & Rutter, W. J. (1985) *Cell* **40,** 747–758.
30. Yarden, Y., Escobedo, J. A., Kuang, W. J., Yang-Feng, T. L., Daniel, T. O., Tremble, P. M., Chen, E. Y., Ando, M. E., Harkins, R. N. & Francke, U. (1986) *Nature (London)* **323,** 226–232.
31. Hindley, J. & Phear, G. A. (1984) *Gene* **31,** 129–134.
32. Boulton, T. G., Nye, S. H., Robbins, D., Ip, N. Y., Radziejewska, E., Morgenbesser, S. D., DePinho, R. A., Panayotatos, N., Cobb, M. H. & Yancopoulos, G. D. (1991) *Cell* **65,** 663–675.
33. De Bondt, H. L., Rosenblatt, J., Jancarik, J., Jones, H. D., Morgan, D. O. & Kim, S. H. (1993) *Nature (London)* **363,** 595–602.
34. Hunter, T. (1987) *Cell* **49,** 1–4.
35. Kuret, J. & Pflugrath, J. W. (1991) *Biochemistry* **30,** 10595–10600.