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Stathmin interaction with a putative kinase and coiled–coil-forming protein domains

(two-hybrid/regulatory cascades/BiP)

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ABSTRACT **Stathmin is a ubiquitous, cytosolic 19-kDa protein, which is phosphorylated on up to four sites in response to many regulatory signals within cells. Its molecular characterization indicates a functional organization including an N-terminal regulatory domain that bears the phosphorylation sites, linked to a putative α-helical binding domain predicted to participate in coiled–coil, protein–protein interactions. We therefore proposed that stathmin may play the role of a relay integrating diverse intracellular regulatory pathways; its action on various target proteins would be a function of its combined phosphorylation state. To search for such target proteins, we used the two-hybrid screen in yeast, with stathmin as a "bait." We isolated and characterized four cDNAs encoding protein domains that interact with stathmin *in vivo*. One of the corresponding proteins was identified as BiP, a member of the hsp70 heat-shock protein family. Another is a previously unidentified, putative serine/threonine kinase, KIS, which might be regulated by stathmin or, more likely, be part of the kinases controlling its phosphorylation state. Finally, two clones code for subdomains of two proteins, CC1 and CC2, predicted to form α-helices participating in coiled–coil interacting structures. Their isolation by interaction screening further supports our model for the regulatory function of stathmin through coiled–coil interactions with diverse downstream targets via its presumed α-helical binding domain. The molecular and biological characterization of KIS, CC1, and CC2 proteins will give further insights into the molecular functions and mechanisms of action of stathmin as a relay of integrated intracellular regulatory pathways.**

Protein phosphorylation and dephosphorylation are among the most frequent molecular mechanisms at the basis of multiple regulations implicated in cellular processes (1). Analysis of protein phosphorylation in response to signals affecting cell activities led to the identification of stathmin as a major phosphorylation substrate in numerous cell types and in response to the activation of various intracellular regulatory pathways (2). Stathmin is a highly conserved, 19-kDa cytoplasmic and soluble phosphoprotein, also designated p19 (3), prosolin (4), p18 or Op18 (5, 6), Oncoprotein 18 (7), and pp20-pp21-pp23 (8). It is phosphorylated in response to hormones (9), to growth and differentiation factors (10), to neurotransmitters (11), or upon activation of T lymphocytes (4, 12, 13). Furthermore, stathmin phosphorylation is also regulated in association with the stress response (14) and during the cell cycle (refs. 15–17; L. Beretta, E. Bailly, M. Bornens, and A.S., unpublished work). The expression of stathmin in cells and tissues is also regulated, in particular during development (18, 19) and tissue regeneration (20). This expression is characterized by a peak around birth in mammals in every tissue analyzed (18). In the adult, stathmin is detected in all tissues, the highest level being in brain, where it is mostly present in neurons (21, 22), in testis (18), and in activated or leukemic lymphocytes (5, 23). On the basis of its overall regulatory and molecular features, we proposed that stathmin could act as a general integrator and relay of signals controlling cell proliferation, differentiation, and functions, during development and adult life (2, 24).

Phosphorylation studies (25–27), sequence analysis (28–31), interspecies comparisons (31), and circular dichroism (32) indicate that stathmin is composed of (*i*) an N-terminal signal-integrating regulatory domain, with four residues that can be phosphorylated *in vivo*, and (*ii*) a C-terminal, mainly α-helical domain that may participate in coiled–coil interactions with downstream-regulated proteins. This stathmin structure is at the basis of a conserved protein family because at least two brain-specific proteins, SCG10 and XB3, have been shown to possess a conserved stathmin domain linked to an additional N-terminal putative membrane anchor (31, 33).

On the basis of this model predicting the interaction of stathmin with diverse downstream target proteins through its putative α-helical domain, we applied the two-hybrid screen in yeast (34, 35) to search for proteins interacting with stathmin *in vivo*. We isolated cDNAs coding for domains of four distinct proteins that are thus likely to be associated with stathmin function: the heat-shock protein BiP (for review, see ref. 36); KIS, a previously unidentified, putative serine/threonine kinase; and two proteins predicted to participate in protein–protein interactions by forming coiled–coil structures and that are therefore good candidates as functional targets for stathmin.§ Altogether, the identification of these proteins by interaction screening further supports our model for the structural organization and regulatory function of stathmin. Their molecular and biological characterization will give further insights into the functions and mechanisms of action of stathmin as a relay of integrated intracellular regulatory pathways.

MATERIALS AND METHODS

Construction of the LexA Stathmin Fusion Vector pLST. To express stathmin hybrid proteins in yeast for interaction screening, we inserted the cDNA for human stathmin in plasmid pVJL10 (37), which allows constitutive expression of protein domains fused to the LexA DNA-binding domain; it also contains the *TRP1* gene as a selectable marker. Briefly, plasmid HST19.6 containing the complete cDNA for human stathmin (1429 bp) (38) in Bluescript SK+ was digested with *Nco* I, made blunt by filling with Klenow fragment, and cut at the *Bam*HI site of the polylinker of Bluescript downstream of the 3′ end of stathmin cDNA. The resulting fragment was cloned downstream of the LexA DNA-binding domain by

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§The sequences reported in this paper have been deposited in the GenBank data base [accession nos. X82318 (CC1), X82319 (CC2), and X82320 (KIS)].

ligation into pVJL10 that had been digested with *Eco*RI, filled in with Klenow fragment, and digested with *Bam*HI.

Fusion Protein-Encoding Plasmids Used for Controls; Fusion Library. pLexA–RAS^{V12} encoding the Ras^{V12} mutated form of Ras fused to the DNA-binding domain of LexA, and pGAD–raf encoding Raf fused to the activation domain of GAL4 are from A. Vojtek (Fred Hutchinson Cancer Research Center, Seattle) (39) and L. Van Aelst (Cold Spring Harbor Laboratory) (40), respectively. pLAM5, referred to in the text as pLexA–Lamin, was provided by A. Vojtek and R. Sternglanz (State University of New York, Stony Brook). This plasmid encodes human lamin C (aa 66–230) as a fusion to the DNA-binding domain of LexA. The mouse embryonic day-9.5 to day-10.5 random-primed cDNA library in pVP16 is from A. Vojtek (39). pVP16 allows expression of fusion proteins to the activation domain of VP16; it contains the *LEU2* gene as a selectable marker.

Two-Hybrid Screen. The yeast reporter strain L40 (39) was used as host for expression of the hybrid proteins. It contains the reporter genes *LacZ* and *HIS3* downstream of the binding sequences for LexA. A *Saccharomyces cerevisiae* L40 reporter strain containing pLST was transformed with the pVP16 fusion library using the lithium acetate method and subsequently treated as described (39). His$^+$ colonies were picked up 4–7 days later, plated on medium lacking tryptophan, leucine, and histidine, and LacZ expression was tested by a filter method as described (39). Plasmid DNA was prepared from colonies displaying a His$^+$/LacZ$^+$ phenotype and used to transform *Escherichia coli* HB101 by electroporation. Library plasmids were selected by incubating the transformation mix for 40 hr in minimal medium (M9) lacking leucine and containing ampicillin, thiamine, and proline. Plasmid DNA was prepared from the growing bacteria and used to transform again L40 yeasts containing pLST to confirm the His$^+$/LacZ$^+$ phenotype conferred by the library plasmids. In our hands, several different library plasmids are often recovered from a single yeast colony; thus, this second passage in yeast is also a "purification" step to correlate the His$^+$/LacZ$^+$ phenotype with a single library plasmid. Briefly, for each bacterial preparation used in this second yeast transformation, one His$^+$/LacZ$^+$ yeast colony was picked for yeast plasmid preparation. HB101 bacteria were transformed with this latter plasmid preparation, and cells were plated on M9 medium containing proline, tryptophan, thiamine, and ampicillin. A single colony was picked to prepare library plasmid DNA. To assay for stathmin dependency of the His$^+$/LacZ$^+$ phenotype conferred by these plasmids, they were used for double transformation of L40 yeasts together with either pLST or pLexA-lamin. pVP16 library plasmids yielding His$^+$/LacZ$^+$ phenotype when used for cotransformation with the LexA–stathmin-encoding plasmid, but not with the LexA–lamin-encoding one, were further tested for specificity by using other plasmids encoding proteins fused to LexA, such as Ras. Positive controls included interactions of Ras^{V12} with Raf in L40 (39).

Sequencing and Sequence Analyses. Nucleotide sequences were determined on both strands with double-stranded plasmid DNA, primers hybridizing with pVP16 upstream and downstream of the *Not* I insertion site, and the Sequenase kit (Amersham). Sequences were analyzed with the Bisance (41) and Genetics Computer Group (42) software packages.

RNA Blot. Total RNAs were isolated from neonate mouse tissues as described (43), electrophoresed in a formaldehyde-agarose gel, and blotted to a Hybond-N filter (Amersham). The blot was checked for RNA integrity and transfer by staining in 0.04% methylene blue/0.5 M sodium acetate, pH 5.2, for 2 min, washing 1 min with water, and then destaining 10 min in 40% (vol/vol) ethanol. Hybridization was done at 42°C in 50% (vol/vol) formamide/5× standard saline citrate/0.1% SDS/2× Denhardt's solution, using the multiprime labeled insert of clone 112 as a probe. Last washes were at 60°C in 0.2× standard saline citrate.

RESULTS

Interaction Screening of a Mouse Embryonic cDNA Library with Stathmin. The molecular and functional characterization of stathmin and of its regulations indicate that it is composed of an N-terminal domain bearing its phosphorylation sites and of a C-terminal, α-helical domain predicted to form coiled–coil interacting structures with downstream target proteins (see Fig. 5). We therefore searched for proteins interacting with stathmin *in vivo* by interaction screening with the two-hybrid system in yeast (34, 35). The DNA-binding domain of LexA was fused to stathmin, and its interaction with a protein X fused to the activation domain of VP16, yielding to the assembly of a functional transcription activator, was then revealed by the expression of the reporter genes *HIS3*, conferring histidine prototrophy, and the β-galactosidase *LacZ* gene.

The host cells L40 were transformed with pLST, encoding stathmin fused to the LexA DNA-binding domain. The stable expression of the fusion protein was checked by immunoblotting of protein extracts.

Because stathmin is highly expressed and markedly regulated at various stages of embryonic development (18, 19, 31), we screened a 9.5- to 10.5-day mouse embryonic cDNA library in the plasmid pVP16, by transformation of the *S. cerevisiae* L40 reporter strain, previously transformed with the pLST construction directing the expression of the stathmin "bait." pVP16 allows constitutive expression of protein domains fused to the nuclear-localized VP16 transcription activator. An estimated 14×10^7 Trp$^+$/Leu$^+$ transformants were plated for selection (the stathmin-encoding plasmid contains the *TRP1* gene; the library plasmids contain the *LEU2* gene), among which ≈800 grew after 7 days in the absence of histidine. Seventy percent of these His$^+$ colonies were also positive in the 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal) test. Plasmid DNA was prepared for 276 of these His$^+$/LacZ$^+$ colonies.

L40 yeasts were retransformed with selected library plasmids and with pLST or with plasmids directing the expression of other proteins fused to LexA. Specific clones yielded a positive β-galactosidase signal only when coexpressed with the stathmin fusion protein. To reduce the number of clones to be tested for specificity, redundant clones were eliminated by dot hybridizations of the DNA from the 276 originally positive clones with mixed probes corresponding to characterized false-positive or specific ones. Altogether, the present screening led to the isolation of six specific clones encoding domains of four distinct proteins, as shown by sequence analysis (see below) and by the absence of double-positive clones when 1.2×10^6 phages of a mouse neonate brain random-primed cDNA library in λgt10 were plated and hybridized with DNA probes corresponding to the clones isolated through the two-hybrid screen (data not shown).

A Protein of the hsp70 Family. Clone 434 encodes aa 421–538 in the C-terminal region of the heat-shock protein BiP/GRP78 (36) (amino acid numbering of the rat sequence). Interestingly, three other His$^+$/LacZ$^+$ clones out of the 276 positive clones of the pVP16 library correspond to different in-frame ligations of BiP cDNA having in common its region corresponding to aa 421–533.

A Putative Protein Kinase. Clone 112 encodes a 199-aa domain. The nucleotide sequence of clone 112 was not found in the GenBank data base (release 80). However, the corresponding amino acid sequence can be aligned with those of protein kinase catalytic cores, from domain VIa to XI (44) (Fig. 1), with an additional 16-residue C-terminal extension (data not shown). The characteristic 10 residues conserved in almost all serine/threonine kinase sequences within these

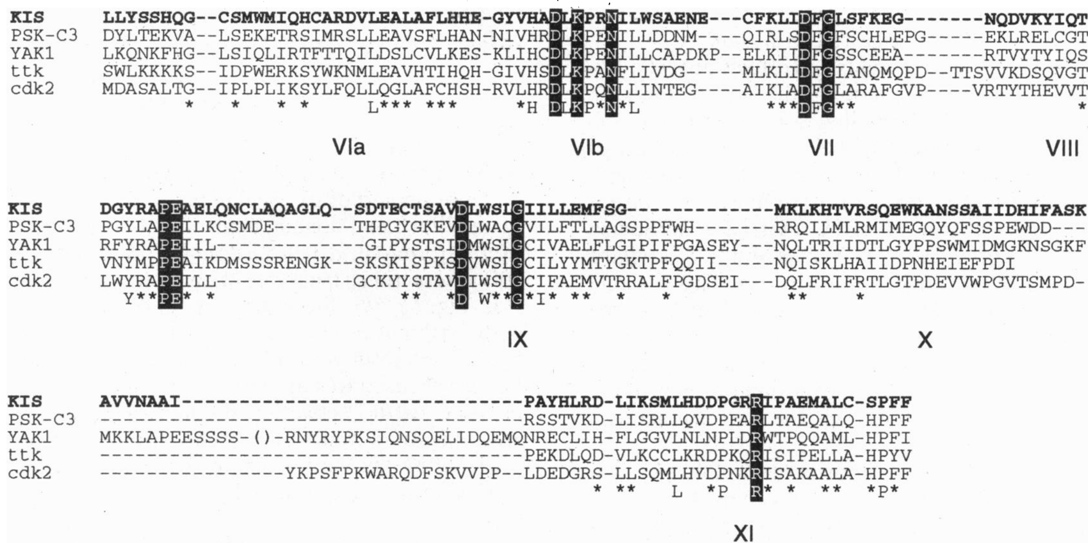

FIG. 1. Sequence alignments indicate that KIS is a previously unidentified, putative serine/threonine kinase. The amino acid sequence of KIS deduced from clone 112 was aligned with the protein sequences yielding the highest homology scores by scanning the National Biomedical Research Foundation data base with FASTA (accession nos. for PSK-C3, YAK1, TTK, and cdk2 are, respectively, A42034, A32582, A42861, and A41227). Residues conserved in almost all serine/threonine kinases are shown by black boxes with white letters. Residues conserved in the five sequences appear under the alignment, and stars indicate conservation of a residue in three or four sequences.

domains are all present in the sequence of the protein domain encoded by clone 112 (Fig. 1). Thus, clone 112 codes for the larger lobe of the catalytic domain of a putative serine/threonine kinase. The best homology scores found by scanning the GenBank data base with FASTA are for the human phosphorylase kinase psk-c3, YAK1, ttk (PYT), and cdk2 with identities between 27% and 32%, indicating that this is a previously unidentified kind of kinase. We designate the corresponding protein KIS, for "kinase interagissant avec la stathmine." RNA blot analysis revealed that a single mRNA corresponding to KIS is expressed, like stathmin, predominantly in the brain (Fig. 2).

Two Independent Domains Predicted to Form Coiled–Coil, Protein–Protein Interacting Structures. Clones 152 and 189 are identical and encode a 142-aa domain (Fig. 3) that does not show strong homology with any sequence of the GenBank data base. The best alignments are with protein domains rich in charged amino acids and/or coiled–coil domains. The Chou and Fasman algorithm (45) predicts the presence of α-helices. Using the algorithm of Stock and colleagues (46), two domains are strongly predicted (>97% probability) to be forming coiled–coils, although they do not present a perfect regularity in the heptad repeats of hydrophobic amino acids. We therefore designate the corresponding protein as CC1 for coiled–coil-forming protein 1.

Clones 257 and 262 are identical and encode an 83-aa domain. Sequence comparisons gave results similar to those with CC1. Structure predictions include mostly an α-helix from residue 1 to 70. As for the CC1 protein, the sequence is rich in charged amino acids and the algorithm of Stock and colleagues (46) predicts, with a probability >99%, that the helix will be involved in a coiled–coil structure. Fig. 4 shows clearly the cluster of hydrophobic residues on one side of the helix and the concentration of charged residues all along the putative helix. The heptad repeats include a leucine-zipper motif with four leucines at 7-residue intervals, further indicating the protein–protein interaction potential of this domain. By analogy with the CC1 protein, we designate the protein corresponding to these clones as the CC2 protein.

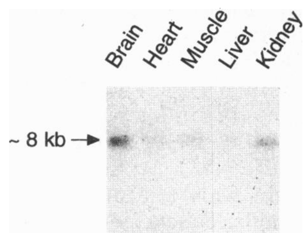

FIG. 2. Tissue distribution of KIS mRNA. KIS mRNA was detected in mouse neonate tissues by hybridization of total RNA (5 μg per lane) with a cDNA probe derived from clone 112. The apparently unique mRNA species detected migrated with an apparent size of ≈8 kb. A faint signal was visible on the original autoradiogram in the heart, muscle, and liver samples.

clone 152 / CC1

```
GCGGCCGCTCAAG CAG AAG GAA ATG CTG AAG TCT TTA CTT    40
               Q   K   E   M   L   K   S   L   L      9

GAA CAA GAG ACC GAA AAC TTA AGA ACA GAA ATA AGT AAA   79
 E   Q   E   T   E   N   L   R   T   E   I   S   K    22

CTA AAC CAA AAA ATT CAT GAT AAT AAT GAG AGT TAC CAG  118
 L   N   Q   K   I   H   D   N   N   E   S   Y   Q    35

GTG GGT TTG TCA GAG TTA AGA GCT TTA ATG ACA ATT GAA  157
 V   G   L   S   E   L   R   A   L   M   T   I   E    48

AAA GAT CAG TGC ATT TCA GAG TTA ATC AGT AGA CAT GAA  196
 K   D   Q   C   I   S   E   L   I   S   R   H   E    61

GAA GAA TCT AAT ATA CTT AAG GCT GAA TTA GAC AAT GTT  235
 E   E   S   N   I   L   K   A   E   L   D   N   V    74

ACA TCT TTG CAT CGC CAA GCA TAT GAA ATA GAA AAA AAA  274
 T   S   L   H   R   Q   A   Y   E   I   E   K   K    87

CTG AAA GAA CAA ATA GTT GAA TTG CAG ACT AGA TTG AAC  313
 L   K   E   Q   I   V   E   L   Q   T   R   L   N   100

TCA GAA TTG AGT GCT CTT GAA AAA CAG AAA GAT GAA AAA  352
 S   E   L   S   A   L   E   K   Q   K   D   E   K   113

ATA ACC CAA CAA GAA GAG AAG TAT GAA GCA CTT ATC CAG  391
 I   T   Q   Q   E   E   K   Y   E   A   L   I   Q   126

AAC CTT GAG AAA GAC AAG GAG AGA CTG GTC AAG AAC CAC  430
 N   L   E   K   D   K   E   R   L   V   K   N   H   139

GAG CAA GCT GAGCGGCCGC                               449
 E   Q   A                                          142
```

FIG. 3. CC1 sequence of clone 152 and structure predictions. The nucleic acid sequence of clone 152 encoding a domain of protein CC1 was translated in-frame with the upstream VP16-coding sequence, yielding a 142-aa sequence (linker sequences are in italics). With the Chou and Fasman (45) algorithm, several α-helices were predicted, within which two coiled–coil-forming regions were predicted (underlined) with the Stock and colleagues (46) algorithm and >97.6% (N-terminal) and 99.8% (C-terminal) confidence.

clone 257 / CC2

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GCGGCCGCTCAGG CGG ATG AAG GAG GAA ATG GAT GGT GCC    40
              R   M   K   E   E   M   D   G   A       9

CAG GCA GAG CTT AAT GCC TTG AAA CGA ACA GAG GAA GAT   79
Q   A   E   L   N   A   L   K   R   T   E   E   D     22

CTG AAA AAA GGC CAC CAG AAA CTG GAA GAG ATG GTC ACC  118
L   K   K   G   H   Q   K   L   E   E   M   V   T     35

CGC TTA GAT CAA GAA GTA GCT GAA GTT GGT AAA AAC ATA  157
R   L   D   Q   E   V   A   E   V   G   K   N   I     48

GAA CTT TTG AAA AAG AAG GAT GAA GAA CTA AGT TCT GCT  196
E   L   L   K   K   K   D   E   E   L   S   S   A     61

CTG GAG AAA ATG GAA AAT CAA TCT GAA AAT AAT GAT ATT  235
L   E   K   M   E   N   Q   S   E   N   N   D   I     74

GAT GAA GTT ATC ATT CCC ACA GCC CAA  TGAGCGGCCGC     273
D   E   V   I   I   P   T   A   Q                     83
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CC2 : alpha helix (Coiled-coil, p>99%)

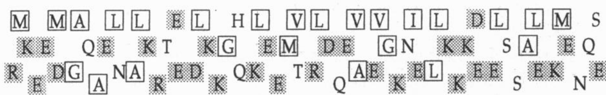

FIG. 4. CC2 sequence of clone 257 and analysis of its predicted α-helix. (*Upper*) The nucleic sequence of clone 257, encoding a domain of protein CC2, was translated in-frame with the upstream VP16-coding sequence, yielding an 83-aa sequence (linker sequences are in italics). The Chou and Fasman (45) algorithm predicted an α-helix structure from residue 1 to 70. The coiled–coil probability of this helix using the algorithm of Stock and colleagues (46) is >99%. (*Lower*) The projection of its amino acid sequence on a plane containing the axis of the putative helix reveals that it is amphipathic; hydrophobic residues are in white boxes, and charged ones are shaded.

DISCUSSION

In agreement with the predicted functional structure of stathmin, the present two-hybrid screen led us to identify several proteins interacting with stathmin *in vivo*. The characterization of their molecular domains involved in the interaction indicate that some, such as KIS, are most likely acting upstream, whereas others, such as CC1 and CC2, are acting rather downstream of stathmin in its proposed role of a relay integrating diverse intracellular regulatory pathways.

A protein domain of BiP, a member of the hsp70 family localized in the endoplasmic reticulum (for a review, see ref. 36), was found to interact with stathmin in the present screen. Interestingly, independent biochemical and immunoprecipitation studies led us to similar results, as they demonstrate specific interactions of stathmin with BiP and with hsc70, the constitutively expressed member of the hsp70 family (V. Manceau, P. Curmi, and A.S., unpublished data). As stathmin is mostly localized in the cytoplasm, it is most likely that, in physiological conditions, it interacts with hsc70 or other cytoplasmic proteins of the hsp70 family.

Proteins of this family are thought to be acting essentially as molecular chaperones (36). Clone 434 described here corresponds to a 118-aa region of the peptide-binding domain (47), indicating that stathmin could be a target of an hsp70 family member. The domain encoded by clone 434 lacks the sequences corresponding to the predicted first β-strand and last α-helix of the peptide-binding domain of hsp70 (48). Therefore, an alternative interaction of the first α-helix presenting hydrophobic residues with the α-helical domain of stathmin could occur corresponding then to a regulatory action of stathmin on the functional properties of hsp70-like proteins. Such a putative interaction could be regulated during the stress response because stathmin has been shown to be highly phosphorylated in response to heat shock in HeLa cells (14).

The amino acid sequence of the domain of KIS deduced from clone 112 shows all the sequence characteristics of the larger lobe of the catalytic domain of a serine/threonine kinase. Preliminary results based on cDNA cloning from a brain cDNA library indicate that this domain is prolonged on its N-terminal end by a sequence showing hallmarks of the small lobe of protein kinases, thus confirming the identification of KIS as a protein kinase. Furthermore, a C-terminal extension was also identified that showed sequence homologies to RNA recognition motifs (49), and thus, this putative protein kinase contains a distinctive regulatory domain.

The observation that, as for stathmin, KIS mRNA is most highly expressed in the brain strengthens the hypothesis that the interaction of KIS with stathmin is physiologically relevant. Because residues involved in substrate recognition are thought to be mainly located at the cleft surface on the larger lobe of kinases, as in the case of protein kinase A interacting with the pseudosubstrate protein kinase inhibitor PKI (for review, see ref. 50), it seems likely that stathmin might be a substrate for KIS. Four serine residues can be phosphorylated *in vivo* on stathmin, Ser-16, -25, -38, and -63 (26), and phosphorylation of stathmin is regulated in response to diverse regulatory signals, to heat shock, and during the cell cycle. Ser-25 is phosphorylated by mitogen-activated protein (MAP) kinase in PC-12 cells in response to nerve growth factor stimulation (27) and in HeLa cells in response to stress (14); stathmin is phosphorylated in response to cAMP increase in neurons (21) and protein kinase A phosphorylates stathmin *in vitro* on Ser-63 (26); Ser-38 is a substrate of cdc2 and cdk2 (26, 51); finally Ser-16 is phosphorylated in response to Ca^{2+} increase (7, 16). Altogether, stathmin appears as an integrator of kinase-activating signals. It is thus likely that KIS belongs to the kinases whose activation is relayed through stathmin toward downstream interacting proteins (Fig. 5). However, the regulation of KIS activity by stathmin cannot be excluded.

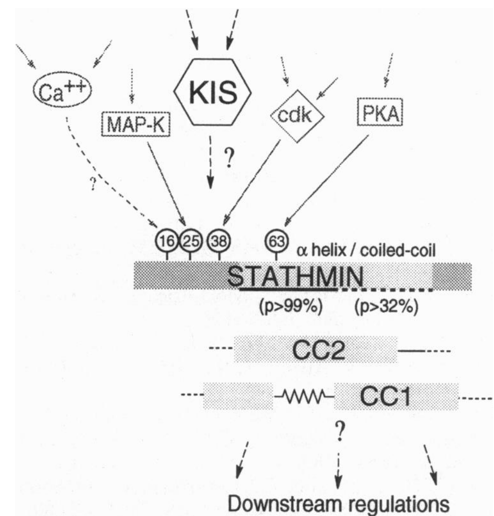

FIG. 5. Model showing the likely relationships of stathmin-interacting molecules with stathmin and its action within intracellular regulatory pathways. Phosphorylation of stathmin on up to four serines is a function of concurrent signaling pathway activations. The second-messenger pathways and kinases most probably implicated are indicated. KIS, identified as a stathmin-interacting protein and predicted to have kinase activity, might be part of these. Stathmin is predicted to interact with other proteins through its middle and C-terminal, mostly α-helical, domain by forming coiled–coil structures [probabilities calculated using the Stock and colleagues (46) algorithm are indicated for two regions of the α-helical domain]. CC1 and CC2 proteins are also predicted to interact with protein partners by forming coiled–coil structures, which suggests that they are good candidates for being stathmin downstream-regulated protein targets. MAP-K, mitogen-activated protein kinase; PKA, protein kinase A; cdk, cell-cycle-dependent kinase.

CC1 and CC2 are good candidates as protein targets being regulated through stathmin phosphorylation. Previous studies based on sequence analysis (28–31) and CD studies (32) indicated that stathmin could be involved in regulation of proteins through interactions yielding coiled–coil structures. Analysis of CC1- and CC2-predicted structures suggests that they are also involved in coiled–coil formation and thus could interact with the C-terminal helical region of stathmin. This observation agrees well with our model (see Fig. 5) that stathmin may be a relay between kinases activated within intracellular regulatory cascades and diverse proteins interacting with its C-terminal domain by forming coiled–coil structures.

Stathmin has been proposed as a general integrator and relay of signals affecting cell state and activities, mostly because of its phosphorylation being ubiquitously associated with cell regulations. Accordingly, this study of putative stathmin-interacting proteins with the two-hybrid system led to the identification of four proteins of likely physiological relevance. Other such regulatory and target proteins of stathmin might be further identified with a similar approach, corresponding to the diversity of regulatory functions of stathmin expected from its wide distribution and regulation at various cell stages. Further characterization of the proteins identified here will contribute to the understanding of the many regulations that occur both upstream and downstream of stathmin and of other members of its protein family, as well as the diverse functions and mechanisms of action of stathmin within the regulatory cascades controlling cell activities.

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