Expression of transfected stathmin cDNA reveals novel phosphorylated forms associated with developmental and functional cell regulation

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Stathmin is a ubiquitous, highly conserved phosphoprotein, which most likely acts as an intracellular relay integrating various transduction pathways triggered by extracellular signals. Two post-translational isoforms (α and β) have been previously identified whose increasingly phosphorylated forms migrate as a set of isoelectric variant spots (molecular mass 19 kDa; pI 6.2–5.6) on two-dimensional electrophoretic gels. In parallel with the phosphorylation of these forms of stathmin, two sets of three proteins migrating with slightly higher apparent molecular masses (21 and 23 kDa respectively) also incorporated radioactive phosphate in response to cell regulation through various transduction pathways. These phosphoproteins, previously referred to as proteins '16' and '17', share several biochemical properties with stathmin and are recognized by antibodies directed to stathmin or to stathmin peptides. Furthermore, when rat stathmin cDNA was transfected into mouse myogenic C2 cells, it directed the expression of protein sets 16 and 17 together with the 19 kDa forms of stathmin, as detected with a species-specific anti-stathmin antiserum. Proteins 16 and 17 are thus novel phosphorylated derivatives of stathmin, encoded by the same cDNA as its previously identified 19 kDa forms. These results increase the known complexity and diversity of stathmin patterns, which may yield the molecular support for its proposed role as a relay integrating various signals which regulate the proliferation, differentiation and functions of cells during development and adult life.

INTRODUCTION

Extracellular signals such as hormones, growth and developmental factors, and neurotransmitters act on their specific target cells through a variety of transduction pathways. In the subsequent cascade of intracellular events, protein phosphorylation–dephosphorylation reactions are known to play a major role in cell regulation [1].

Stathmin [2], also referred to as p19 [3], prosolin [4], Op18 [5], pp20 [6] and 19K [7], is a ubiquitous, highly conserved [8–10] 19 kDa cytoplasmic protein whose phosphorylation appears to be associated, in many biological systems, with the regulation of cells by their specific effectors (reviewed in [11]). We thus proposed that this protein may act as a general intracellular relay, possibly integrating extracellular signals triggering various second messenger pathways [2]. In good agreement with this model, the expression of stathmin is also highly regulated during early and late embryonic and postnatal development [8,12–15], as well as in relation to cell proliferation and differentiation [15–18].

At least eight molecular forms of stathmin have been identified so far, which migrate as unphosphorylated (N1, N2) and increasingly phosphorylated (P1–P3) spots (molecular mass 19–20 kDa; pI 6.2–5.6) on two-dimensional electrophoretic gels [19]. They are all derived from two isoforms, α and β [19], which are themselves post-translational variants encoded by the same mRNA [14].

In addition, we have identified two other sets each comprising three phosphorylated spots, designated respectively proteins '16' and '17' [12,20–22], which migrate at slightly higher apparent

molecular masses (21 and 23 kDa respectively) than the N1–P3 stathmin spots. Proteins corresponding to these two sets were, like stathmin, present in all the biological systems examined, and their phosphorylation pattern in response to extracellular regulatory agents also reflected, in a way similar to that of the 19 kDa forms of stathmin, the corresponding intracellular pathways activated [12,17,20–22]. Furthermore, proteins which are most likely identical to at least some of the forms of protein sets 16 and 17 were shown to have one-dimensional [6] or two-dimensional [23] peptide maps similar to those obtained with stathmin.

In this study we show that, in addition to the similar regulation of their phosphorylation, proteins of sets 16 and 17 share common biochemical and immunological properties with the 19 kDa forms of stathmin. Furthermore, stathmin cDNA transfection experiments demonstrate that these two sets of proteins are actually additional phosphorylated forms of a single mRNA translation product, namely stathmin itself. This result extends the diversity of stathmin forms and thus further supports the model in which stathmin may act as an intracellular relay globally reflecting the general state of activation of cells.

MATERIALS AND METHODS

Cell culture

Primary culture of striatal neurons from 16-day-old mouse embryos was performed as described previously [12]. PC12 cells were grown as previously described [21]. C2 cells were grown as myoblasts at 37 °C with 5% CO₂ in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. Jurkat T

Abbreviations used: 1D-/2D-PAGE, one-/two-dimensional PAGE; NGF, nerve growth factor; PMA, phorbol 12-myristate 13-acetate; PHA, phytohaemagglutinin; PKA, cyclic AMP-dependent protein kinase; PKC, protein kinase C.

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lymphoblastoid cells were maintained in suspension culture at $(5-10) \times 10^5$ cells/ml in RPMI 1640 supplemented with 10% fetal calf serum, 1 mM-pyruvate, 4 mM-glutamine, 50 units of penicillin/ml and 50 μ g of streptomycin/ml.

$^{32}\text{PO}_4^{3-}$ labelling

Labelling was performed by preincubating cells for 4 h with 0.3 mCi of $^{32}\text{PO}_4^{3-}$ in 250 μ l of phosphate-free medium per 16 mm well, the effectors being added to the radioactive medium for the last 10 or 15 min. The labelling was stopped as previously described [2] and samples were prepared for electrophoresis according to Garrels [24], with some modifications [25].

Tissue and cell extracts

Mouse or rat forebrain was homogenized in 9 vol. of homogenization buffer (25 mM-sodium phosphate, pH 7.8, 10 μ g of leupeptin/ml, 25 μ g of aprotinin/ml, 10 μ g of pepstatin/ml, 0.5 mM-EDTA sodium salt) and centrifuged at 11600 *g* for 3 min, thus providing the 'low-speed supernatant' extracts. Stathmin was further purified by a succession of high-speed centrifugation, and DEAE and phenyl-Sepharose chromatographies. The detailed purification procedure will be described elsewhere (T. Dobransky, L. Beretta, V. Manceau, J. Koppel and A. Sobel, unpublished work; details available on application to authors).

PC12 or C2 cells were harvested in cold phosphate-buffered saline ($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ 2.16 g/l, KH_2PO_4 0.2 g/l KCl 0.2 g/l, NaCl, 8 g/l, pH 7.4) and centrifuged (400 *g*, 10 min). Jurkat cells, activated or not for 30 min at 37 °C (10^6 cells/ml) with a combination of phytohaemagglutinin (PHA; 37.5 μ g/ml) and phorbol 12-myristate 13-acetate (PMA; 10 ng/ml), were washed twice with RPMI 1640. Cells were resuspended in homogenization buffer and lysed by sonication.

Soluble tissue or cell extracts were obtained by centrifugation at 100000 *g* for 6 min in a Beckman TL100 ultracentrifuge. For heat extraction, samples were adjusted to 100 mM-NaCl, treated at 100 °C for 2 min, and centrifuged again as above to give rise to the 'boiled supernatant'.

Protein concentration was estimated by the method of Bradford [26], using BSA as standard.

PAGE and autoradiography

One-dimensional PAGE (1D-PAGE) was performed according to Laemmli [27] on 13% acrylamide gels. Two-dimensional PAGE (2D-PAGE) was performed as previously described [25]. The isoelectric focusing gels contained 2% total Ampholines pH 5-7 and 3-10 in the proportions 4:1, and the second dimension was run on 13% acrylamide gels. In the case of ^{32}P -labelled samples, the gels were fixed and exposed for autoradiography with Kodak XAR-5 films. For protein detection, gels were silver-stained according to Morrissey [28].

Western blot analysis

Proteins from 1D- and 2D-PAGE were transferred on to 0.2 μ m nitrocellulose (Schleicher & Schuell) as described [8]. The blots were saturated with casein (2.5%) and probed with a rabbit polyclonal anti-stathmin antibody directed either against an internal peptide (serum I) or against the entire rat stathmin extracted from 2D gels (serum P) [2,8]. Bound antibodies were detected with ^{125}I -Protein A (Amersham) and autoradiography.

Plasmid construction and transfection

The *EcoRI*-*DraI* fragment of stathmin rat cDNA (nucleotides

1-809) [14] was inserted into the *EcoRI* cloning site of the pSG5 vector (Stratagene), downstream of the simian virus 40 early promoter. The properties of protein encoded by the resulting plasmid pSG5-stath was checked by *in vitro* transcription using the T3 promoter of the pSG5 vector, followed by *in vitro* translation in a rabbit reticulocyte lysate. The translation products were then analysed on 2D-PAGE as previously described [14].

C2 cells were plated at a density of 6×10^5 cells/100 mm dish and were transfected 24 h later by the calcium phosphate precipitate procedure of Davis *et al.* [29]. Transfections were performed with 100 μ g of pSG5-stath and 10 μ g of pSV2neo DNA. At 2 days after transfection, geneticin (G418) was added to the culture medium at a concentration of 0.4 mg/ml. Colonies of G418-resistant cells were selected for clonal growth about 3 weeks after transfection and grown in 24-well dishes; 9 days later, cells were dissociated and subcultured into two 35 mm dishes. One dish was used to assay the expression of the transfected rat stathmin using one-dimensional Western blot analysis with the anti-stathmin antiserum P, which discriminates between the transfected rat stathmin and the endogenous mouse stathmin in C2 cells (see Fig. 4) [8]. Among the selected colonies, one clone stably expressed the exogenous stathmin and was further used in our study. C2 cells stably transfected with the pSV2neo vector alone were used as a control.

RESULTS AND DISCUSSION

Phosphorylation of stathmin and proteins 16 and 17 in PC12 cells and in striatal neurons in culture

We previously reported that the stimulation by extracellular regulatory agents of the phosphorylation of the 19 kDa isoforms of stathmin (cDNA-derived calculated molecular mass of 17269 Da) was often associated with the incorporation of ^{32}P into two other protein sets, which we designated 16 (21 kDa) and 17 (23 kDa) [12,20-22], migrating in the same area as stathmin on two-dimensional gels.

In $^{32}\text{PO}_4^{3-}$ -labelled embryonic striatal neurons in culture, two phosphorylated spots of protein 17 were detected in the control unstimulated sample (Fig. 1a). Interestingly, a mixture of pharmacological effectors known to mimic or activate distinct second messenger pathways (PMA, forskolin and ionomycin) stimulated, together with the phosphorylation of the P2 and P3 spots of stathmin, the incorporation of ^{32}P into the sets of spots of proteins 16 and 17: into the originally described spots 16 and 17 (designated here 16₁ and 17₁) and with an even higher intensity into the more acidic spots (16₂, 16₃ and 17₂, 17₃), corresponding probably to more phosphorylated forms of proteins 16 and 17 (Fig. 1a).

In $^{32}\text{PO}_4^{3-}$ -labelled PC12 cells, nerve growth factor (NGF) also elicited the incorporation of ^{32}P into the sets of spots 16 and 17 (Fig. 1b). Interestingly, in a soluble heat-stable fraction originally designed for the enrichment of stathmin, silver-stained protein spots co-migrating with the ^{32}P -labelled spots 16 and 17 became detectable on two-dimensional gels of samples from NGF-stimulated PC12 cells, whereas they were not visible in the control sample (Fig. 1b). Thus, as in the case of the previously described conversion of the unphosphorylated isoforms of stathmin (N1, N2) towards their phosphorylated forms (P1-P3) [21] (Fig. 2), this result indicates that the increased ^{32}P labelling of proteins 16 and 17 corresponds not only to an enhanced phosphate turnover but to an actual increase of their level of phosphorylation. Similar results were also obtained with Jurkat leukaemic lymphocytes (see below, Fig. 3).

Altogether, it appears clear from the above-mentioned results

Fig. 1. Phosphorylation of stathmin and proteins 16 and 17

(a) Striatal neurons in culture were prelabelled with $^{32}\text{PO}_4^{3-}$ for 4 h and then treated or not for 10 min with a mixture of pharmacological effectors: PMA (100 ng/mg) + forskolin (10 μM) + ionomycin (0.2 μM). The areas of stathmin and proteins of the sets 16 and 17 on the 2D-PAGE autoradiograms are presented. Three phosphorylated spots (1–3) could be detected for each of the two sets 16 and 17 in the stimulated neurons. (b) PC12 cells, prelabelled or not with $^{32}\text{PO}_4^{3-}$, were stimulated with 7 S NGF (200 ng/ml) for 15 min. ^{32}P -labelled proteins were detected on the 2D-PAGE autoradiograms, whereas proteins from a boiled supernatant (5 $\mu\text{g}/\text{sample}$; see the Materials and methods section) were detected by silver staining. Note the NGF-induced appearance of spots 16₁ and 17₁ together with the previously described conversion of stathmin from its unphosphorylated (N1 and N2) to its 19 kDa phosphorylated (P1–P3) forms.

Fig. 2. Proteins of sets 16 and 17 co-purify with stathmin and are detected with an anti-stathmin antiserum

Proteins from (a) a heat-stable supernatant (see the Materials and methods section) and (b) a purified stathmin fraction obtained by classical chromatography from neonatal mouse brain were electrophoresed on two-dimensional gels and detected either by silver staining (a, 80 μg of protein; b, 7.5 μg of protein) or by immunoblotting with antiserum I, directed against an internal peptide of stathmin [8] (a, 400 μg of protein; b, 15 μg of protein). For a better detection of proteins 16 and 17, the same immunoblot (a) was exposed for autoradiography for a short (top) and a long (bottom) period. The unlabelled spot on the immunoblot in (b) is a minor degradation product appearing during purification.

that stathmin and proteins of sets 16 and 17 share similar regulatory properties of their phosphorylation.

Stathmin and phosphoproteins of sets 16 and 17 share common biochemical and immunological properties

To further assess the extent of the similarities of proteins 16 and 17 with stathmin at the molecular level, we examined if they were recognized by anti-stathmin antibodies. Two-dimensional Western blot analysis of a partially purified, heat-stable extract from neonatal rat forebrain, with an antiserum directed against an internal peptide of stathmin (serum I), showed that this serum reacted with several proteins migrating as the phosphorylated spots of set 17 and, although more weakly, set 16 (Fig. 2a). Similar results were obtained with antiserum P, directed against the whole stathmin molecule (not shown), thereby indicating that the proteins of sets 16 and 17 share at least several common epitopes with stathmin.

Since proteins of the sets 16 and 17 could be detected in boiled cell and tissue extracts (Figs. 1b and 2a), they also share the characteristic heat stability of stathmin. In addition, when stathmin was further purified (see the Materials and methods section) from neonatal mouse forebrain, proteins 16 and 17 were co-purified with stathmin, as they were still detected in a purified stathmin fraction (Fig. 2b). This result thus further stresses the molecular similarities between these proteins and stathmin, as was also suggested by the similar one-dimensional phosphopeptide maps observed with stathmin and the respective a, b and c forms of proteins pp21 and pp23 [6], and the similar two-

dimensional peptide maps found for proteins D and E [23], corresponding to the least acidic forms of protein sets 16 and 17.

As proteins of sets 16 and 17 are all phosphorylated (see Fig. 1), it was of interest to examine from which unphosphorylated precursor(s) they may derive. In human T lymphocytes, stathmin is expressed in large amounts, as are proteins 16 and 17, which strongly incorporate radioactive phosphate in response to various activating agents [6,23,30,31]. In Jurkat leukaemic T cells, the phosphorylation of these proteins in response to a combination of PHA and PMA, known to generate a strong activating signal, was clearly detected even on silver-stained two-dimensional gels (Fig. 3a). The intensities of spots in protein sets 16 and 17 were increased, together with those of the P1 and P2 phosphorylated forms of stathmin, concomitant with a relative decrease in the N1 and N2 unphosphorylated forms. On one-dimensional immunoblots of the same samples, the anti-stathmin antiserum revealed two bands of 21 and 23 kDa which were very weak in the controls but were greatly enhanced in response to the same pharmacological treatment with PHA and PMA (Fig. 3a); they corresponded thus respectively to sets 16 and 17 identified on silver-stained two-dimensional gels. Since no other immunoreactive band was detected on the rest of the gel of the control sample (Fig. 3b), this result clearly indicates that proteins of sets 16 and 17 most likely derive by phosphorylation from 19 kDa stathmin-immunoreactive precursors, possibly from one of the previously described 19 kDa stathmin forms.

The cDNA for stathmin directs the expression of proteins 16 and 17 *in vivo*

We next examined if proteins of sets 16 and 17 could actually

Fig. 3. Generation of proteins of sets 16 and 17 in response to activation of Jurkat leukaemic T cells

Jurkat cells in culture were treated (stimulated, S) or not (control, C) with a combination of PHA (37.5 $\mu\text{g/ml}$) and PMA (10 ng/ml) as indicated in the Materials and methods section. The patterns of stathmin forms were analysed by 2D-PAGE and silver staining (a, left) or one-dimensional immunoblotting with anti-stathmin antiserum I (a, right; b). (a) The areas of stathmin are presented, clearly showing that the 21 and 23 kDa immunoreactive bands, whose intensity increased in stimulated cells, correspond to proteins of sets 16 and 17 respectively, generated in response to the activation of the cells. (b) The entire one-dimensional immunoblots show that no immunoreactive band was detected other than the 19 kDa and the 21 and 23 kDa bands, indicating that the latter derive from the former.

be novel states of stathmin itself, derived from its 19 kDa forms solely by post-translational modifications.

We already demonstrated by *in vitro* transcription and translation of stathmin cDNA that the various 19 kDa isoforms of stathmin are encoded by the same cDNA [14]. However, since only some of the physiological post-translational modifications take place in the rabbit reticulocyte lysate, we examined here the various forms expressed from the same unique cDNA *in vivo*, after transfection into cells in culture.

Cells from the mouse C2 muscle cell line were thus stably transfected with the pSG5 plasmid containing the rat stathmin cDNA inserted under the control of the simian virus 40 early promoter, allowing the constitutive expression of rat stathmin in the recipient mouse cells. The C2 cell line was chosen (1) because the basal expression level of endogenous stathmin is relatively low, and (2) because of its mouse origin, since we have an anti-stathmin antiserum (serum P) that reacts much better with rat stathmin than with the mouse protein (Fig. 4a) [8]. It was thus possible to discriminate the product of the transfected rat stathmin cDNA from the endogenous mouse stathmin present in C2 cells.

Following transfection a stable clone expressing rat stathmin was isolated. Although stathmin normally decreases during muscle cell fusion and differentiation, cells of this clone did not display any perturbation in their differentiation properties, probably because of the low level of the constitutive expression of the exogenous stathmin (less than the level of endogenous protein), as measured with antiserum I recognizing both mouse and rat stathmin (results not shown). However, the use of antiserum P allowed us to detect more specifically the expression of rat stathmin above the endogenous level of the mouse protein, as the latter is poorly recognized by this antiserum. As shown in Fig. 4, only forms N1, P1 and P2 of stathmin were detected with antiserum P in the control cells transfected only with the *neo* resistance gene. In the cell line transfected with the vector containing the rat stathmin cDNA, rat stathmin was indeed expressed, as revealed by a much stronger immunological reaction on immunoblots (Fig. 4b). As expected, the exogenous cDNA directed the expression of all the previously described 19 kDa forms of stathmin, namely the increasingly phosphorylated states of its α and β isoforms migrating as spots N1, N2 and P1–P3. However, proteins of the sets 16 and 17 were also clearly detected with antiserum P in the stathmin-cDNA-transfected cells: spots 17₁ and 17₂ were relatively abundant, whereas a weaker signal was observed for spot 16₁. Since the overexpression of stathmin is less than 2-fold after transfection, and since all

these spots are well recognized by the anti-(rat stathmin) antiserum, we can exclude the hypothesis of an artefactual or secondary overexpression of an endogenous mouse protein. The exogenous rat stathmin cDNA thus also directs the expression of proteins 16 and 17. This result clearly demonstrates that the two sets of proteins, previously described as stathmin-related proteins, are encoded by the same cDNA as the 19 kDa stathmin forms, and thus represent additional phosphorylated variant forms of stathmin.

Multiple molecular forms of stathmin as a support of its biological function(s)?

Following the previous identification of the α and β isoforms of stathmin and of their 19 kDa phosphorylated derivatives [14,19], the present results extend the number of stathmin forms present in cells in various biological situations to up to 14 distinct post-translational variants. Since the newly identified variants of the sets 16 and 17 are all phosphoforms of stathmin, derived from 19 kDa stathmin-immunoreactive forms, they might, in principle, derive from the known α and/or the β isoforms, or from an as yet unidentified unphosphorylated post-translational variant of stathmin. Preliminary results indicate, however, that alkaline phosphatase-mediated dephosphorylation of proteins 17 yields a single spot migrating as spot N1 on two-dimensional gels and thus corresponding to the unphosphorylated state of the α isoform of stathmin (L. Beretta, T. Dobransky & A. Sobel, unpublished work). Also, no higher-molecular mass intermediary unphosphorylated form could be detected by either silver staining or immunochemical detection, indicating that $\alpha 0$ and $\beta 0$ (i.e. spots N1 and N2) are most likely the only unphosphorylated isoforms of stathmin, and that phosphorylation alone is responsible of the higher apparent molecular masses of the forms of sets 16 and 17.

Indeed, the primary sequence of stathmin displays a number of potential phosphorylation sites on serine residues located within consensus sites for diverse kinases (reviewed in [11]), such as protein kinase A (PKA) [14,32], proline-directed protein kinases [10] such as cdc2, or Ca^{2+} /calmodulin kinase II and casein kinase II [33], and possibly protein kinase C (PKC). Stathmin was also shown to be a good substrate *in vitro* for PKA [12,19] and for cdc2 (L. Beretta, T. Dobransky & A. Sobel, unpublished work), but its substrate properties for PKC *in vitro* remain controversial [7,34]. Phosphorylation by some of these and/or additional kinases on a combination of sites might thus yield the diverse forms of stathmin with slightly distinct two-dimensional migration properties corresponding to forms of apparent molecular

Fig. 4. Transfected rat stathmin cDNA directs the expression of proteins 16 and 17 in mouse C2 cells

(a) The same amounts of low-speed supernatant proteins (140 μ g/sample) from mouse or rat forebrain were resolved by 1D-PAGE and immunoblotted with anti-stathmin serum P [2,8]. The 19 kDa stathmin band is shown. Note that this antiserum recognizes rat stathmin much better than mouse stathmin. (b) C2 cells stably transfected with the rat cDNA for stathmin were obtained as described in the Materials and methods section. Heat-stable supernatant proteins (150 μ g/sample) from control or stathmin-transfected cells were analysed by 2D-PAGE and immunodetected with antiserum P. With regard to species-specificity of the antiserum, the additional immunoreactive spots in the transfected cells correspond to stathmin forms encoded by the exogenous rat cDNA.

mass 19, 21 and 23 kDa. The absence of some of these kinases from the reticulocyte lysate most likely explains why only the 19 kDa forms of stathmin were generated from the stathmin mRNA in an *in vitro* translation system [14], whereas the 21 and 23 kDa forms could be generated from the transfected sequence *in vivo*. It should be noted that the same proteins could be detected on two-dimensional gels of neonatal mouse brain (Fig. 2), indicating that corresponding phosphorylations actually take place in physiological conditions.

Interestingly, the activation of diverse second messenger pathways *in vivo* is known to yield patterns of stathmin forms reflecting the corresponding putative kinase cascades triggered, and even the interaction of several concomitant pathways (reviewed in [11]). For example, the activation of the cyclic AMP-dependent pathway alone stimulated the phosphorylation of only the 19 kDa forms of stathmin, whereas agents acting via cyclic AMP-independent pathways, such as growth factors (as shown here for NGF), known to activate a proline-dependent protein kinase [35], or agents stimulating PKC or Ca^{2+} -dependent kinases, also induced the phosphorylation of the higher-molecular-mass forms designated here as 16₁ and 17₁ [20,21,31]. On the other hand, the combined action of both cyclic AMP-dependent and -independent effectors leads to the incorporation of phosphate into the more acidic forms (2 and 3) of proteins 16 and 17 [21,22]. These forms could also be generated by forskolin alone in striatal neurons in culture, in which proteins 16₁ and 17₁ are already present in unstimulated cells [22]. The more acidic forms of the sets 16 and 17 might thus be generated through the activation of PKA from their least phosphorylated forms, 16₁ and 17₁. Phosphorylation of stathmin isoforms on various sites, and in particular phosphorylations leading to the three forms of sets 16 and 17, thus appear to be interdependent and even sequential [11,21,22] or hierarchal [35], i.e. phosphorylation of one site favouring phosphorylation of another one in response to distinct regulatory signals [21,22]. Together with the variety of identified stathmin forms, this feature may yield the molecular basis for the proposed integrating role of stathmin as a relay for extracellular and intracellular signals regulating the proliferation, differentiation and functions of cells [2,11].

The mechanism by which the various forms of stathmin present in the cell at a given time regulate and/or modulate the cell's behaviour and responses, however, remains to be elucidated.

More potent expression systems, as well as perturbation experiments aiming at inhibiting stathmin expression and/or function within cells, will be necessary to characterize its functional properties. The analysis of its amino acid sequence led us previously to propose that this protein may fulfill its functional role by interacting with other proteins through coiled-coil structures [14]. Since phosphorylations are known to regulate this kind of interaction, it is tempting to speculate that stathmin, in its diverse phosphorylated forms, either may interact with diverse affinities with a single kind of substrate, or may interact with diverse subclasses of intracellular proteins.

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