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# Nuclear Import of Protein Kinases and Cyclins

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**Abstract** Karyophilic and acidic clusters were found in most nonmembrane serine/threonine protein kinases whose primary structure was examined. These karyophilic clusters might mediate the anchoring of the kinase molecules to transporter proteins for their regulated nuclear import and might constitute the nuclear localization signals (NLS) of the kinase molecules. In contrast to protein transcription factors that are exclusively nuclear possessing strong karyophilic peptides composed of at least four arginines (R) and lysines (K) within an hexapeptide flanked by proline and glycine helix-breakers, protein kinases often contain one histidine and three K + R residues; this is proposed to specify a weak NLS structure resulting in the nuclear import of a fraction of the total cytoplasmic kinase molecules as well as in their weak retention in the different ionic strength nuclear environment. Putative NLS peptides in protein kinases may also contain hydrophobic or bulky aromatic amino acids proposed to further diminish their capacity to act as strong NLS. Most kinases lacking karyophilic clusters (c-Mos, v-Mos, sea star MAP, and yeast KIN28, SRA1, SRA3, TPK1, TPK2) also lack acidic clusters, which is in contrast to most kinases containing both acidic and karyophilic peptides; this and the presence of R/K clusters in the transporter proteins supports a role of acidic clusters on kinases in nuclear import. Cyclins B lack karyophilic signals and are proposed to be imported into nuclei via their association with Cdc2. 

**Key words:** protein kinases, cyclins, nuclear import, NLS, acidic domains, cell cycle, phosphatases, $p34^{cdc2}$

## INTRODUCTION

Protein kinases regulate enzymatic activities as well as the DNA-binding, transactivating potential, and nuclear import of transcription factors and other kinases; these processes occur in response to extracellular stimuli, emergence of cells from quiescence or the commitment to differentiate, the decision to replicate DNA and to enter mitosis. Kinases might play a notable role in establishing memory pathways through their activation by opening ion-gates in neurojunctions [reviewed by Boulikas, 1995]. Nuclear import of proteins is mediated by short karyophilic peptides that electrostatically anchor the protein molecule to cytoplasmic or nuclear pore transporter proteins [reviewed by Boulikas, 1993]. Indeed, transporter molecules contain clusters of karyophilic but also of acidic amino acids. For example, the yeast NSR1 possesses the motifs *D***KKRK***SEDAEEEEDEE*SS and $\mathbf{Rx_3Rx_4Rx_3}$ $\mathbf{Rx_3Rx_4Rx_3}$ $\mathbf{Rx_2Rx_3R}$ [Lee et al., 1991]; the rat Nopp140 has ten motifs and twelve amino acids each composed entirely of S, D, and E like SSSSS*EDSSEEE*, each followed by a basic oligopeptide; S residues are highly phosphorylated in the functional transporter Nopp140 protein molecule [Meier and Blobel, 1992].

We have proposed that not only karyophilic but also acidic clusters on cytoplasmic proteins to be imported into nuclei mediate their binding to transporter molecules [Boulikas, 1993, 1994]. An examination of the primary structures of a number of transcription factors known to function exclusively in the nucleus has revealed the presence of karyophilic motifs; of the sixteen possible forms of putative NLS structures the θθθθ, θθθxθ, θθxθθ, and θθxθxθ (where θ is R or K) were predominating, together accounting for about 70% of all karyophilic clusters on transcription factors. These are expected to have their complementary acidic clusters on transporter molecules where E or phosphoserine might complement K and D might complement R [Boulikas, 1994a]. Evidently more transporter protein structures and predictions of the three-dimensional conformation of both transporters and transcription factors are needed to resolve these issues.

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Are transcription factors phosphorylated already in the cytoplasm by cytoplasmic kinases after their synthesis on polyribosomes and prior to their nuclear import or some protein kinases enter the nucleus and phosphorylate transcription factors in the nucleoplasm? The second possibility is favored since phosphorylations and dephosphorylations of transcription factors occur during the cell cycle and in response to mitogenic signals; the vast majority of transcription factors are strictly nuclear proteins that are supposed to see the cytoplasm only once in their lifetime, during their birth on polyribosomes.

*In spite of* the possibility that nuclear factors are phosphorylated after their synthesis in the cytoplasm by cytoplasmic kinases to promote their nuclear import, like rNFIL-6 [Metz and Ziff, 1991] or ISGF3 [Fu, 1992; Fu and Zhang, 1993; Shuai et al., 1993], the issue on whether some protein kinases enter the nucleus is important to our comprehension of the involvement of protein phosphorylation in the control of gene expression, DNA replication, cell cycle control, and neoplasia. For several protein kinases it has been established that they shuttle between the nucleus and the cytoplasm like casein kinase II [Lin et al., 1992; Krek et al., 1992]; the catalytic subunit of cAMP-dependent protein kinase II following stimulation of cells with cAMP [Nigg et al., 1985]; the MAP kinases ERK1 and ERK2 following stimulation of cells with growth factors or mitogens [Anderson et al., 1990; Ahn et al., 1991; Chen et al., 1992; Kyriakis et al., 1992]; the RSK or p90$^{rsk}$ after its phosphorylation by MAP kinase [Chen et al., 1992]; and protein kinase C (PKC) after stimulation of cells with the phorbol ester TPA [Leach et al., 1989]. Questions on whether other kinases are imported to the nucleus might be answered by searching for nuclear localization signals (NLS) on protein kinases.

## RESULTS

### Putative Nuclear Localization Signals on Nonmembrane Protein Kinases

Ambiguities as to whether a kinase enters the nucleus or stays as a strictly cytoplasmic enzyme exist for several kinases. The primary structure of a significant number of protein kinases as well as their function are known; this has allowed the search for karyophilic hexapeptides in their sequences that might function in their nuclear import (Table I) as a possible clue to their intracellular location. Acidic clusters on protein kinases are also given since these might also function in the anchoring of the molecules to karyophilic clusters on transporter proteins (see Introduction). Acidic clusters are arbitrarily defined as four aspartic (D) and glutamic acid (E) residues over a stretch of six amino acids; evidently due to our ignorance of the sites of phosphorylation of most kinases, we cannot predict the contribution of phosphoserines, phosphothreonines, and phosphotyrosines in increasing the acidity of a vicinal D/E-rich cluster.

One type of protein kinase C playing a central role in signal transduction and acting as the principal receptor of the phobol ester TPA translocates to the nucleus from the cell membrane following stimulation of NIH 3T3 cells with TPA [Leach et al., 1989]. TPA treatment seems to reduce the levels of PKC in the cytoplasm with a concomitant increase in membrane and nuclear levels of PKC. However, a number of other similar studies that have examined nuclear localization of PKC gave varying results, raising the interesting possibility that different isoforms of PKC might follow different cell trafficking paths [see Leach et al., 1989, for references]. Type α, β, or γ protein kinase C molecules from human, rat, rabbit, or bovine cells all display three karyophilic peptides conserved in all molecules of the type **HKRCH**, or **HRRCH** around position 71, **RSKHKFK** or **RNKHKFR** around position 95, and **KH**PG**KR** or **KH**PA**KR** around position 577 or 591 (Table I). These signals, although rather weak because of the histidine residue, are flanked by Pro, Glu, Asp, or Gly and are proposed to act for the nuclear import of PKC under special circumstances.

GSK-3 was originally described as a cytoplasmic enzyme involved in glycogen metabolism [Hemmings et al., 1982]. Later, however, GSK-3 was shown to phosphorylate nuclear oncoproteins in vitro such as c-Jun [Boyle et al., 1991] and c-Myb [Woodgett, 1991]. Furthermore, molecular cloning of *GSK-3* [Woodgett, 1990] has shown sequence similarity to the *zeste-white/shaggy* gene of *Drosophila* coding for a protein involved in neural cell fate determination and known to be a nuclear transcription regulator [Siegfried et al., 1990]. These observations make it likely that GSK-3 is localized also in the nucleus. Our search on GSK-3α and β rat isoforms encoded by different genes and highly expressed in brain shows the presence of the

**TABLE I. Karyophilic and Acidic Clusters on Non-Membrane Protein Kinases**

| Non-membrane protein kinase | Species | Karyophilic peptides | Acidic stretches | Features | Reference |
|---|---|---|---|---|---|
| Protein kinase C (673 aa) | Bovine, human β type | 73FVV**HKRCH** E<br>96DDP**R SKHK** F**KIH**<br>577T**KH**PG **KR**LG | 374KK**D**$x_4$**DDD** V**E**$x_4$ **E**KR<br>540PF**EGEDE D** LF | Known to translocate to the nucleus following treatment of cells with mitogens | Parker et al., 1986; Coussens et al., 1986a |
| Protein kinase C (697 aa) | Bovine, human γ type | 71F**VV HRRCH** EF<br>95DDP**RNKH K** F**R**LH<br>591T**KH**PA**KR**L G | 383KK**D**$x_4$**DDD** V**D**$x_4$ **E**KR<br>552PPF**DGED EEE** LF | | Parker et al., 1986; Coussens et al., 1986a |
| Protein kinase C (673 aa) | Rabbit type α and β | 72FVV**HKRCH** E<br>96DDP**RSKHK** F**KIH**<br>577T**KH**PG**KR**L G | 374KK**D**$x_4$**DDD** V**E**$x_4$ **E**KR<br>540PF**EGEDE DE** LF | | Ohno et al., 1987 |
| PKC-I (701 aa) | Rat brain | 71F**VV HRRCH** E<br>95DDP**RNKHK** F**R**L**H**<br>594T**KH**PG**KR**L G | 386KK**D**$x_4$**DDD** V**D**$x_4$ **E**KR<br>556PPF**DGED EEE** LF | | Knopf et al., 1986 |
| Protein kinase C (639aa, 75 kDa) | *Drosophila* | 22GEN**KMKSR** L**RK**G (not conserved)<br>80SYV**VHKR**C **HE**YVT (conserved)<br>211PDDKDQ**SK KKTR**TIK (not conserved)<br>614PPF**KPKIK HRK** MCP (not conserved) | 300**ADDEQD**L<br>379**QDDDVE**C<br>540F**DGEDEE EL** | 14 exons, 20 kb; 3 transcripts in adult flies; not expressed in 0–3 h *Drosophila* embryos; the VVHKRCHE motif (or VVHRRCHE) is conserved among all PKC known | Rosenthal et al., 1987 |
| Glycogen synthase kinase 3<br>GSK-3α (483 aa)<br>GSK-3β (420 aa) | Rat brain | 148**KK**VLQD**K-RF**K**NR-**ELQIM**R-**KL**D | None (181SSGEK-**KDE**, after phosphorylation at S) | Phosphorylates glycogen synthase, c-Jun, c-Myb; two isoforms encoded by discrete genes; highly expressed in brain; both α and β forms are cytosolic but also associated with the plasma membrane consistent with their role in signal transduction from the cell surface | Woodgett, 1990 |

*Table I continues on next page*

**TABLE I.** ***(continued)***

| Non-membrane protein kinase | Species | Karyophilic peptides | Acidic stretches | Features | Reference |
|---|---|---|---|---|---|
| Zw3 zeste-white 3 | *Drosophila* | LQD**RRFKNRE** LQ | None | Product of the segment polarity gene *zw3*; the protein encoded has 34% homology to cdc2; mutations in *zw3* give embryos that lack most of the ventral denticles, differentiated structures derived from the most anterior region of each segment | Siegfried et al., 1990 |
| $Ca^{2+}$/calmodulin-dependent protein kinase II (CaM kinase II) β subunit (542aa, 60.3 kDa) | Rat brain | 289ECLKKFNA **RRK**LKGAIL | 402I**EDED**A | Composed of nine 50 kDa α-subunits and three 60 kDa β-subunits; both are catalytic; calmodulin- and ATP-binding domains; highly expressed in forebrain neurons, concentrated in postsynaptic densities; acts as a $Ca^{2+}$-triggered switch and could be involved in long-lasting changes in synapses | Bennett and Kennedy, 1987 |
| CaM kinase II (478 aa, 54 kDa) α-subunit | Rat brain | 290L**KK**FNA**RR** **KLK**GAILTTM 450EET**RVWH** **RR**DG**K** | 338I**EDED**TKV R | This particular isoform is exclusively expressed in the brain; high enzyme levels in specific brain areas; might be involved in short- and long-term responses to transient stimuli | Lin et al., 1987 |
| cADPK catalytic subunit (349 aa, 40.6 kDa) | Bovine (cardiac muscle) | 185 GFA**KR**V-**K**G**R**T WTLCG | 327**DDYEEEE** $x_6$**E**$x_4$ **E**$x_2$**E**Fend | By Edman degradation of protein fragments; mediates the action of and is activated by cAMP; consists of two regulatory (R) and two catalytic (C) subunits; cAMP releases the C subunit from the inactive $R_2C_2$ cADPK; two cDNAs were cloned encoding two isoforms of the catalytic subunit of cADPK in mouse | Shoji et al., 1981; Uhler et al., 1986a,b |
| cADPK (catalytic sununit) (350 aa) | Bovine | 186GFA**KR**V**K**G **R**TWTLCG | 338DDYEEEDIR $x_4$E$x_4$E$x_2$EFend | cDNA was isolated by screening a bovine pituitary cDNA library; 93% sequence similarity to known bovine cADPK; represents the second gene for the catalytic subunit of cADPK | Showers and Maurer, 1986 |

*Table I continues on next page*

**TABLE I.** ***(continued)***

| Non-membrane protein kinase | Species | Karyophilic peptides | Acidic stretches | Features | Reference |
|---|---|---|---|---|---|
| cGDPK (670 aa, 76.3 kDa) | Bovine lung | 29EEEIQEL**KR** **KLHK**CQSVLP 389KIL**KKRH**-IVDTR | 1AcS**EL**E**EDE** 20**EL**E**K**RLS **EK** **EEE**IQE 652P**ED**N **DE**P PP**DD** | By protein sequencing; composed of two identical subunits activated in an allosteric manner by binding of cGMP and not by dissociation of catalytic subunit as in cADPK; sequence similar to cADPK | Takio et al., 1984 |
| TPK3 (398 aa) cADPK | *S. cerevisiae* | 117**KTL**K**KH**TI VK | None | cAMP-DPK is a tetrameric protein with two catalytic and two regulatory subunits; cAMP activates the kinase by dissociating the catalytic subunits from the tetramer; all three TPK 1, 2, 3 are catalytic subunits | Toda et al., 1987 |
| SNF1 (633aa, 72 kDa) | *S. cerevisiae* | 16$S_2$**H**$_{13}$G**H**$G_2$ 166EYC**HRH**KI V**HR**DL**K**P 495PLVT **KKSK** T**R**W**H**FG | 323P**EE**N**EN**N **DS**K**KD**$x_4$ **DN**D **EIDD** | Ser/Thr kinase; autophosphorylated; plays a central role is carbon catabolite repression in yeast required for expression of glucose-repressible genes; region 60–250 shows high sequence similarity to cAMP-dependent protein kinase (cADPK) | Celenza and Carlson, 1986 |
| Casein kinase II (α-subunit, catalytic) (336aa) | *Drosophila melanogaster* | 70PV**KKKK**I**KR** **EIK** 269DILQ **RHSR** **KR**WERF | None | CKII is composed of α and β sununits in a $\alpha_2\beta_2$ 130–150 kDa protein; the α-subunit is the catalytic and the β is autophosphorylated | Saxena et al., 1987 |
| CKII (β-subunit, regulatory) (215aa) | *Drosophila melanogaster* | 146P**K**SS**RHH** **H**TDG | 24**EVDEDY**IQ**D** 55DL**E**P **EDE** L **ED**NP | | |
| CKII (β-subunit, regulatory) (209aa, 24.2 kDa) | Bovine (lung) | 142P**K**SS**RHH** **H**TDG | 20**EVDED** 51**DLEPDEE** L **ED** | | Takio et al., 1987 |
| KIN1 (1064 aa, 117 kDa) | *S. cerevisiae* | 108P**KQRHRK** SLG 129GSMC**K**V **KL** A**KHR**YTNE 506D**RKH**A**K**I **R** NQ 638GNIF**RK**LS QR**RKK**TI EQ 773PPLNVA**K**G **RK**L**H**P | 433**EF**I**DDVEE** TR 762GS**DDD** EN | 30% aa similarity to bovine cADPK and 27% (KIN1) or 25% (KIN2) aa similarity to v-Src within the kinase domain; the catalytic domains of KIN1 and KIN2 are near the N-terminus and are structural mosaics with features characteristic of both Tyr and Ser/Thr kinases | Levin et al., 1987 |

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**TABLE I. *(continued)***

| Non-membrane protein kinase | Species | Karyophilic peptides | Acidic stretches | Features | Reference |
|---|---|---|---|---|---|
| KIN2 (1152 aa, 126 kDa) | *S. cerevisiae* | 87EL**RQFHRR** SLG 111G**KVK** LV**K** **HRQ**TKE 217GSLKE**HH**A **RK**FARG 807LSVP**KGRK** L**HP** | 412**EFIDDIEDT** RR | | Levin et al., 1987 |
| STE7 (515 aa) | *S. cerevisiae* | 60FL**RRGIKKK** LTLD 472PS**K**DD **KFR** **HWCRKIKSKIK** ED**KRIKR**E | | Implicated in the control of the three cell types in yeast; (a, α, and a/α) of which a and α cells are haploid and are specialized for mating, whereas a/α cells are diploid and are specialized for meiosis and sporulation; with the exception of the mating type locus, *MAT*, all cells contain the same DNA sequences; *STE7* gene produces insensitivity to cell-division arrest induced by the yeast mating hormone, α-factor | Teague et al., 1986 |
| S6KIIα (733aa) | *Xenopus* | 722-**QRRVKK**LPST TLend | | | Jones et al., 1988 |
| S6KIIβ | *Xenopus* | **QRRVKK**LPSIT L end | | | Jones et al., 1988 |
| S6KII (752 aa) | Chicken | 742 **QRRVKK**LPST TLend | | | Alcorta et al., 1989 |
| S6KII (724aa) | Mouse | 713**QRRVRK**L PSTTLend | | | Alcorta et al., 1989 |
| CDC2Hs (297aa) $p34^{cdc2}$ | Human | 16GVVY**KGRH** **K**TTG 120FCHS **RR**VL **HR**DLKP | 33KKIRL**ESEE** **E**GVP | Isolated by expressing a human cDNA library in *S. pombe* and selecting for clones that complement a mutation in the *cdc2* yeast gene; the human CDC2 gene can complement both the inviability of a null allele of *S. cerevisiae CDC28* and *cdc2* mutants of *S. pombe; CDC2* mRNA appears after that of CDK2 | Lee and Nurse, 1987; Ninomiya-Tsuji et al., 1991 |
| cdc2 (297aa) | *S. pombe* | GVVY**KARHK**L SGR | KKIRL**EDESE** PG**DSEIDE** I G**EED**AIEL | High homology to *S. cerevisiae* CDC28 | Hindley and Phear, 1984 |

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**TABLE I.** ***(continued)***

| Non-membrane protein kinase | Species | Karyophilic peptides | Acidic stretches | Features | Reference |
|---|---|---|---|---|---|
| CDK2 (cell division kinase 2) (298 aa) | Human | 119HS**HRVLH RD**LKP | None | The human CDK2 protein has 65% sequence identity to human p34$^{cdc2}$ and 89% sequence identity to *Xenopus* Eg1 kinase; human *CDK2* was able to complement the inviability of a null allele of *S. cerevisiae CDC28* but not *cdc2* mutants in *S. pombe.* CDK2 mRNA appears in late G1/early S | Ninomiya-Tsuji et al., 1991; Tsai et al., 1991 |
| Eg1 (297aa) | *Xenopus* | 109FCHS**HRVL HRD**LKP | 254PLDEDGRD LL | Cdk2-related | Paris et al., 1991 |
| CDC28 (298a) | *S. cerevisiae* | 125GIAYC**HSH RILHRD**LK P | 37**KKIRLESED EG**VP | The homolog of *S. pombe* Cdc2 | Lörincz and Reed, 1984 |
| cdk3 (305aa) | Human | 119**HSHRVIHR** DL**K**P | None (287 SSP**E**PSPx$_8$RF RH. C-terminus) | | Meyerson et al., 1992 |
| PSSALRE (291 aa) | Human | 56**KE**L**KHK**NIV **R** | 33KRVRL**DDD DE**GVPSS | cdc2-related kinase | Meyerson et al., 1992 |
| PCTAIRE-1 (496 aa) | Human | 1MD**RMKKIKR** Q (N-terminus) 141D**K**PLS**RR**L **RR**V | | cdc2-related kinase | Meyerson et al., 1992 |
| PCTAIRE-2 (523 aa) | Human | 1M**KKFKRR** 129**RNRIHRR** IS 172S**RRSRR**A S 304**HRRK** VL**H R** 512G**H**G**K**N **RR** QSMLF end | | cdc2 related kinase | Meyerson et al., 1992 |
| PCTAIRE-3 (380 aa) | Human | 163**HTRKILHR** 369PG**RGK NR RQ**SIF end | | cdc2 related kinase | Meyerson et al., 1992 |
| KKIALRE (358 aa) | Human | 69EVF**RRKRR LH** 302D**K**PT**RK** TL **RKSRKHH** | 243**D**PED**M**EP LE**L** | cdc2-related kinase | Meyerson et al., 1992 |
| nim1$^+$ gene product (new inducer of mitosis); protein kinase (370 aa) | *S. pombe* | 1MV**KRHK**NT 87DGELF**HYIR K H**GP 114DAVA**HCH RFRF RHR**D 295**KK**SSS**KK** VV**RR** LQQRDD | 309R**DDNDE**K | | Russel and Nurse, 1987a |
| Wee1$^+$ gene product (877aa) | *S. pombe* | 194PA**Q**K**LRK KN**NFD 388**K Q**H**RPRK** NTNFTPLPP 592KYAV**KK**L **K** V**K**FSGP | 538M**EEEADV** | The *Wee1*$^+$ gene functions as a dose-dependent inhibitor that delays the initiation of mitosis until the yeast cell has attained a certain size; Wee1 has a protein kinase consensus probably regulating cdc2 kinase | Russel and Nurse, 1987a |

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**TABLE I. *(continued)***

| Non-membrane protein kinase | Species | Karyophilic peptides | Acidic stretches | Features | Reference |
|---|---|---|---|---|---|
| CDC7 (497 aa) | *S. cerevisiae* | 266PNET**RRIK** **R**ANRAG | 480**DGESTDE** **DDVVSSSE AD** LLD**K**D**V**LLI**SE** end | Required for mitotic but not meiotic DNA replication presumably to phosphorylate specific replication protein factors; implicated in DNA repair and meiotic recombination; some homology with CDC28 and oncogene protein kinases but differs in a large region within the phosphorylation receptor domain | Patterson et al., 1986 |
| ERK1 (MAP kinase) (367 aa; 42 kDa) | Rat | 48YD**HVRKTR** VAI**KK** | **FDMELDDL** P**KERLKE** $x_4$**D** | Known to translocate to the nucleus following their activation by phosphorylation at T-190, and Y-192 (T-183, Y-185 in ERK2) | Boulton et al., 1990 |
| FUS3 (353aa) | *S. cerevisiae* | 59IL**KHFKH**E | | MAP-(ERK1)-related | Boulton et al., 1990 |
| KSS1 (368 aa) | *S. cerevisiae* | 252Q**IKSKRAK** EY | 343RP**EEEEE** VP | MAP-(ERK1)-related | Boulton et al., 1990 |
| SWI6 (803aa, 90kDa) | *S. cerevisiae* | ELV**KHLVKH**G SN GK**AKKIRSQ**LL | **EFEEPETDQD EEDP EEEEESFRE ESKK** SSEDPQDIDT **DE** MQD | Activator of CACGA-box with sequence similarity to cdc10; required at START of cell cycle | Breeden and Nasmyth, 1987 |
| cdc10 | *S. pombe* | EQ**RL**K**RHR**ID VSDED | | | Breeden and Nasmyth, 1987 |
| CTD kinase (528 aa) 58 kDa subunit (catalytic) | *S. cerevisiae* | SNI**KSKCRRV**V 37PP**KRIRTD** (suggested by the authors) 492**KLARKQK** **R** P | $N_8$**DDDD**K (N-terminus) | Consists of 3 subunits of 58, 38, and 32 kDa; disruption of the 58 kDa gene gives cells that lack CTD kinase, grow slowly, are cold sensitive, but have different phosphorylated forms of RNA pol II | Lee and Greenleaf, 1991 |
| Phosphorylase kinase (catalytic subunit) (386aa) | Rabbit (skeletal muscle) | 29GVSSVV**RR** CI**HK**P | None | | Reimann et al., 1984 |
| Myosin light chain kinase (MLCK) (669 aa) | Chicken gizzard | 489KKYMA**RR** **KWQK**TGHAV | 176**EKQEEEL** **KEEEAELSDD** **EGKETE** 304**DED FELTE** **RE** 431**DFDD EAF** **DEISDDAKD** 606**D YDEE** 653**EGEGEGE** **GEEDE**$_6$end | $Ca^{2+}$ /calmodulin-activated; phosphorylated by cADPK; first described as responsible for the phosphorylation of a specific class of myosin light chains; required for initiation of contraction in smooth muscle | Guerriero et al., 1986 |

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**TABLE I. (*continued*)**

| Non-membrane protein kinase | Species | Karyophilic peptides | Acidic stretches | Features | Reference |
|---|---|---|---|---|---|
| Myosin light chain kinase (partial 368 carboxy-terminal aa sequence) | Rabbit (skeletal muscle) | 314PWLNNLAE KA**KR**CN**RR**LK SQ333 334ILLKKYLM-**KRR**W**KK**N-FIAVS | 260G**DDDTE** 275WYF**DEE**TF **E** | By protein sequencing | Takio et al., 1985 |
| Phosphorylase kinase (PhK) (catalytic γ subunit) (389 aa) | Mouse (muscle) | 28GVSSVV**RR** CI**HK**P | 384A**EEED**Fend | Glycogenolytic regulatory enzyme; undergoes complex regulation; composed of 16 subunits containing equimolar ratios of α, β, γ, and δ subunits; high levels in skeletal muscle; isoforms in cardiac muscle and liver; cDNA probe does not hybridize to X chromosome in mice and is thus distinct from the mutant recessive PhK deficiency that results in glycogen storage disease | Chamberlain et al., 1987 |

D**KRFKNRE** signal in both isoforms; because of the phenylalanine (F) residue might be a weak NLS.

$Ca^{2+}$/calmodulin-dependent kinases I and II (CaM kinases) are bulky enzymes especially expressed in brain consisting of α and β subunits both possessing catalytic kinase activity; these enzymes are partly bound to membranes or cytoskeletal structures and partly soluble [reviewed by Edelman et al., 1987]; yet CaM kinases phosphorylate the transcription factor CREB [Sheng et al., 1990, 1991] and mediate the effect of $Ca^{2+}$ influx to the nucleus [Colbran et al., 1989; Sheng et al., 1991]. The rat enzyme has the **KK**FNA**RRKLK**G signal (Table I) which, although flanked by hydrophobic amino acids, is among the few found in this study not to contain histidine; furthermore, this signal is flanked to its C-side by glycine that makes it a relatively strong NLS.

cGDPK and cADPK are cytoplasmic enzymes but phosphorylate histone H2B [Glass and Krebs, 1979; Martinage et al., 1980], whereas cADPK phosphorylates SRF [Janknecht et al., 1992], c-Fos [Tratner et al., 1992], and the transcription factor ADR-1 in yeast [Cherry et al., 1989]. The phosphorylation of the transcription factor rNFIL-6 (related to c/EBP) by cADPK is known to occur in the cytosol and promotes the nuclear import of rNFIL-6 [Metz and Ziff, 1991]. Is the phosphorylation of the other nuclear proteins executed by cADPK or cGDPK in the cytosol or do these kinases enter the nucleus? Initial studies on the localization of these two kinases showed them to be cytosolic [reviewed by Edelman et al., 1987]; however, there is substantial evidence to suggest that these kinases spend some of their time in the nuclear compartment [Cherry et al., 1989; Janknecht et al., 1992]. cADPK is composed of catalytic and regulatory subunits; cAMP binds to the regulatory subunit and activates the dissociation of the catalytic subunit. Nigg and coworkers [1985] have demonstrated that cAMP elevation by treatment of cells with forskolin causes dissociation of the catalytic subunit of cADPK from the Golgi complex and its translocation to the nucleus; during the experiment the regulatory subunit remained associated with the Golgi; the catalytic subunit returned to the Golgi after drug withdrawal. Table I shows that the catalytic subunit of bovine cADPK possesses the karyophilic peptide **KRVKGR** that could specify nuclear import.

The distribution of casein kinase I (CKI) in rat liver was found to be cytosol (72%), microsomes (18%), mitochondria (10%), and nuclei

(1%); CKII in rat liver was found to be predominantly cytosolic (90%), whereas the remaining activity was divided among the nuclear, mitochondrial, and microsomal fractions [reviewed by Edelman et al., 1987]. CKII has an $\alpha_2\beta_2$ structure of 130 kDa with the $\alpha$ subunit containing the catalytic site. The catalytic subunit in *Drosophila* has the strong putative NLS **KKKKIKR** and a second signal **RHSRKR** proposed here to specify nuclear import; the best karyophilic peptide, however, I could find on both the bovine and *Drosophila* regulatory subunits of CKII is the **KSSRHHH**, which looks like an extremely weak signal; thus the regulatory subunit of the *Drosophila* CKII might either depend on its association with the catalytic subunit for its nuclear import or might function only in the cytoplasm. CKII modifies a significant number of transcription modulators after its activation by MAP kinases, including c-Jun, c-Fos, c-Myb, p53, T antigen, E7 of human papilloma virus, and SRF [reviewed by Hunter and Karin 1992; Boulikas, 1995]. This is consistent with findings that CKII shuttles between the nucleus and the cytoplasm [Lin et al., 1992; Krek et al., 1992].

The Cdc2 ($p34^{cdc2}$) and the Cdk family of Ser/Thr kinases associate with cyclins B and G1 cyclins (A, D1, E), respectively, to control the G2/M and G1/S checkpoints of the cell cycle, respectively, [reviewed by Boulikas, 1995]; these kinases are expected to spend most of their time in the nucleus. All Cdc/Cdk kinases examined contain the rather weak histidine-containing NLS as, for example, Y**K**G**RHK**T (human CDC2Hs), **KARHK**L (*S. pombe* Cdc2), **HR**VL**HR** (human CDK2 and *Xenopus* Eg1), or **KEL**K**HK** (human PSSALRE). However, some of the strongest putative NLS found are within this family: **RRKRR** in the KKIALRE molecule (the temporary name is derived from diversions in a conserved motif), **KKIKR** and **RRLRR** in PCTAIRE-1, and **KKF**K**RR** and **RRSRR** in PICTAIRE-2 (Table I). On the other hand, the related molecule PLSTIRE lacks four positively-charged amino acids over a stretch of six (Table II).

### Karyophilic and Acidic Clusters on Other Kinases, Cyclins, and Phosphatases

Putative NLS on cyclins are shown in Table III. Karyophilic signals in tyrosine kinases without a transmembrane domain are shown in Table IV and those with a transmembrane domain are shown in Table V. Finally, Table VI shows putative NLS on some phosphatases.

## CONCLUSIONS AND DISCUSSION

### Histidine in Karyophilic Clusters

Nonmembrane protein kinases supposedly free to follow different subcellular trafficking paths very frequently use one histidine out of the four positively charged amino acids in the karyophilic hexapeptide (Table I); this is in contrast to transcription factors that use arginines and lysines [see Boulikas, 1994a]. The conclusion that most protein kinases that function in both cytoplasmic and nuclear compartments possess a putative NLS with a histidine residue within the karyophilic amino acid tetrad of the hexapeptide is supported by the fact that protein kinase C has three karyophilic signals, all three containing one or two histidines but never four arginies plus lysines: **HKRCH**, **RSKHK**, and **KHPG**K**R**. Some exceptions to this rule include signals on CaM kinase and CKII (see above), the Cdc2-related human PCTAIRE-1 (**KKIKR** and **RRLRR**), PCTAIRE-2 (**KKF**K**RR** and **RRSRR**), KKIALRE (**RRKRR**), and myosin light chain kinase (**KR**CN**RR** and **RRWKK**). Since histidines are protonated to a much lesser extent than lysines and arginines at physiological pH and since their protonation changes depending on the three-dimensional protein microenvironment that harbors histidine residues, it is proposed that histidine-containing NLS found in kinases are weak NLS giving to the protein the characteristic of being present in both cytoplasmic and nuclear compartments. However, protein kinases that need to be constitutively present in nuclei might contain at least one strong NLS composed exclusively of K and R in a helix turn or otherwise exposed part of the protein in addition to containing proper acidic clusters as well as to interact with nuclear components resulting in a strong nuclear retention.

### Number of Karyophilic Clusters per Protein Molecule

Most kinases possess a single karyophilic (putative NLS) hexapeptide (Table I); this is in contrast to protein transcription factors that possess a second and occasionally a third or fourth karyophilic peptide [see Boulikas, 1994a]. The higher the number of NLS on a protein, the more readily this protein is transported to the nucleus [reviewed by Boulikas, 1993]. The presence of a single karyophilic hexapeptide containing more often one histidine and three arginines/lysines on most protein kinases argues for their

**TABLE II. Nonmembrane Ser/Thr Kinases Lacking Four Karyophilic Amino Acids Over a Stretch of Six Amino Acids**

| Non-membrane protein kinase | Species | Karyophilic peptides | Acidic stretches | Features | Reference |
|---|---|---|---|---|---|
| v-Mos (374 aa) | Mouse sarcoma virus MuSV12 7 | None | None | Ser/Thr kinase; v-*mos* is the Moloney murine sarcoma virus (MSV) transforming gene; only a few base substitutions with its cellular homolog; however, c-*mos* (human) was unable to transform mouse cells; considerable homology with *src* of avian sarcoma virus | van Beveren et al., 1981a,b |
| c-Mos | Rat | None | None | Not expressed in normal mouse tissues; sequences with high mutation rates by comparison of human, mouse, and rat cDNA | van der Hoorn and Firzlaff, 1984 |
| c-Mos (346aa) | Human | None | None | | van Beveren et al., 1981 a,b; |
| c-Mos | Mouse | None | None | | Watson et al., 1982 |
| PLSTIRE (326 aa) | Human | None (**FRRK**PL-F**R**GSEDVD) | None | cdc2-related kinase | Meyerson et al., 1992 |
| KIN28 (306aa) | *S. cerevisiae* | None (265PPPS**R** DEL**RKR**F) | | Related to CDC28 and Src, Erb, Abl, EGFR, as well as to Ser/Thr kinases; essential for cell proliferation; unable to complement *cdc28* mutations | Simon et al., 1986 |
| SRA1 (cADPK regulatory subunit) | *S. cerevisiae* | None (38**KR**$x_4$**R**$x_3$-**KAR**$x_4$ | | *SRA1* gene is not essential; its deletion results in reduction in glycogen accumulation, temperature sensitivity, inability to grow on galactose; cAMP controls the meiotic versus mitotic decision in yeast | Cannon and Tatchell, 1987 |
| SRA3 (cADPK catalytic subunit) | *S. cerevisiae* | $x_4$**KAK**) None (**K**$x_2$**KK**$x_4$**RLK**$x_3$**H**$x_4$**R**) | | | |
| MAP Myelin basic protein (MBP) kinase pp44$^{mpk}$ | Sea star oocyte | None | None | Ser/Thr kinase; functions for meiotic maturation; p42 in *Xenopus;* related to MAP kinases of mammals; activated by phosphorylation at Tyr | Posada et al., 1991 |
| MAP p42$^{mpk}$ (361 aa) | *Xenopus* ovary cDNA library | None | 333KFEM-**ELDD**LP | Ser/Thr kinase related to sea star pp44$^{mpk}$ and mammalian MAP kinase ERK1 | Posada et al., 1991 |
| TPK2 | *S. cerevisiae* | None | None | cADPK-related (catalytic subunit) | Toda et al., 1987 |
| TPK1 | *S. cerevisiae* | None (**RVHLIRSRH**) | None | cADPK-related (catalytic subunit) | Toda et al., 1987 |
| PK-25 *cdc*25-suppressing protein kinase (397 aa) | *S. cerevisiae* | None (82**HLIRSRH**NG**R**YY) | None | cAMP-independent protein kinase involved in the control of the START point of the cell cycle; 48% sequence similarity to the mammalian cADPK; 27–31% sequence similarity to CDC28 | Lisziewicz et al., 1986 |

**TABLE III. Karyophilic and Acidic Clusters on Cyclins**

| Cyclin | Species | Karyophilic peptides | Acidic stretches | Features | Reference |
|---|---|---|---|---|---|
| Cyclin A | Human | 65**QRPKTRR**V AP | 104V**DEAEKE** | | Wang et al., 1990; Koff et al., 1991 |
| Cyclin B | Human | None | **PEPEPEPEPE** PY **KEEK DVDAED** | Mitotic cyclin; suggested here to enter the nucleus in association with Cdc2 | Pines and Hunter, 1989; Koff et al., 1991 |
| Cyclin E | Human | S**ARSRKRK**AN V SS**KLKH** F**R**G D**KARKK**AM | **D**KE**DDD**R | G1 cyclin; in association with cdk2 controls the G1/S transition | Koff et al., 1991 |
| Cyclin B1 (398a; 44.6 kDa) | *Xenopus* | None | **DVDADDD** | Cutting the mRNA of cyclin B1 and B2 with antisense oligos blocks entry into mitosis | Minshull et al., 1989 |
| Cyclin B2 | *Xenopus* | None | **EDIDADD** | | Minshull et al., 1989 |
| Cyclin A (422aa) | Clam *S. solidissima* | None | 136**D**LP**EEE**K P 153P**EYEED**IY 195**EVSEEDK** | | Swenson et al., 1986; Minshull et al., 1989 |
| cdc13 (cyclin) | *S. pombe* | P**KKRH**ALD PAS**KKRR**QP P**KK** L**KK**DVDE **R** | **DWDDLDAED** GGY**DEEE** IL G**DDADEDY HDN KDEEW** | | Minshull et al., 1989 |
| Cyclin D1 (295aa) | Human | None (169PEAEEN-**KQIIRKH**) | 272$\mathbf{E_9VD}$ | G1 cyclin | Xiong et al., 1991 |
| Cyclin A (491 aa) | *Drosophila* | 212RESE**KKH RPKPRYMRR** | None | Accumulates in the cytoplasm of cellularized fly embryos during interphase, relocates to the nuclear region early in prophase, completely degreaded within metaphase; expressed in dividing cells throughout development | Lehner and O'Farrel, 1989 |
| CLN1 (546aa) | *S. cerevisiae* | None (87$\mathbf{Rx_7Hx_2Rx_3 Rx_3KRx_3Kx_3}$ **K**) | 265M**DEDEEL** | Related to cyclins; mutation in *CLN2* gene advances the G1 to S transition and impairs the ability of yeast cells to arrest in G1 in response to external signals | Hadwiger et al., 1989 |
| CLN2 (545aa) | *S. cerevisiae* | None (89$\mathbf{Rx_7Hx_2Rx_3 Rx_3KRx_3Rx_3K}$ ) | 250N**EEEEEE D** LK | | |
| Cyclin | Sea urchin *A. punctulata* | GS**KKVVKKD** | **EDIDKDD** G | | Minshull et al., 1989 |

role in both cytoplasmic and nuclear compartments. Exceptionally the human PCTAIRE-2 (Cdc2-related) and the yeast KIN1 contain five positively charged clusters; the yeast KIN2 and Nim1 contain four; the mammalian PKC and yeast SNF1, SWI6, and Wee1 contain three; whereas the a-subunit of rat CaMK, the cGDPK, the catalytic subunit of *Drosophila* CKII, the yeast STE7, the human CDC2Hs and its related PCTAIRE-1, 3, and KKIALRE contain two (Table I).

### Protein Kinase Import After its Phosphorylation

The cell might have invented ways of translocating a protein kinase to the nucleus at a certain stage or in a narrow window during the cell cycle or after cell stimulation. Evidently, nuclear import of kinases could be affected a) via exposure of a cryptic kinase NLS by phosphorylation or b) by stimulation of association of the kinase with a protein in the cytoplasm possessing a NLS, then phosphorylation/dephosphorylation reactions on their transactivating interfaces will be followed by nuclear import of the complex. For example, TPA treatment causes translocation of PKCα to the nuclear envelope [Leach et al., 1989].

Phosphorylation of a kinase at a specific site is proposed to either increase the acidity of a peptide augmenting the anchoring of the kinase molecule to the transporter protein or to expose by a change in protein conformation a cryptic karyophilic NLS. This might explain activation and nuclear import of cADPK, MAPK, PKC, RSK, and CKII immediately following activation of the cells with extracellular stimuli (growth factors, phorbol esters) or by an increase in the intracellular concentration of $Ca^{2+}$ or cAMP.

### Hydrophobic and Bulky Amino Acids in the Vicinity of Karyophilic Peptides

Putative nuclear localization signals in some protein kinases, in addition to containing histidine, may also be embedded in an immediate short hydrophobic (L,I,V,C,M) or bulky aromatic (F,Y,W) amino acid environment; these environments add a strong negative factor to the capacity of the karyophilic peptide to act as a NLS [Boulikas, 1993, 1994a]; however, such short domains are broken by acidic (D,E), basic (K,R), or amino acids with uncharged small groups (A,G,S,T,P), especially including the α-helix breakers G and P. For example, the *nim1+* gene product of *S. pombe* has the putative signal KKSSS**KK**VV**RR**LQQRDD (Table I). Such signals are very unusual as unique signals on protein transcription factors [Boulikas, 1994a]. Also a mixture of charged and hydrophobic amino acids are signals for the mitochondrial import of proteins [see Boulikas, 1993]. It is noteworthy that an examination of the subcellular distribution of CKI showed it to be localized in mitochondria (10% of total), as well as in cytoplasmic, endomembrane, and nuclear compartments [reviewed by Edelman et al., 1987]; this might be true for other kinase molecules and karyophilic signals with hydrophobic amino acids described here (Table I) may specify mitochondrial import of the kinase molecule.

### Nuclear Import of Cyclins

Several cyclins lack karyophilic signals and are proposed to depend on their association with other nuclear proteins in the cytoplasm for their nuclear import. These include cyclins B and D1 in humans, B1 and B2 in *Xenopus,* cyclin A in the clam *S. solidissima,* and CLN1 and CLN2 in *S. cerevisiae* (Table III). Human cyclin B might enter the nucleus in association with CDC2. Cyclins are indeed known to form strong complexes with Cdc2 or Cdks in a cell cycle-dependent manner. The import of a cyclin in association with its proper kinase might have been invented by the cell to ensure that precise stoichiometric amounts of some cyclins and kinases are present in nuclei. In addition cyclins may associate with transcription factors or other nuclear proteins and such an association already in the cytoplasm might be of physiological importance. For example, cyclin A associates with E2F regulating the *DHFR*, *c-myc*, and *RB* genes to control the onset and progression of DNA replication [Mudryj et al., 1991; reviewed by Boulikas, 1994b].

### NLS-Less Protein Kinases

Few kinases lack putative NLS and are proposed to never enter nuclei or to do so in tight association in the cytoplasm with proteins possessing a NLS. These include the *Xenopus* $p42^{mpk}$ (related to mammalian ERK1), the sea star MAP kinase, c-Mos, v-Mos; the human Cdc2-related PLSTIRE; the yeast KIN28, TPK1 and 2, and PK-25; and the *S. cerevisiae* SRA1 and SRA3 related to mammalian cADPK regulatory and

**TABLE IV. Tyr Kinases Without a Transmembrane Domain**

| Protein kinase | Species | Karyophilic peptides | Acidic stretches | Features | Reference |
|---|---|---|---|---|---|
| c-Abl (1130 aa) | Human | 601SALI**KKKK** **K**TAPTPPKR 868PAEES**R** V**R** **RHKH**SSESPG | **KED**TM**E** V**EEF** KKN**EE**AA **DE**V F**K**D | Tyr kinase associating with DNA; neither transmembrane nor signal peptide regions; N-terminal M is cleaved leaving a G that might be myristoylated for membrane-association; all motifs are conserved between human and mouse c-Abl; c-*abl* is the prototype of v-*abl* of Abelson leukemia virus; c-*abl* is translocated from chromosome 9 to 22 next to the *bcr* gene (Philadelphia chromosome) in chronic myeloid leukemias | Shtivelman et al., 1986; Koch et al., 1991 |
| Tck p56$^{tck}$ (509 aa) | Mouse | 176GVV**KH**Y**K**I **R**NLD | 9P**EDD**WME**N**I **D**V**CE** 232PWW**EDE**W **E**VPR**E** 478K**E** R**PEDR** PTF**D**$x_6$**DD** | Overexpression in thymomas is caused by insertion of the M-MuLV about 200 bp upstream from the 5′ end of the transcription initiation site of c-*tck* gene; p56 primary structure is similar to p60$^{c\text{-}src}$, p90$^{v\text{-}yes}$, p70$^{v\text{-}fgr}$; does not possess a transmembrane domain; its association with the membrane seems to depend on the attachment of a myristyl moiety to the α-amino group of Gly-2 after removal of Met-1 | Voronova and Sefton, 1986 |
| Met (454aa) | Human | 256GMKYLAS**K** **K**F**V**H**R**DLAAR | 49K**EE**L**EAE**K **R**D | Tyrosine kinase present in MNNG-transformed human cells; *met* can transform NIH3T3 mouse fibroblasts; absence of transmembrane domain; believed to have arisen by fusion of the Tyr-kinase domain of a transmembrane protein with a relative of laminin B1 in a mechanism reminiscent of *trk* and v-*fgr* | Chan et al., 1987 |

*Table IV continues on next page*

**TABLE IV. (*continued*)**

| Protein kinase | Species | Karyophilic peptides | Acidic stretches | Features | Reference |
|---|---|---|---|---|---|
| c-Yes (543aa) | Human | 206GDNV**KHYK** **IRK**LDNGG 320EAQI M**KKL** **RHDK**LVPLY | None | Neither membrane-spanning region nor ligand-binding domain; resembles $p60^{c\text{-}src}$ | Sukegawa et al., 1987 |
| c-Lyn (512 aa) | Human | 79PDDLSF**KKH** E**KMK**VLEE | None | Tyr kinase similar to $p56^{lck}$, v-Yes, v-Fgr, v-Src; no transmembrane domain | Yamanashi et al., 1987 |
| v-Raf (318 aa) Pks (248 aa) v-Mht/v-Mil (317 aa) | | 63EMQVL**RK**T **RH**VNILP | None | Ser/Thr kinases; Pks is associated with the cell membrane by myristilation; transformation by Raf protein, unlike v-Src, does not require myristilation | Mark and Rapp, 1984; Mark et al., 1986; Bonner et al., 1986 |
| A-Raf (606 aa) | Murine | 286DD**KKK**V**K** NLG 354EMQVL**RK**T **RH**VNILL | None | The first putative NLS motif is characteristic of A-Raf; expressed especially in epididymis followed by intestine; a *gag*/A-*raf* fusion gene causes transformation in vitro and induces tumors in newborn mice | Huleihel et al., 1986; Beck et al., 1987 |
| c-Raf-1 (648 aa) | Human (fetal liver cDNA library) | 100RLL**HEHK**G **KKAR**LD 393EVAVL**RK**T **RH**VNILLFMG | None | Eleven exons homologous to *mil,* nine of which are also homologous to v-*raf* | Bonner et al., 1986 |
| Jak1 (1142aa, 132 kDa) | Human | 113SVW**RH**SP **KKQK**NGYE**KK** **K**IP 313**RHK**PNVV SVE**K**E **KNK**L**K** **RKK**LEN**K**D**KK** DEE**KNKI**REE 856PT**H**FE**KR**F L**KRIR**D | 591M**DYK**D**DE** GTS**EE**KK | Tyrosine kinase activated by IFN-γ to phosphorylate the STAT91 subunit of ISGF3α at Tyr-701 causing its translocation to the nucleus | Velazquez et al., 1992; Shuai et al., 1993 |
| Tyk2 IFN-Tyk (1187 aa, 134 kDa) | Human | 216P**RS**TF**RRH** **IRQH**S 888GPTTF**HKR** YL**KKIR**DLG | 614G**DPEE**GK M**DDED**PLVP | Links the IFNα/β receptor to the cytoplasmic ISGF3 activating IFN-responsive genes | Velazquez et al., 1992 |
| Src $p60^{c\text{-}src}$ (533aa) | Chicken | 196GLNV**KHYK** **IRK**LD 313VM **KKLRH** EK | None | $p60^{c\text{-}src}$ is myristylated through an amide bond to the α-amino group of Gly-2, after cleavage of Met-1 in the mature protein; this modification is crucial for its interaction with cellular membranes | Takeya and Hanafusa, 1983 |

**TABLE V. Putative NLS on Transmembrane Tyrosine Kinases**

| Non-membrane protein kinase | Species | Karyophilic peptides | Acidic stretches | Features | Reference |
|---|---|---|---|---|---|
| Insulin receptor (1355aa, 1382aa precursor) | Human | 261DL**HHKCK** NS**RR** 730PS**RK RR**S LG 1124NA**KK**FV**H R** D | 149K**DDNEEC** G**D** 1280**EE** NKAPE **SEELEMEFED** M**E** | Contains signal N-terminal hydrophobic peptide for entering ER; a transmembrane domain; insulin stimulates glucose uptake and acts as a growth and maintenance factor for vertabrate cells in culture | Ullrich et al., 1985; Ebina et al., 1985 |
| c-Kit (976 aa) | Human | 680NFL**RRKR**D SF 737D**KRR**SVRI G | 749ER**D**$x_6$**EDD** **EL** AL**D**L**ED** | Product of c-*kit* proto-oncogene; cell surface receptor for an unidentified ligand tyrosine kinase | Yarden et al., 1987 |
| EGFR (1210 aa) v-*erb*B | Human (placenta) | 297GV**RK**C**KK** CEG (extracellular domain) 640IGLFM**RRR** **HIVRKR**TVR**R**-LL (intracellular, flanking the transmembrane domain) 805ED**RR**LV**H** **R**DLAA (intracellular) | 290**DSYEMEE** **DG** 979 **DEEDMDD** **VVDADE** 1055 **EDSIDD** | 23aa-transmembrane domain; EGFR gene is amplified and rearranged in A431 carcinoma cells generating a truncated mRNA which encodes only the extracellular EGF-binding domain | Ullrich et al., 1984 |
| c-Ros-1 (471 aa) | Human | 90TFVW**HRR**L **K**NQKSA (intracellular, immediately after the transmembrane domain) | None | Transmembrane domain and Tyr kinase domains are present; absence of signal peptide; ten exons, 26 kb; UR2 sarcoma virus of chickens encodes a fusion p68$^{gag\text{-}ros}$ protein; v-*ros* of UR2 is derived from c-*ros* of chickens | Matsushime et al., 1986 |
| c-Fms; CSF-1R (972aa) | Human | 703HLE**KK**YV**R** **R**DSG (intracellular domain) | 936G$S_4$E**L**EEE $S_3$**E** (near C-terminus) | Has a signal peptide for membrane translocation and a transmembrane domain; in FeSV v-*fms* is in fusion with *gag* produced by homologous recombination that truncated the *gag* gene and fused it in-frame with the pre-c-*fms* sequence | Coussens et al., 1986b |

*Table V continues on next page*

catalytic subunits, respectively (Table II). Furthermore, all of these kinases except *Xenopus* MAP also lack acidic clusters (Table II) proposed to aid the anchoring of the protein to the transporter molecule. This is in contrast to the non-membrane protein kinases containing putative NLS most of which also contain acidic clusters (Table I).

The strictly cytoplasmic function of some protein kinases with a specificity for phosphorylating a certain type of peptide motif is expected to be of physiological significance by maintaining

**TABLE V. (*continued*)**

| Protein kinase | Species | Karyophilic peptides | Acidic stretches | Features | Reference |
|---|---|---|---|---|---|
| c-Fes/c-Fps (825aa, 93.4 kDa) | Human | 196AQL**HHQH HHQ** 818QSI**RK RHR** end | None | Malignant oncogene; the feline *c-fes* proto-oncogene has been captured by feline sarcoma virus (FeSV); the Fujinami sarcoma virus (FSV) of chickens has acquired similar sequences from the avian counterpart *c-fps;* both v-*fps* and v-*fes* encode for Tyr-kinases; the human c-*fes*/c-*fps* has exons 2–9 homologous to v-*fps* of FSV, exon 1 corresponds to exon 2 of chicken v-*fps;* Alu repeats represent 70% of intron between exons 18 and 19; may function in myeloid cell differentiation; associated with a 150 kDa cellular Ser/Thr kinase | Coussens et al., 1986b |
| Ret (805aa) | Human | 121VCD**RS**RE **HRGH**SVLP 150QLD**HLKR**V **K**D 159DL**KKR RR** ANG | None | Fusion protein; the *ret* transforming gene encompasses ~34 kb of DNA and is generated by recombination of two ~17 DNA fragments which are unlinked in both normal human cells and T-cell lymphomas; the recombination takes place during secondary transfection of NIH3T3 cells with T-cell lymphoma DNA; all three karyophilic motifs are in the extracellular part of the transmembrane protein | Takahashi and Cooper, 1987 |
| Met (1408aa) | Human | 300ILTE**KRKK RS**T**KK**EV (extracellular domain) 970FLWL**KKR KQ**IKDL (immediately following the membrane-spanning segment) | 1390**ED**NA**DD EVD** | Contains a 24aa signal peptide, and a 23aa membrane-spanning segment; the 435aa intracellular domain has sequence similarity with the SRC family of Tyr kinases, insulin receptor, and v-Abl | Park et al., 1987 |

**TABLE VI. Putative NLS on Protein Phosphatases**

| Protein phosphatase | Species | Karyophilic peptides | Acidic stretches | Features | Reference |
|---|---|---|---|---|---|
| PP2C (382 aa, 42.4kDa) | Rat (kidney) | 149GLLC**RNRK** V**H**FFTQD**HK**P | 218**EPEVHDIE** RS**EEDD** | Cytosolic, distantly related to yeast adenylate cyclase; not related to PP1 and to PP2A | Tamura et al., 1989 |
| PPZ (348 aa) | Rabbit (brain cDNA library) | 10P**KKF**K**K**PIDI D | None | Ser/Thr phosphatase | Cohen et al., 1990 |
| PPY (314 aa) | *Drosophila* | 134DEI**KRRH**T VKLW | None | Related to PP1 (66% identity in catalytic domain) but different than *Drosophila* PP1; all three isoforms of *Dr.* PP1 are 94% identical to mammalian PP1 | Cohen et al., 1990 |
| PP1α (330 aa) | *Drosophila;* mammals | None (314PGG**R**-PITP**RR**N-SA**KAKK**end) | None | Perhaps the most evolutionarily conserved enzyme; 94% identity between *Drosophila* and mammalian enzymes | Cohen et al., 1990 |
| PPX (307aa) | Rabbit (liver cDNA library) | None | None | Related to but distinct from PP2A | Cohen et al., 1990 |
| PP2Aα | Rabbit | None (104**KVRYR**E-**R**ITILR**G**N**R** ES**R**) | None | Involved in chromosome separation in mitosis; suggested here to gain access to chromatin after dissolution of the nuclear envelope or via association with other proteins that possess NLS | Cohen et al., 1990 |

such available peptides in the nuclear compartment nonphosphorylated.

### Transmembrane Tyrosine Kinases

The transmembrane tyrosine kinases, most of which are growth factor receptors, may possess putative NLS in their primary structure (Table V); in their case the signal N-terminal hydrophobic peptide directing them to the lumen of the endoplasmic reticulum and then from the Golgi to the cell membrane is a stronger determinant than their NLS-like peptides for their subcellular localization as was first proposed by Boulikas [1994b].

### Are Src and Raf Proteins Imported Into Nuclei?

The Raf family of serine/threonine kinases comprises c-Raf-1, v-Raf, Pks, v-Mht, and A-Raf; these proteins are related to the Src family of tyrosine kinases. Their association with the cell membrane like that of the Src family of tyrosine kinases depends on their myristilation. However, unlike v-Src, which requires myristilation for transforming activity, transformation by Raf protein does not [for references see Huleihel et al., 1986]. Raf proteins seem to be cytosolic; v-Raf is expressed by the 3611 murine sarcoma virus (3611-MSV) in the form of two differentially modified Gag-fusion proteins, a myristilated/phosphorylated p79 and a glycosylated p90 [see Huleihel et al., 1986].

All Raf proteins possess the weak **RKTRH** NLS embedded in a 15aa hydrophobic segment; however, A-Raf possesses the DD**KKKVK**NLG motif and c-Raf-1 the **HKGKK** motif in addition to the **RKTRH** which is conserved in all Raf members. We suggest that A-Raf enters the nuclei and that the other Raf members may do

so to a lesser extent. It is interesting to note that the Src family of kinases, which comprises members with either Tyr or Ser/Thr kinase activity, do not possess a transmembrane domain and that their association with the cell membrane depends on their myristilation; we suggest that a population of nonmyristilated kinase molecules may shuttle between the cytoplasm and the nucleoplasm.

### Inert Karyophilic Signals

The fact that most cytoplasmic protein kinases possess a NLS-like karyophilic signal does not necessarily mean that they have to spend some time in the nucleus. This signal, especially on those molecules containing the bulky amino acids W, F, or Y in the hexad or those embedded in a short hydrophobic segment of the protein, may be unavailable for binding to the transporter molecules; one or more phosphorylations at S, T, or Y residues vicinal to the NLS may unmask such cryptic NLS, rendering the kinase amenable to nuclear import. If the four amino acids of the hexapeptide are arginines and lysines whose $pK_a$ is 9.5 to 12.5, it will be harder to be burried or hidden in the protein structure unless brought into electrostatic interaction with a complementary segment containing four aspartic, glutamic, or phosphoserine/phosphothreonine/phosphotyrosine residues within a cleft of the protein.

Evidently, proteins possessing functional NLS may remain strictly cytoplasmic if strongly associating with large cytoplasmic protein complexes or structures like filaments, cell membrane, or endoplasmic reticulum membrane proteins [Boulikas, 1994a].

### Mechanisms of Protein Transportation

It has been proposed [Boulikas, 1993] 1) that the anchoring of the protein to be transported to the nucleus to the transporter protein vehicle is electrostatic and 2) that not only karyophilic but also acidic amino acid clusters on the protein molecule to be imported contribute to its binding to the transporter proteins; this is justified from presence of $(K/R)_4$ as well as $\mathbf{Rx_3Rx_4Rx_3}$ $\mathbf{Rx_3Rx_4Rx_3}$ $\mathbf{Rx_2Rx_3R}$ type of motifs displaying a positive charge to the same side of the α-helix on the small number of transporter proteins whose primary structure is known [Lee et al., 1991; Meier and Blobel, 1992]. Thus, electrostatic binding might be disrupted either in the higher ionic milieu of the nucleoplasm [Mazzanti et al., 1990] or by competition by RNA molecules for binding to the same transporter protein molecule for their export to the cytoplasm. In this model the transporter protein molecules shuttle between the nucleus and the cytoplasm through the pore complexes of the nuclear envelope and are used not only for nuclear protein import but also for RNA export.

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