Stathmin Family Proteins Display Specific Molecular and Tubulin Binding Properties*

Received for publication, November 26, 2000, and in revised form, February 14, 2001
Published, JBC Papers in Press, February 15, 2001, DOI 10.1074/jbc.M010637200

Elodie Charbaut, Patrick A. Curmi, Sylvie Ozon‡, Sylvie Lachkar, Virginie Redeker§, and André Sobel¶

From the INSERM U440, Institut du Fer à Moulin, 17 Rue du Fer à Moulin and §CNRS, UMR 7637, Ecole Supérieure de Physique et de Chimie Industrielles de la Ville de Paris, 10 Rue Vauquelin, 75005 Paris, France

Stathmin family phosphoproteins (stathmin, SCG10, SCLIP, and RB3/RB3*) are involved in signal transduction and regulation of microtubule dynamics. With the exception of stathmin, they are expressed exclusively in the nervous system, where they display different spatio-temporal and functional regulations and hence play at least partially distinct and possibly complementary roles in relation to the control of development, plasticity, and neuronal activities. At the molecular level, each possesses a specific “stathmin-like domain” and, with the exception of stathmin, various combinations of N-terminal extensions involved in their association with intracellular membrane compartments. We show here that each stathmin-like domain also displays specific biochemical and tubulin interaction properties. They are all able to sequester two αβ tubulin heterodimers as revealed by their inhibitory action on tubulin polymerization and by gel filtration. However, they differ in the stabilities of the complexes formed as well as in their interaction kinetics with tubulin followed by surface plasmon resonance as follows: strong stability and slow kinetics for RB3; medium for SCG10, SCLIP, and stathmin; and weak stability and rapid kinetics for RB3*. These results suggest that the fine-tuning of their stathmin-like domains contributes to the specific functional roles of stathmin family proteins in the regulation of microtubule dynamics within the various cell types and subcellular compartments of the developing or mature nervous system.

Formation, plasticity, and activities of the mature nervous system require numerous coordinated events such as cell proliferation and migration, neurite extension, guidance toward targets, synapse formation, and stability. Intracellular signaling and cytoskeleton dynamics are key processes for these events, in which stathmin family phosphoproteins are good candidates for playing a significant role. The generic element of this phylogenetically conserved family (reviewed in Ref. 1) is stathmin, also designated Op18 (2), a ubiquitous cytosoluble phosphoprotein most highly expressed in the nervous system. The stathmin family further includes SCG10, SCLIP, RB3, and its splice variants RB3’ and RB3**, expressed exclusively in various cell types and populations of the nervous system, and possessing a stathmin-like domain (SLD) with various N-terminal extensions (3–6). Their involvement in signal transduction and regulation of microtubule dynamics, in relation with their different spatio-temporal expression patterns, suggests that stathmin family proteins may play related but distinct and likely complementary roles in the regulation of differentiation, activities, and plasticity of the nervous system.

During rat development, expression of stathmin family proteins is highest from late embryogenesis until a week after birth (3, 7). They are likely involved in neural differentiation: SCG10 was shown to be induced in neural crest cells when they differentiate into sympathetic neurons (7); in the PC12 cell model of neuron-like differentiation, SCG10, SCLIP, and to a lesser extent RB3* are induced in response to nerve growth factor (3, 7, 8), and overexpression of SCG10 potentiates neurite extension (9).

All proteins of the stathmin family are expressed in the adult brain, some of them at a reduced level (3, 7), indicating that they also have a role in the mature nervous system. This role might be in relation with differentiated neural activities or with regeneration or plasticity, as suggested by the induction of SCG10 with neurite regrowth following corticostriatal deafferentation (10), or the up-regulation of RB3/RB3* (but not SCG10) after neuronal activation in the hippocampus (8). Interestingly, the various stathmin family proteins are expressed in different but overlapping cell populations (3, 11–15). SCG10 and SCLIP are expressed only in neurons, whereas stathmin and RB3/RB3*/RB3** are also expressed in glial cells (3, 7). Stathmin family proteins may thus play related but distinct roles in different physiological environments.

Stathmin (reviewed in Ref. 16) has been originally identified as a relay protein integrating diverse intracellular signaling pathways (17) through combinatorial phosphorylation of its four phosphorylation sites (18). Several target/partner candidates have been identified (19, 20), among which tubulin (21) is the protein whose interaction with stathmin has been best characterized. Stathmin interacts with two αβ tubulin heterodimers in vitro to form a T2S complex (22, 23). The two tubulin heterodimers bind mostly the predicted α-helical “interaction” domain of stathmin (24), likely forming a curved complex whose three-dimensional structure has been recently revealed with the stathmin-like domain of RB3 (25). Stathmin displays a microtubule destabilizing activity both in vitro and in vivo (21, 26–29), which is stoichiometrically accounted for in

* This work was supported by INSERM, ARC, and AFM. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
† Present address: CNRS FRE2371, 9 quai Saint Bernard, 75005 Paris, France.
‡ To whom correspondence should be addressed: INSERM U440, Institut du Fer à Moulin, 17 Rue du Fer à Moulin, 75005 Paris, France. Tel.: 33 1 45 87 61 30; Fax: 33 1 45 87 61 32; E-mail: sobel@ifm.insERM.fr.

** The abbreviations used are: SLD, stathmin-like domain; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; SPR, surface plasmon resonance; Mes, 4-morpholineethanesulfonic acid; Pipes, 1,4-piperazinediethanesulfonic acid; FPLC, fast protein liquid chromatography.
vitrō by a free tubulin sequestration mechanism (22, 27), although it has been proposed to be also due in part to a direct catastrophe-promoting activity (21, 30, 31). Interestingly, the activity of stathmin toward tubulin and microtubules is diminished when it is phosphorylated on various site combinations (28, 29, 32). Being phosphorylated during mitosis and in response to numerous extracellular signals, stathmin appears as a phosphorylation-dependent microtubule destabilizing factor, which may play important roles in proliferating as well as postmitotic cell regulations, in particular in the nervous system (1).

The various stathmin-like domains (3, 4) of the stathmin family proteins display 65–75% amino acid identity with stathmin, including the predicted α-helix, and several of the four stathmin phosphorylation sites. Besides their stathmin-like domain, the neural members of the stathmin family are characterized by additional N-terminal domains. The A domain is common to SCG10, SCLIP, and RB3/RB3‘/RB3″ with 56–68% amino acid sequence identity. As demonstrated in the case of SCG10 and very likely in the case of SCLIP and RB3/RB3‘/RB3″, it is responsible for the membrane attachment and targeting of the proteins to the Golgi area (33); the presence of SCG10 is also demonstrated in neuritic processes and in the growth cone (3, 6, 28, 34). RB3/RB3‘/RB3″ also possesses a specific additional A′ domain between the A and the stathmin-like domains. RB3″ further possesses another additional A′ domain, between A and A′. Finally, RB3′ differs from RB3 within its stathmin-like domain by an alternative splicing thus resulting in a different C terminus. The roles of these specific domains are still unknown, but they likely participate in extending and specifying the properties and functions of the various stathmin family proteins.

Like stathmin, the other members of its phosphoprotein family were also shown to destabilize the microtubule network when overexpressed in cultured cells (28, 35). All these proteins thus form a family involved in the regulation of microtubule dynamics in the nervous system, in relation to differentiation, activities, and plasticity. Their diversity, partly originating in their different spatio-temporal expression and in the presence of specific combinations of N-terminal domains, suggests that they play related and likely complementary roles. The stathmin-like domains of stathmin family proteins might also have different contributions to the control of microtubules, especially as they show a molecular variability that could result in different tubulin binding and hence functional properties. This would be particularly relevant in cells of the nervous system and in their subcompartments such as axons, dendrites, or the growth cone, which display specific and distinctive microtubule organization and dynamics. To test this hypothesis, we examined the specific properties of the various stathmin-like domains. We show that they each interfere with microtubule polymerization and interact with two α/β tubulin heterodimers. However, the T2S complexes formed have different stabilities and display distinct tubulin-SLD interaction kinetics, suggesting that each stathmin family protein is involved in microtubule dynamics regulation in a specific, distinctive fashion. The specific functional properties of the various proteins of the stathmin family in the nervous system thus appear to be determined not only by their characteristic N-terminal extensions but also through the fine-tuning of their stathmin-like domains.

**Experimental Procedures**

**Plasmid Constructs for Prokaryotic Expression of Stathmin-like Domains**

Standard recombinant DNA techniques were carried out as described (36). The full-length cDNA of human stathmin (37) within the pET-8c vector (38) was used for stathmin expression. Rat SCG10 (6), mouse SCLIP (3), rat RB3, and rat RB3′ (4) cDNA clones were used for polymerase chain reaction amplification of the stathmin-like domain coding region. At the 5′ end, the primers (Genset, Paris, France) were designed to introduce a NotI site including an initiating ATG codon, a following alanine codon, and the appropriate sequence for each stathmin-like domain, starting at the residue corresponding to amino acid 5 in the stathmin sequence. At the 3′ end, a BamHI site was introduced in the non-coding region. Vent DNA polymerase and restriction enzymes were from New England Biolabs (Surrey, British Columbia, Canada). The resulting digested polymerase chain reaction fragments were subcloned into the corresponding sites of the pET-8c plasmid (Novagen, Madison WI) (39). For protein expression, the various plasmids were used to transform the Escherichia coli strain BL21(DE3) (Strategene, La Jolla, CA), which provides an inducible expression system suitable for the pET-8c vector.

**Recombinant SLD Expression, Characterization, and Purification**

**Prokaryote Expression**—An overnight preculture was used to seed 1 liter of Luria-Bertani medium containing 50 μg/ml ampicillin, which was grown at 37 °C. At exponential phase, recombinant protein expression was induced for 3 h by the addition of 0.4 μM isopropyl-β-D-thiogalactopyranoside. Bacteria were then pelleted by sedimentation at 4 °C, resuspended in 20 mM Tris-HCl, 1 mM EDTA, pH 8.0, containing the antiprotease mixture Complete (Roche Molecular Biochemicals), and sonicated three times for 1 min on ice.

**Heat Stability and Protein Purification**—Bacterial extracts were centrifuged at 100,000 rpm (Optima MAX ultracentrifuge, rotor TLA 100.1 Beckman Instruments, Fullerton, CA) for 6 min at 4 °C to yield the S2 supernatants and P2 pellets. The S2 supernatants were then heated to 100 °C for 5 min in the presence of 100 mM NaCl, and the samples were centrifuged again as above to yield the S3 supernatants and P3 pellets (17). S3 extracts were adjusted to 20 mM Tris-HCl, 1 mM EDTA, pH 8.0, using Centriprep 10 (Millipore, Bedford, MA), loaded on a Q-Sepharose FF anion exchange column (Amersham Pharmacia Biotech), and eluted with a 0–200 mM NaCl linear gradient in 20 mM Tris-HCl, 1 mM EDTA, pH 8.0. The eluted fractions were analyzed by SDSPolyacrylamide gel electrophoresis and Coomassie Blue staining. Stathmin-like domain-positive fractions were pooled, concentrated with Centriprep 10, and loaded on a Superose 12 HR 10/30 FPLC gel filtration column (Amersham Pharmacia Biotech) equilibrated with phosphate-buffered saline, 1 mM EDTA. Stathmin-like domain-positive fractions were pooled and concentrated as above. The precise masses of the purified protein products were checked by MALDI-TOF (see below), and their protein concentrations were accurately determined by amino acid analysis. In the case of RB3 scr5 and RB3 scr4, purification and subsequent experiments were performed in the presence of 1 mM dithiothreitol to avoid disulfide bond formation.

**Mass Spectrometric Analysis**—Purified recombinant proteins were analyzed with a matrix-assisted laser-desorption/ionization time-of-flight (MALDI-TOF) mass spectrometer (Voyager-DE STR Biospectrometry Work station, PE Biosystems Inc., Framingham, MA). The spectra of positive ions were recorded in linear mode with an accelerating voltage of 25 kV and a delayed extraction of 400 ns. The samples were mixed with a saturated solution of sinapinic acid (3,5-dimethoxy-4-hydroxycinnamic acid, Aldrich) in 30% acetonitrile and 0.1% aqueous trifluoroacetic acid. MALDI-TOF mass spectra of the peptide mixture were performed in positive ion reflector mode with an accelerating voltage of 25 kV and a delayed extraction of 400 ns. External calibration was performed with horse aprotinin using the monoprototated and diprototated ions with average mass-to-charge ratios of 16,952.56 and 8476.78, respectively.

**Results**

**Stathmin**—Stathmin (8) was purified to homogeneity on a MonoQ HR 5/5 column (Amersham Pharmacia Biotech) equilibrated with 50 mM Tris-HCl, 100 mM NaCl, pH 7.5. Stathmin purity was verified by SDS-PAGE and mass spectrometry, which showed a single protein band that was analyzed by MALDI-TOF-MS with a mass-to-charge ratio of 16,952.56 Da, corresponding to the predicted molecular mass of 17,020 Da. The expected peak was present in all samples, but a peak at 17,109 Da corresponding to the addition of two extra masses was also observed in some samples. The optimal concentration of stathmin for the experiments was determined to be 0.4 μM, as lower concentrations led to incomplete binding of tubulin to stathmin, whereas higher concentrations resulted in more than 95% inhibition of tubulin polymerization. The time course of tubulin polymerization inhibition was monitored by the decrease in tubulin lattice density as a function of time after the addition of stathmin.

**Tubulin**—Detyrosinated tubulin was prepared by growing the tubulin-producing mammalian cell line 221B at 32 °C to allow tyrosination of tubulin. Cells were harvested by treatment with 0.25% Triton X-100 and centrifugation at 100,000 × g for 30 min. Tubulin was extracted from the supernatant by treatment with 100 mM NaN3 and 5 mM MgCl2 and was purified by low-speed centrifugation followed by sucrose density gradient centrifugation. The purified tubulin was then dialyzed against 50 mM Tris-HCl, 150 mM NaCl, pH 7.5.

**Tubulin Polymerization Assay**—The ability of stathmin to inhibit tubulin polymerization was measured by the increase in turbidity of the tubulin solution at 350 nm as a function of time after the addition of stathmin. Tubulin was mixed with a solution of stathmin at a final concentration of 0.4 μM, and the change in turbidity was monitored at 350 nm as a function of time.

**Effect of Stathmin on Tubulin Network Stability**—Tubulin was mixed with a solution of stathmin at a final concentration of 0.4 μM, and the change in turbidity was monitored at 350 nm as a function of time. The temperature was then increased to 35 °C, and the change in turbidity was monitored as a function of time at 35 °C.

**Effect of Stathmin on Tubulin Network Stability**—Tubulin was mixed with a solution of stathmin at a final concentration of 0.4 μM, and the change in turbidity was monitored at 350 nm as a function of time. The temperature was then increased to 35 °C, and the change in turbidity was monitored as a function of time at 35 °C.
Stathmin Family, Molecular and Tubulin Binding Properties

EGTA, pH 6.5, at a 0.5 ml/min flow rate. The standard proteins used to calibrate the column are as follows: ribonuclease A ($R_s = 16.4$ Å), chymotrypsinogen A ($R_s = 20.9$ Å), ovalbumin ($R_s = 30.5$ Å), bovine serum albumin ($R_s = 35.5$ Å), aldolase ($R_s = 48.1$ Å), catalase ($R_s = 52.2$ Å), and ferritin ($R_s = 61$ Å). The void volume $V_v$ was measured as the blue dextran elution volume and the total volume of the gel bed $V_b$ as the acetone elution volume. The data were plotted according to Siegel and Monty (40). At least two runs were performed for each protein.

**Bovine Brain Tubulin Preparation**

Tubulin was purified from bovine brain crude extracts by two cycles of polymerization (41), followed by phosphocellulose chromatography (42). Tubulin was stored at $-80^\circ$C in either 25 mM Mes-KOH, 0.25 mM dithiothreitol, 0.25 mM EGTA, 0.125 mM MgCl$_2$, 0.025 mM EDTA, pH 6.8, for short term use, or in 50 mM Mes-KOH, 0.5 mM dithiothreitol, 0.5 mM EGTA, 0.25 mM MgCl$_2$, 0.05 mM EDTA, 3.4 mM glycerol, 0.1 mM GTP, pH 6.8, for long term storage. In the latter case, an additional cycle of polymerization was performed before use, at the end of which tubulin was resuspended in 12.5 mM Mes-KOH, 0.25 mM EGTA, 0.25 mM MgCl$_2$, pH 6.8. Tubulin concentration was determined by amino acid analysis.

**In Vitro Tubulin Polymerization Assay**

Samples containing varying concentrations of either tubulin alone or tubulin plus stathmin-like domain in 50 mM Mes-KOH, 30% glycerol, 6 mM MgCl$_2$, 0.5 mM GTP, 1 mM EGTA, pH 6.8, were loaded in 100-μl quartz cuvettes with a 1-cm light path. Tubulin polymerization was turbidimetrically monitored at 350 nm (43) in an Ultrospec 3000 thermostated spectrophotometer (Amersham Pharmacia Biotech). The temperature was raised from 3 to 37 °C, and the increase in turbidity was recorded until a plateau was reached. The temperature was then set back at 3 °C, and the decrease in turbidity recorded until depolymerization was complete. The polymerized stationary state level was defined as the difference between the plateau value at 37 °C and the baseline after return at 3 °C.

**Gel Filtration Assay**

100-μl samples containing 10 μM tubulin, either alone or with varying concentrations of each stathmin-like domain, were analyzed by gel filtration on a Superose 12 HR 10/30 FPLC column (Amersham Pharmacia Biotech) equilibrated with 80 mM Pipes-KOH, 1 mM EGTA, 5 mM MgCl$_2$, pH 6.5, at a 0.5 ml/min flow rate. The elution profile of the samples was recorded either at 278 nm (only tubulin is visible, as the stathmin-like domains have very few aromatic amino acids) or at 226 nm (both species monitored). When necessary, fractions were collected and analyzed by Western blot with an anti-α-tubulin monoclonal antibody (Amersham Pharmacia Biotech) and a rabbit polyclonal serum against SCG10 (4).
domains, as well as their activities toward tubulin and microtubules. For clarity, we use the generic term “stathmin-like domains” that includes stathmin and the related stathmin-like domains, unless specified otherwise. Stathmin was produced in bacteria as described previously (38), and four other constructs were generated to produce the recombinant stathmin-like domains of SCG10, SCLIP, RB3, and RB3', respectively, designated SCG10SLD, SCLIPSLD, RB3SLD, and RB3'SLD (not shown); RB3SLD-A' is recovered in the insoluble P2 fraction.

**Solubility and Heat Stability of Stathmin-like Domains**—To examine the solubility of the various recombinant proteins, the corresponding bacterial extracts were submitted to high speed centrifugation. As shown for RB3SLD in Fig. 2, all stathmin-like domains were recovered, like stathmin, in the soluble “S2” fraction. Furthermore, after heat treatment at 100 °C in the presence of 100 mM NaCl, the recombinant stathmin-like domains were also recovered in the subsequent high speed soluble fraction “S3,” which indicates that they all possess the characteristic heat stability property of stathmin (17). Interestingly, the presence of the additional A’ domain common to all RB3 proteins (Fig. 1) resulted in the production of an insoluble RB3SLD-A’ protein, essentially recovered in the insoluble “P2” fraction (Fig. 2). Due to the low yield of soluble RB3SLD-A’, its biochemical and functional properties were not further investigated in this study.

The molecular masses of the recombinant proteins were checked by MALDI-TOF MS analysis. For stathmin, SCG10SLD, and SCLIPSLD, the measured masses corresponded to the calculated masses of the proteins without N-terminal methionine. For RB3SLD and RB3'SLD, they correspond to the masses of the proteins without N-terminal methionine and a mass increment of about 42 ± 3 Da. Since RB3SLD and RB3'SLD are identical except for their C-terminal amino acid sequence, we assumed that both could bear the same post-translational acetylation that would account for a 42-Da mass increment. A tryptic digestion followed by MALDI-TOF MS analysis of the generated peptides showed that the N-terminal peptide of both RB3SLD and RB3'SLD was indeed acetylated. This result was confirmed by the fact that their N-terminal sequencing was 80–90% blocked. Furthermore, tandem mass spectrometric fragmentation of the N-terminal peptides using a Q-TOF mass spectrometer revealed that N-terminal peptides of both RB3SLD and RB3'SLD were N-α-acetylated on their N-terminal alanine.

**Shape Characterization by Stokes Radius Measurement**—We analyzed the various stathmin-like domains by gel filtration, and their elution volumes were used to calculate their Stokes radii and apparent molecular masses (Table I). As expected, stathmin displayed an atypical behavior, since it eluted like a globular protein of about 110 kDa, i.e. about 6-fold its actual molecular mass. This high apparent molecular mass reveals an asymmetrical shape (44). The other stathmin-like domains also displayed abnormally high Stokes radii (Table I), implying that they are asymmetrical proteins as well. However, by using the MM<sub>app</sub>/MM<sub>MS</sub> ratio (apparent molecular mass determined by gel filtration/molecular mass measured by MALDI-TOF MS) as a measure of this asymmetry, it appears that stathmin is more elongated than the other stathmin-like domains, followed by SCG10SLD and SCLIPSLD and then by RB3SLD and RB3'SLD (Table I). Altogether, all stathmin-like domains share an elongated form but to significantly different extents, suggesting that they possess specific structural features that might be functionally relevant.

**Activity on Tubulin Polymerization in Vitro**

In order to compare the functional properties of the various stathmin-like domains, we assessed their activity on tubulin polymerization in vitro. The addition of any stathmin-like domain to the tubulin polymerization reaction resulted in a decreased microtubule amount at steady state. For example, 4 μM RB3SLD lowered the amount of microtubules normally formed with 20 μM tubulin to that obtained with 12 μM tubulin, as if 4 μM RB3SLD prevented 8 μM tubulin to enter the polymerization reaction (Fig. 3A). The effects of increasing concentrations of stathmin-like domain on microtubule assembly with 20 μM tubulin revealed that the presence of any stathmin-like domain at a concentration C induced the same effect as a 2C tubulin decrease (Fig. 3B). Therefore, everything occurs as if each stathmin-like domain molecule were able to sequester two α/β tubulin heterodimers. It thus seemed very likely that they might interact directly with tubulin, like stathmin which forms a T₄S complex preventing the corresponding tubulin to enter the polymerization reaction (22, 27).

**Interaction of Stathmin-like Domains with Tubulin**

**Characterization of the Tubulin-Stathmin-like Domain Complexes**—In order to assess the existence of a direct tubulin-SLD interaction, samples containing 10 μM bovine brain tubulin and a concentration range of each stathmin-like domain (1, 2.5, 5, 10, and 20 μM) were loaded on an FPLC gel filtration column. Monitoring at 278 nm was used to follow tubulin elution only, whereas both tubulin and stathmin-like domains were monitored either at 226 nm or in outflow fractions by Western blotting. As illustrated in Fig. 4A for SCG10SLD, we observed for each stathmin-like domain a second tubulin peak with a smaller elution volume, which probably corresponds to tubulin within a larger molecular complex. The latter is probably a tubulin-SLD-complex because its amount increased with stathmin-like domain concentration. Moreover, the gel elution profiles obtained with 5, 10, and 20 μM stathmin-like domain are superimposable and do not reveal any free tubulin. Thus, 5 μM stathmin-like domain is sufficient to complex 10 μM tubulin, which is consistent, as in the case of stathmin, with a 2:1 tubulin:SLD stoichiometry.

Western blot analysis (Fig. 4B) confirmed the stoichiome-
The Stokes radii \( R_s \) and apparent molecular masses \( M_{app} \) of the proteins were deduced from their gel filtration elution volumes, after column calibration using standard globular proteins (see "Experimental Procedures"). The \( M_{app}/M_{inst} \) ratio is presented as an indication of the protein asymmetry.

| Protein      | \( R_s \) | \( M_{app} \) | \( M_{inst} \) | \( M_{app}/M_{inst} \) |
|--------------|-----------|---------------|---------------|------------------------|
| Stathmin     | 41        | 112           | 17.2          | 6.5                    |
| SCG10SLD     | 38        | 86            | 16.4          | 5.2                    |
| SCLIPSLD     | 37        | 81            | 16.7          | 4.9                    |
| RB3SLD       | 33        | 60            | 16.7          | 3.6                    |
| RB3 SLD      | 31        | 49            | 15.1          | 3.2                    |
| Tubulin      | 44        | 132           | 96.7          | 1.4                    |

\( ^* \) The actual molecular masses \( M_{inst} \) were measured by MALDI-TOF MS analysis, except for tubulin for which only the predicted masses is available.

**FIG. 3.** Inhibition of tubulin polymerization by stathmin-like domains in vitro. A, polymerization kinetics of 20 \( \mu M \) tubulin (\( T \) \( \) thin dashes), 12 \( \mu M \) tubulin (thick dots), or 20 \( \mu M \) tubulin in the presence of 4 \( \mu M \) RB3SLD (full line). The amount of microtubules formed is measured by absorbance at 350 nm. It reaches a steady state value that is linearly dependent on the initial tubulin concentration (as seen in B). B, microtubule amounts at steady state obtained with 20 \( \mu M \) tubulin in the presence of increasing concentrations of the various stathmin-like domains (bottom scale). To relate these results directly to the amounts of tubulin alone leading to the same steady state values, the microtubule amounts obtained with decreasing concentrations of tubulin (\( T \) alone (upper scale) are figured on the same plot (diamonds, experimental data; line, linear regression: \( r^2 = 0.99985 \)). It appears that a concentration \( C \) of any stathmin-like domain has the same effect as a 2\( C \) decrease in tubulin concentration.

Finally, monitoring the elution outflow at 226 nm (which allows the detection of both tubulin and stathmin-like domains) revealed a small peak corresponding to free stathmin-like domain in excess, as shown for RB3SLD in Fig. 4C. This peak was present when the tubulin:SLD ratio was lower than 2:1, i.e. when there was more than 5 \( \mu M \) stathmin-like domain for 10 \( \mu M \) tubulin. Altogether, our results are consistent with a 2:1 stoichiometry of the tubulin-SLD complexes.

**DIFFERENT STABILITIES OF THE TUBULIN-STATHMIN-LIKE DOMAIN COMPLEXES—**Besides the overall similarities of the various stathmin-like domains in their interference with microtubule assembly and their interaction with tubulin, the comparison of the gel filtration elution profiles points out that the various tubulin-SLD complexes have actually distinct properties. Indeed, the resulting shifted elution peaks had different shapes, as seen, for example, for 10 \( \mu M \) tubulin in the presence of 10 \( \mu M \) of any stathmin-like domain (Fig. 5A). The shifted peaks can be sorted in three types as follows: tubulin peaks obtained with SCG10SLD and RB3SLD were the most shifted toward the smaller elution volumes and displayed a narrow and relatively symmetrical shape. The peaks with stathmin and SCLIPSLD were less shifted and had an asymmetrical shape. This is probably due to the dissociation along the column of some originally complexed molecules, as they underwent dilution during their migration and were separated from the slower
migrating monomers. Finally, the peak obtained with RB3' SLD was eluted at an intermediary position between the other complexes and free tubulin; this late elution position likely results from a more effective dissociation. Indeed, if the dissociation on the column is extensive from the beginning of the elution, it is possible that no tubulin molecule originally complexed in the sample remains associated, all being eluted at an average intermediate position between the volumes corresponding to the free and complexed forms of tubulin.

The dissociation of the complex is even more striking in more dilute concentration conditions, such as 10 μM tubulin + 1 μM stathmin-like domain, for example (Fig. 5B). In the case of stathmin and SCLIP SLD, there was no more individualized complex peak but only a shoulder in front of the free tubulin peak. On the other hand, the tubulin-RB3 SLD complex peak remained individualized and was eluted at the same position as observed with 10 μM RB3 SLD (about 10.5 ml). Therefore, it appears that the tubulin-RB3 SLD complex is far less sensitive to dissociation along the column than tubulin-stathmin and tubulin-SCLIP SLD. The tubulin-SCG10 SLD complex seems to have an intermediate stability, as its elution began at an early position but was spread out toward the free tubulin peak. As mentioned above, the tubulin-RB3' SLD complex is the least stable, since the tubulin peak was even less shifted at 1 μM than at 10 μM RB3' SLD.

As the tubulin-RB3 SLD complex remained totally associated at 1 μM RB3 SLD, we tried even more dilute concentration conditions to test to what extent the stability of this complex was different from the others. The elution profile of a stoichiometric mixture of 1 μM tubulin + 0.5 μM RB3 SLD (Fig. 5C) reveals clearly that the complex did not dissociate during migration, as the peak position was not delayed and no trail appeared. We did not try lower concentrations, as the signal:noise ratio was too low.

In conclusion, we found that the complexes of tubulin with the various stathmin-like domains display different stabilities in the following order: RB3 SLD→ SCG10 SLD, stathmin and SCLIP SLD, and finally RB3' SLD.

**Different Interaction Kinetics of Tubulin with the Various Stathmin-like Domains**

We further characterized the kinetics of the interaction between the various stathmin-like domains and tubulin by using the SPR BIAcore technology, which monitors the mass concentration variation at the vicinity of a surface. A constant tubulin concentration flow was applied (association phase) on flow cells coupled with the various stathmin-like domains, followed by a flow of buffer containing no tubulin (dissociation phase). The SPR signals were then corrected for nonspecific signal using a reference flow cell coupled with bovine serum albumin.

An example of the corresponding interaction kinetics is given in Fig. 6A, where the signals corresponding to a 4 μM tubulin run have been normalized (the maximum signal corresponding to 100%), to allow their more direct comparison. We see clearly that the various tubulin-SLD complexes displayed distinct association and dissociation kinetics. As a measure of the kinetics differences, we represented the half-association and dissociation kinetics. As the tubulin-RB3 SLD complex remained totally associated to RB3 SLD was much slower and to RB3' SLD dissociated even slower, whereas tubulin-RB3 SLD dissociated even slower, whereas tubulin-
RB3\textsubscript{SLD} dissociated extremely fast as compared with all the other stathmin-like domains. Altogether the various kinetics of tubulin association and dissociation with stathmin-like domains are in good agreement with the stabilities of the formed complexes as revealed by gel filtration chromatography: intermediate stability for stathmin and SCLIP\textsubscript{SLD}; slightly higher stability for SCG10\textsubscript{SLD} due to slower dissociation; and strong stability for RB3\textsubscript{SLD}, with both slow association and dissociation, as opposed to weak stability with RB3\textsubscript{SLD} with both fast association and dissociation.

**DISCUSSION**

The biological expression and regulation of phosphoproteins of the stathmin family suggest that they play different, possibly complementary roles in development, plasticity, and activities of the nervous system. In the case of stathmin, its proposed signal integration and relay functions are at least in part mediated by its interaction with tubulin and hence its involvement in the control of microtubule dynamics (reviewed in Ref. 1). Interestingly, the other phosphoproteins of the stathmin family share a similar but distinct stathmin-like domain and have been shown to interfere also with microtubule assembly *in vivo* and *in vitro* (9, 28, 35). We demonstrate here that all known stathmin-like domains interact with tubulin and inhibit microtubule assembly *in vitro* through tubulin sequestration. We further report that each stathmin-like domain interacts with tubulin in a distinctive way, most likely contributing to the specific biological roles of the various stathmin family phosphoproteins, particularly in the differentially regulated control of microtubule assembly in the diverse cell and subcellular compartments of the nervous system, at the various stages of development and adult life.

**Biochemical Properties of Stathmin-like Domains**—All recombinant stathmin-like domains display characteristic stathmin-like biochemical properties, such as high solubility and heat stability, as well as high Stokes radius indicating an elongated shape. This illustrates their genuine stathmin-like character, consistent with their extensive amino acid sequence identity (65–75%), and their similar predicted secondary structure including a long α-helix (3, 4, 45). However, beside these overall similarities, the various stathmin-like domains display distinctive structural features as suggested by differences in their measured Stokes radii, which most likely reveal different conformations possibly related to specific biological roles.

The biochemical characterization of recombinant stathmin-like domains revealed an unexpected feature, the N-α-acetylation of RB3\textsubscript{SLD} and RB3\textsubscript{9SLD}. Although N-α-acetylation is rarely observed in prokaryote systems, it was reported previously for the endogenous ribosomal proteins L12, S5, and S18 in *E. coli*, as well as for some recombinant proteins (46). SCG10\textsubscript{SLD} and SCLIP\textsubscript{SLD} did not undergo this modification, although they share the same (M)ADMEV N-terminal sequence as RB3\textsubscript{SLD}, the first difference arising only at the sixth residue (a lysine for SCG10\textsubscript{SLD} and SCLIP\textsubscript{SLD} instead of an isoleucine for RB3\textsubscript{SLD} and RB3\textsubscript{9SLD}). This further indicates that the determinants for N-α-acetylation are far broader than the very first N-terminal residues (47).

**Microtubule Destabilizing Activity of Stathmin Family Proteins**—Our present observation that all stathmin-like domains of the family inhibit tubulin polymerization *in vitro*, in a way similar to stathmin (21, 27) and SCG10 (9), confirms their stathmin-like character at the functional level. These results demonstrate *in vitro* an action on microtubule assembly for all members of the family, in agreement with *in vivo* observations of microtubule interference of entire proteins of the family when overexpressed in HeLa cells (28). Moreover, this is the first demonstration of microtubule assembly inhibition by RB3\textsubscript{9}, whose activity could not be evidenced *in vivo*.

Quantitative analysis of the activities of stathmin-like domains toward tubulin polymerization reveals that the presence of any stathmin-like domain at a concentration C inhibits the polymerization of a concentration 2C of tubulin. In the case of stathmin, the existence of a T\textsubscript{S} complex (22, 23) is sufficient to account for the stoichiometric effect of stathmin on tubulin polymerization *in vitro*, if one considers that one stathmin sequesters two α/β tubulin heterodimers and prevents them from entering polymerization (27). As we demonstrate here the

![Image](http://www.jbc.org/)

**FIG. 6.** Different interaction kinetics of tubulin with stathmin-like domains. A, surface plasmon resonance "net" sensorgrams (bovine serum albumin reference signal subtracted) revealing an interaction between soluble tubulin and the various stathmin-like domains immobilized on CM5 sensorchips. The association phase (from 0 to 720 s) corresponds to a constant 4 μM concentration of free tubulin in the flow, whereas the dissociation phase (from 720 s to the end) corresponds to buffer without tubulin. The signals were normalized (100% = maximal signal) in order to highlight the kinetics differences. B and C, the mean half-association times (B) and mean half-dissociation times (C) were measured for each stathmin-like domain at various tubulin concentrations. Error bars correspond to the standard deviation obtained for two to seven different measurements, whereas the absence of an error bar indicates a single measurement.
existence of complexes between tubulin and stathmin-like domains, it is likely that all stathmin-like domains inhibit tubulin polymerization in vitro by tubulin sequestration. Recently, the tubulin-RB3SLD complex was actually crystallized, and x-ray diffraction analysis revealed a three-dimensional structure compatible with tubulin sequestration (25).

Stathmin has been shown also to increase microtubule catastrophe frequencies in some conditions (21, 30, 31). Although such an effect is expected to some extent as a consequence of tubulin sequestration, it has been proposed that it might also result from a direct interaction of stathmin with microtubule ends, which has not been observed so far. Quantitative comparison of the tubulin binding and catastrophe-promoting activities of stathmin-like domains would give clues to determine to what extent tubulin sequestration and catastrophe promotion are two independent actions of these proteins on microtubule stability.

**Distinct Tubulin Interaction Properties of Stathmin-like Domains**—The interaction between stathmin-like domains and tubulin has been characterized by means of gel filtration, which informs on the complex formed and its stability, and by surface plasmon resonance, which allows us to follow the kinetics of the interaction, as done previously for stathmin (22).

Some limitations preclude the interpretation of SPR results in terms of actual kinetic rate constants. Indeed, the reaction pathway for the formation of the TₕS complexes is not known and very probably involves second-order kinetics. Moreover, heterogeneities of two kinds are likely to result in additional complexity of the interaction kinetics. Brain tubulin is heterogeneous due to the existence of several genes and post-translational modifications of the gene products; a differential interaction of stathmin-like domains with the diverse tubulin isofoms might be actually of physiological significance. A more technical heterogeneity is that of the stathmin-like domains, which may be coupled to the sensorchip through one or several random lysines. In any case, the SPR technology allowed us to compare the relative interaction potencies of the various stathmin-like domains with tubulin and to reveal, at least qualitatively, a true diversity of the tubulin-SLD interactions. Indeed, the interaction of SCLIPSLD with tubulin is very close to that of tubulin, whereas the SLD complex was actually crystallized, and x-ray diffraction analysis revealed a three-dimensional structure compatible with tubulin sequestration (25).

The interaction between stathmin-like domains and tubulin has been characterized by means of gel filtration, which informs on the complex formed and its stability, and by surface plasmon resonance, which allows us to follow the kinetics of the interaction, as done previously for stathmin (22).

Some limitations preclude the interpretation of SPR results in terms of actual kinetic rate constants. Indeed, the reaction pathway for the formation of the TₕS complexes is not known and very probably involves second-order kinetics. Moreover, heterogeneities of two kinds are likely to result in additional complexity of the interaction kinetics. Brain tubulin is heterogeneous due to the existence of several genes and post-translational modifications of the gene products; a differential interaction of stathmin-like domains with the diverse tubulin isofoms might be actually of physiological significance. A more technical heterogeneity is that of the stathmin-like domains, which may be coupled to the sensorchip through one or several random lysines. In any case, the SPR technology allowed us to compare the relative interaction potencies of the various stathmin-like domains with tubulin and to reveal, at least qualitatively, a true diversity of the tubulin-SLD interactions. Indeed, the interaction of SCLIPSLD with tubulin is very close to that of stathmin: SCG10SLD associates similarly but dissociates more slowly from tubulin; RB3SLD displays both the slowest association and dissociation phases; RB3SLD displays the fastest one.

The SPR data can be compared with results following gel filtration, which sort the various complexes according to their stability along the column: interestingly, RB3SLD generates the most stable complex, followed by SCG10SLD, stathmin, and SCLIPSLD, and finally RB3SLD. These results are highly consistent with the SPR results, which mutually strengthen their validity and significance. The fact that the tubulin-SLD stability differences were not revealed by following the action of stathmin-like domains on tubulin polymerization is not surprising because of the high tubulin concentrations necessarily used in this assay, thus leading to full association of low as well as high affinity stathmin-like domains with tubulin.

The predicted α-helix encompassing the “core” region (residues 42–126) (24) is essential for the interaction with tubulin and corresponds most likely to the 91-amino acid α-helix interacting with two α/β tubulin heterodimers in the tubulin-RB3SLD complex (25). This α-helix is made of two duplicated stretches (30–40% identity) of 35 residues (25, 37) (Fig. 1B), whose spacing is consistent with that of the two α/β tubulin heterodimers in the tubulin-RB3SLD complex (25). As the amino acid sequence of the first stretch is significantly more conserved between all stathmin-like domains than that of the second, the two stretches might have different contributions to the binding of the two tubulins.

The characteristic tubulin interaction differences among the various stathmin-like domains result from their 25–35% differences in primary sequences. The two regions (residues 29–39 and 138–149) flanking the interacting α-helix are the most divergent (Fig. 1B) and could contribute to the observed tubulin interaction differences if they were involved in stabilizing the interaction. However, as these regions are also phylogenetically variable, this would not be the case if the characteristic interaction properties of the various members of the stathmin family with tubulin were, as expected, conserved through evolution. It is thus likely that more subtle sequence differences within the more conserved regions of the various stathmin-like domains are responsible for the observed tubulin interaction differences.

The two splice variants RB3SLD and RB3SLD have opposite tubulin interaction properties, although their sequences are identical up to residue 124, but are totally divergent on their C termini. This suggests that either the C-terminal part of RB3SLD (which is absent from RB3SLD) is very important to stabilize the interaction or the C-terminal part of RB3SLD has a strong destabilizing effect. It is interesting that differential splicing can direct the expression of the rb3 gene toward proteins forming a highly stable or unstable complex with tubulin, which might be of physiological importance regarding the regulation of microtubule dynamics in the corresponding cells.

**Biological Significance**—Microtubule dynamics are regulated by many stabilizing and destabilizing factors, such as microtubule-associated proteins or Kin1, whose opposite activities establish a finely tuned balance between microtubule polymerization and depolymerization. The diversity of stathmin family proteins, together with the diversity of MAPs in the nervous system, might enhance the flexibility of this regulation, each protein contributing to it differently with specific tubulin interacting properties and microtubule destabilizing activity. In particular, different tubulin interaction kinetics and complex stability might result in different contributions to microtubule assembly dynamics, allowing a fine-tuning of its regulation according to the local tubulin/microtubule status within the cell, as well as to specific needs in various cells and cell compartments within the nervous system. Moreover, the different intracellular distribution of stathmin family proteins might allow the local regulation of the microtubule network. Indeed, stathmin is cytosolic whereas stathmin family proteins are membrane-bound, with a punctate localization at the level of the Golgi apparatus, neuronal processes, and growth cones (6, 34). The concentration of stathmin-related proteins on some membrane compartments may thus create a local change in microtubule dynamics, which could be important for process elongation or organelle transport. This could explain why most membrane-bound members of the family are expressed in neural cells where stathmin itself is abundant. Furthermore, the activity of stathmin family proteins toward microtubules might be regulated by their local release from the membrane compartment, as it has been suggested in the case of SCG10 (35), this release being possibly controlled differently for the various proteins of the family. It is also possible that the various stathmin family proteins interact differently with the various tubulin isofoms, thus modifying the microtubule composition and stability.

An additional complexity in the regulation of the neural microtubule network might come from the differential phosphorylation of stathmin family proteins. Indeed, stathmin is known to integrate intracellular signaling pathways through combinatorial phosphorylation, and phosphorylation has been
shown to regulate the microtubule destabilizing activity of stathmin and SCG10 (35, 48). As several not all the stathmin phosphorylation sites are present in the various stathmin-like domains, their phosphorylation may occur under different conditions; each protein would thus be able to modulate the microtubule network differently, possibly locally, according to its phosphorylation state. The presence of other additional domains A’ and A” in the RB3 proteins may further broaden the diversity of stathmin family functions, in part through alternative splicing.

Altogether, we show that in addition to their overall structural and functional stathmin-like properties, the various stathmin-like domains display specific molecular and tubulin binding properties most likely of physiological relevance. The stathmin family phosphoproteins thus form a set of microtubule regulators with diverse properties, which may participate in cytoskeleton reorganization in the developing and the mature nervous system. For a better understanding of the role of these proteins, it will be important to continue assessing their diversity, particularly by investigating the role of their N-terminal domains, their phosphorylation, and also their differential expression and localization in tissues and cells. All these properties may participate in defining the specific physiological role of each stathmin family protein.

Acknowledgements—We thank M. F. Carlier and P. Amayed (LEBS, Gif-sur-Yvette, France) for help in preparing bovine brain tubulin. We are grateful to J. M. Camadro and B. Gontero-Meunier (Institut Jacques Monod, Paris, France) for technical help with the BIAcore experiments and stimulating discussions; to J. P. Le Caer and V. Labas (ESPCI, Paris, France) for N-terminal sequencing; and to O. Gavet, V. Labas and A. Labas for discussions and critical reading of the manuscript.

REFERENCES

1. Curmi, P., Gavet, O., Charbaut, E., Ozon, S., Lachkar-Colmerauer, S., Maneveu, V., Sivaswam, S., Mauccur, A., and Sobel, A. (1999) Cell Struct. Funct. 24, 345–357
2. Haillat, N., Strahler, J. R., Melhem, R. F., Zhu, X. X., Brodeur, G., Seeger, R. C., Reynolds, C. P., and Hanash, S. M. (1996) Oncogene 5, 1615–1618
3. Ozon, S., Mauccur, A., and Sobel, A. (1998) J. Neurochem. 70, 2386–2396
4. Ozon, S., Mauccur, A., and Sobel, A. (1997) Eur. J. Biochem. 248, 784–806
5. Schubart, U. K., Das Banerjee, M., and Eng, J. (1989) DNA (New York) 8, 389–398
6. Stein, R., Mori, N., Matthews, K., Lo, L. C., and Anderson, D. J. (1988) Neuron 1, 463–476
7. Anderson, D. J., and Axel, R. (1985) Cell 42, 649–662
8. Beilharz, E. J., Zhukovsky, E., Lanahan, A. A., Worley, P. F., nikolik, K., and Godman, L. J. (1998) J. Neurosci. 18, 9780–9789
9. Riederer, B. M., Pellet, V., Antonsson, B., Di Paolo, G., Stimpson, S. A., Latif, R., Catsicas, S., and Grenningloh, G. (1997) J. Biol. Chem. 272, 5175–5182
10. Steinmetz, M. O., Kammerer, R. A., Jahnke, W., Goldie, K. N., Lustig, A., and van Oostrum, J. (2000) EMBO J. 19, 572–580
11. Redeker, V., Lachkar, S., Slavoshian, S., Charbaut, E., Rossier, J., Sobel, A., and Curmi, P. (2000) J. Biol. Chem. 275, 6841–6849
12. Gigant, B., Curmi, P. A., Martin-Barbery, C., Charbaut, E., Lachkar, S., Lebeau, I., Slavoshian, S., Sobel, A., and Knossow, M. (2000) Cell 102, 809–818
13. Mauccur, A., Camonis, J. H., and Sobel, A. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 3100–3104
14. Belmont, L. D., and Mitchison, T. J. (1996) Cell 84, 63–203
15. Curmi, P. A., Andersen, S. S. L., Lachkar, S., Gavet, O., Karsenti, E., Knossow, M., and Sobel, A. (1997) J. Biol. Chem. 272, 2529–25306
16. Steinmetz, M. O., Kammerer, R. A., Jahnke, W., Goldie, K. N., Lustig, A., and van Oostrum, J. (2000) EMBO J. 19, 572–580
17. Redeker, V., Lachkar, S., Slavoshian, S., Charbaut, E., Rossier, J., Sobel, A., and Curmi, P. (2000) J. Biol. Chem. 275, 6841–6849
18. Gigant, B., Curmi, P. A., Martin-Barbery, C., Charbaut, E., Lachkar, S., Lebeau, I., Slavoshian, S., Sobel, A., and Knossow, M. (2000) Cell 102, 809–818
19. Mauccur, A., Larnson, M., Melander Gradin, H., Brattsand, G., and Gulberg, M. (1996) EMBO J. 15, 5290–5298
20. Jouirdain, L., Curmi, P., Sobel, A., Pantalone, D., and Carlier, M. F. (1997) Biochemistry 36, 10817–10821
21. Gavet, O., Ozon, S., Manuceau, V., Lawler, S., Curmi, P., and Sobel, A. (1998) J. Cell Sci. 111, 3333–3346
22. Horwitz, S. B., Shen, J.-H., He, L., Dittmar, P., Neef, R., Chen, J., and Schubart, U. K. (1997) J. Biol. Chem. 272, 8129–8132
23. Howell, B., Larnson, N., Gulberg, M., and Cassimeris, L. (1999) Mol. Biol. Cell 10, 105–118
24. Arnaud, I., Karsenti, E., and Hyman, A. A. (2000) J. Cell Biol. 149, 767–774
25. Larsson, N., Mauccur, U., Gradin, H. M., Brattsand, G., and Gulberg, M. (1997) Mol. Cell. Biol. 17, 5350–5359
26. Di Paolo, G., Lutijens, R., Pellet, V., Stimpson, S. A., Beuchat, M. A., Catsicas, M., and Grenningloh, G. (1997) J. Biol. Chem. 272, 5175–5182
27. Di Paolo, G., Lutijens, R., Osen-Sand, A., Sobel, A., Catsicas, S., and Grenningloh, G. (1997) J. Neurosci. Res. 50, 1000–1009
28. Antonsson, B., Kassel, D., Di Paolo, G., Lutijens, R., Riederer, B. M., and Grenningloh, G. (1998) J. Biol. Chem. 273, 8439–8444
29. Sambrock, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
30. Mauccur, A., Doye, V., and Sobel, A. (1990) FEBS Lett. 264, 275–278
31. Curmi, P., Mauccur, A., Asselin, S., Lecourtios, M., Chaffotte, A., Schmitter, J. M., and Sobel, A. (1994) Biochem. J. 300, 331–338
32. Studier, F. W., Rosenberg, A. H., Dunn, J. J., and Dubendorff, J. W. (1990) Methods Enzymol. 185, 60–89
33. Siegel, L. M., and Monty, K. J. (1966) Biochim. Biophys. Acta 112, 346–362
34. Shelanski, M. L., Gaskin, F., and Cantor, C. R. (1973) Proc. Natl. Acad. Sci. U. S. A. 70, 765–768
35. Weingarten, M. D., Lockwood, A. H., Hwo, S. Y., and Kirschner, M. W. (1975) Proc. Natl. Acad. Sci. U. S. A. 11576–11580
36. Violand, B. N., Schlittler, M. R., Lawson, C. Q., Kane, J. K., Siegel, N. R., and Curmi, P. (1997) Trends Biochem. Sci. 22, 101–109
37. Maucuer, A., Doye, V., and Sobel, A. (1999) Trends Biochem. Sci. 24, 861–867
38. Weingarten, M. D., Lockwood, A. H., Hwo, S. Y., and Kirschner, M. W. (1975) Proc. Natl. Acad. Sci. U. S. A. 11576–11580
Stathmin Family Proteins Display Specific Molecular and Tubulin Binding Properties
Elodie Charbaut, Patrick A. Curmi, Sylvie Ozon, Sylvie Lachkar, Virginie Redeker and André Sobel

J. Biol. Chem. 2001, 276:16146-16154.
doi: 10.1074/jbc.M010637200 originally published online February 15, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M010637200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 47 references, 21 of which can be accessed free at
http://www.jbc.org/content/276/19/16146.full.html#ref-list-1