Journal of Cellular Biochemistry 80:346–352 (2001)

Differences in Phosphorylation of Human and Chicken Stathmin by MAP Kinase

Bruno Antonsson,[1]* Daniel B. Kassel,[2] Evelyne Ruchti,[3] and Gabriele Grenningloh[3]

[1]Geneva Biomedical Research Institute, Glaxo Wellcome R&D S.A., 14 ch des Aulx, CH-1288 Plan-les-Ouates, Geneva, Switzerland
[2]Glaxo Inc., Research Triangle Park, North Carolina 27709, USA
[3]University of Lausanne, IBCM, Rue du Bugnon 9, CH-1005 Lausanne, Switzerland

Abstract Stathmin/Op18 is a highly conserved 19 kDa cytosolic phosphoprotein. Human and chicken stathmin share 93% identity with only 11 amino acid substitutions. One of the substituted amino acids is serine 25, which is a glycine in chicken stathmin. In human stathmin, serine 25 is the main phosphorylation site for MAP kinase. In this study, we have compared the phosphorylation of human and chicken stathmin. The proteins were expressed in Sf9 cells using the baculovirus expression system and purified for in vitro phosphorylation assays. Phosphorylation with MAP kinase showed that chicken stathmin was phosphorylated 10 times less than human stathmin. To identify the phosphorylation sites we used liquid chromatography/mass spectrometry (LC/MS/MS). The only amino acid found phosphorylated was serine 38, which corresponds to the minor phosphorylation site in human stathmin. Phosphorylation with $p34^{cdc2}$- and cGMP-dependent protein kinases gave almost identical phosphorylation levels in the two stathmins. J. Cell. Biochem. 80:346–352, 2001. © 2001 Wiley-Liss, Inc.

Key words: stathmin; phosphorylation; mass spectrometry; MAP kinase; phosphoprotein

Stathmin/Op18, a 19 kDa ubiquitous cytosolic protein, is an in vivo and in vitro substrate for several serine/threonine protein kinases including the mitogen-activated protein (MAP) kinase family members, cAMP dependent protein kinase (PKA) and cyclin dependent kinases (CDKs) [Beretta et al., 1993; Brattsand et al., 1994; Leighton et al., 1993; Luo et al., 1994; Marklund et al., 1993]. The protein is developmentally regulated with high expression levels during the embryonic and postnatal periods, although a lower expression level persists during adulthood [Koppel et al., 1990; Pampfer et al., 1992]. Particularly high expression is found in the brain, mainly in neurons [Peschanski et al., 1993]. Based on the complex pattern of phosphorylation in response to diverse extracellular signals [Cheiweiss et al., 1992; Cooper et al., 1991; Doye et al., 1990; Gullberg et al., 1990; Sobel, 1991; Toutant and Sobel, 1987], several investigators have proposed a role for stathmin in signal transduction. More recently, stathmin has been identified as a protein that increases the catastrophe frequency of microtubules [Belmont and Mitchison, 1996].

Stathmin exists as two isoforms, with different isoelectric points, encoded by a single mRNA [Doye et al., 1989]. In cell extracts, at least 14 different forms of stathmin have been isolated by 2D gel electrophoresis [Beretta et al., 1993; Sobel, 1991]. Apart from the two unphosphorylated isoforms, the different forms have been shown to correspond to different phosphorylated forms of the protein [Beretta et al., 1993; Leighton et al., 1993; Marklund

Abbreviations used: MAP kinase, mitogen-activated-protein kinase; PKG, cGMP-dependent protein kinase; h-stathmin, human stathmin; c-stathmin, chicken stathmin; ECL, enhanced chemiluminescence.

Grant sponsor: National Science Foundation of Switzerland; Grant number: 31-50948.97.

Bruno Antonsson's present address is Serono Pharmaceutical Research Institute, 14 chemin des Alux, CH-1228 Plan-les Ouates, Geneva, Switzerland.

Daniel B. Kassel's present address is CombiChem Inc., 9050 Camino Santa Fe, San Diego, California 92121, USA.

*Correspondence to: Bruno Antonsson, Serono Pharmaceutical Research Institute, 14, chemin des Alux, CH-1228 Plan-les Ouates, Geneva, Switzerland.
E-mail: bruno.antonsson.ch_gva03@serono.com

Received 19 June 2000; Accepted 25 July 2000

This article published online in Wiley InterScience, November XX, 2000.

et al., 1993]. In intact cells, h-stathmin is phosphorylated on four serine residues, namely serine 16, 25, 38, and 63 [Beretta et al., 1993; Leighton et al., 1993; Luo et al., 1994; Marklund et al., 1993]. Mapping studies have identified serine 25 as the major target for members of the MAP kinase family [Leighton et al., 1993; Marklund et al., 1993, 1993a], and both serines 25 and 38 as the target sites for phosphorylation by CDKs [Luo et al., 1994; Marklund et al., 1993]. During the cell cycle, all four serine residues are phosphorylated by the cyclin dependent kinase system (serines 25 and 38) and an unidentified kinase system (serines 16 and 63). Mutation of serines 25 and 38 interferes with phosphorylation of serines 16 and 63 indicating that there is a cascade of phosphorylation events [Larsson et al., 1995]. Furthermore, mutational analysis suggests that all four phosphorylation sites of stathmin are functionally important during cell division [Larsson et al., 1995].

Studies in a variety of other systems suggest an important role for MAP kinase regulated phosphorylation of serine 25. For example, in PC12 cells, stathmin is a target for MAP kinase in the NGF-stimulated signaling pathway [Di Paolo et al., 1996; Leighton et al., 1993] and in cortical neurons, MAP kinase activation in response to brain-derived neurotrophic factor triggers phosphorylation of stathmin [Cardinaux et al., 1997].

Stathmin is highly conserved between species with only scarce amino acid substitutions. While human and rat stathmin contain only one amino acid substitution, human and chicken stathmin share 93% identity with 11 amino acid substitutions [Godbout, 1993; Sobel, 1991]. However, one of the amino acids substituted in chicken stathmin is serine 25, which is replaced by a glycine. The other three phosphorylation sites are conserved in the chicken protein. Based on the above-mentioned observations that indicate the importance of serine 25, it was of interest to determine how chicken stathmin is phosphorylated by MAP kinase.

In the present study, we analyzed the in vitro phosphorylation of recombinant human and chicken stathmin using mass spectrometry.

MATERIALS AND METHODS

[γ-^{33}P]-ATP was from Amersham, UK. MAP-kinase (p44mpk) purified from sea star oocytes was purchased from Upstate Biotechnology Inc., USA. cGMP-dependent protein kinase, $p34^{cdc2}$ kinase were from Promega, USA, and the trypsin perfusion column was form PreSeptive Biosysteme, MA.

Purification of the Recombinant Human and Chicken Stathmin

Human and chicken stathmin were expressed as secreted proteins with the baculovirus expression system in Sf9 cells (BaculoGold, PharMingen, San Diego, CA). The proteins were purified from 500 ml concentrated culture medium which was diluted with 2 volume of buffer A (20 mM Tris–HCl, 1 mM DTT, 0.1 mM PMSF, 0.1 mM benzamidine, pH 8.0), and applied to a Q-Sepharose column (5×12 cm). The column was eluted with a 1200 ml linear gradient of 0–0.5 M NaCl in buffer A. Fractions containing stathmin were pooled, diluted with 2 vol. of buffer B (20 mM Tris–HCl, 0.2 mM DTT, 0.1 mM PMSF, pH 7.5) and further purified on FPLC Mono Q (HR 5/5). The column was developed with a 500 ml linear gradient of 0–250 mM NaCl in buffer B. Stathmin eluted between 50 and 100 mM NaCl and the fractions containing stathmin were pooled and concentrated on an Amicon pressure concentrator on a YM3 membrane. In the last purification step, the sample was applied to gel filtration on Superdex 75 (2.5×100 cm) equilibrated in 50 mM Tris–HCl, 400 mM NaCl, 0.2 mM DTT, 0.2 mM benzamidine, 0.5 mM EDTA, 0.5 mM EGTA, pH 7.5. Fractions containing stathmin were pooled, concentrated as above, dialyzed against 20 mM Tris–HCl, 0.2 mM DTT, pH 7.5 and stored at -80°C.

In Vitro Phosphorylation

For each phosphorylation assay, 250 pmol (5 μg) of the purified human and chicken proteins were used in a total reaction volume of 50 μl in the buffers as described below. MAP-kinase (p44mpk): 15 ng MAP-kinase in 15 mM MOPS, pH 7.0, 10 mM $MgCl_2$, 0.5 mM EGTA, 50 μM NaF and 1 mM DTT. $pp34^{dcd2}$ kinase: 10 units $pp34^{cdc2}$ in 20 mM MOPS, pH 7.0, 10 mM $MgCl_2$, 0.5 mM EGTA, 50 μM NaF, and 0.5 mM DTT. cGMP-dependent protein kinase: 20 U cGMP-dependent kinase in 40 mM Tris–HCl, pH 7.5, 20 mM $Mg(C_2H_3O_2)_2$, 0.1 mM EDTA and 2 μM cGMP. All reaction mixtures contained 0.2 mM ATP. For the samples analyzed

by SDS-PAGE and autoradiography [γ-^{33}P]-ATP was added at 2.5 Ci/mmol.

After incubation of the samples at 35°C for 30 min, the reactions were stopped by addition of 20 mM EDTA and samples were stored at −80°C until analyzed. Samples for SDS-PAGE were stopped by addition of 1 volume SDS electrophoresis sample buffer, heated at 95°C for 5 min, analyzed on 10–15% gradient Phast-Gels and autoradiographed.

Protein Molecular Weight Determinations by Mass Spectrometry

Electrospray ionization-mass spectrometry (ESI-MS) was performed using an API-III triple quadrupole mass spectrometer (PE-Sciex, Concord, Ontario, Canada) equipped with an HP1090 microbore HPLC (Hewlett-Packard, Palo, Alto, CA). Separations of protein samples (50–100 pmol) were carried out using a Poros R2/H 300 μm × 10 cm capillary perfusion column (LC Packings, San Francisco, CA) equilibrated in buffer C (0.05% trifluoroacetic acid in H_2O). Proteins were eluted using a linear gradient of 15–65% buffer D (0.035% trifluoroacetic acid in 90/10 acetonitrile/H_2O) over 5 min at a flow rate of 50 μl/min. The mass spectrometer was scanned from m/z 800 to 1200 Da every 3 s using a step size of 0.2 Da and a dwell time of 1.5 ms. The resolution of the mass spectrometer was unit resolution up to m/z 1500 (10% valley definition) as determined from the infusion of a (poly)propylene glycol standard calibrant solution (PE-Sciex).

Enzymatic Digestion and LC/MS Characterization

A quantity, 30–60 pmol, of the proteins was loaded onto an immobilized trypsin perfusion column and digested on-column at 37°C for 2 min. Peptides were trapped and separated on a reversed phase C18 column (LC Packings, CA) at a flow rate of 5 μl/min using a 5 min linear gradient of 1–21% buffer D followed by a 15 min linear gradient of 21–41% buffer D. The eluate from the column was fed into the ion source of the mass spectrometer as previously described [Lombardo et al., 1995]. Mass spectra were acquired by scanning the mass spectrometer between m/z 300–1800 Da in 3 s using a 0.5 Da step and a 1.0 ms dwell time. Phosphopeptides were identified from the tryptic digest using the stepped orifice voltage technique [Ding et al., 1994; Huddleston et al., 1993]. An aliquot of protein digest (30–60 pmol) was separated on the C18 column and analyzed by ESI-MS. Peptides were ionized in the negative ion mode and phosphopeptides were identified based on their ability to form a prominent PO_3^- ion at m/z 79.

Identification of Phosphorylation Sites by LC/MS/MS

Phosphopeptides identified by negative ion stepped orifice potential experiments were subjected to on-line LC/MS/MS analysis. The peptides were separated using the same column and gradient as described above. LC/MS/MS spectra of the phosphopeptides were acquired by scanning the mass spectrometer from 50 Da to the mass of the target phosphopeptide in 3 s. The collision gas was set 3×10^{14} collision gas thickness units (cgts). The mass spectrometer resolution was set to 1,000 (full with half maximum).

RESULTS AND DISCUSSION

The four identified phosphorylation sites (serine 16, 25, 38 and 63) are well conserved in the stathmin sequences from human, mouse, and rat. However, a comparison of the human and chicken stathmin sequences revealed an interesting amino acid substitution. Serine 25, which is the major site for MAP kinase phosphorylation in mammalian stathmin, is replaced by a glycine in the chicken sequence (Fig. 1). To examine whether the absence of the MAP kinase phosphorylation site had an effect on the overall phosphorylation of the protein, we produced recombinant human and chicken stathmin (h-stathmin and c-stathmin, respectively) for in vitro phosphorylation assays. The proteins were expressed in Sf9 cells using the baculovirus expression system and purified by sequential chromatography on Q-Sepharose, FPLC MONO Q and Superdex 75. The identity of the purified proteins that appeared homogeneous on SDS-PAGE (Fig. 2) was confirmed by NH_2-terminal amino acid sequencing and mass spectrometry (not shown).

The two proteins were phosphorylated with MAP kinase in the presence of ^{33}P-ATP. At equimolar amounts, the incorporation of radioactivity was considerably higher in h-stathmin than in c-stathmin (Fig. 3A). Further analysis of the phosphorylated samples by mass spectrometry showed that c-sthathmin was 10 times less phosphorylated than h-stathmin. With

```
                      16        25            38
                      ↓         ↓             ↓
  1 MASSDIQVKE LEKRASGQAF ELILSPRSKE SVPEFPLSPP KKKDLSLEEI
    ||.|||||||| |||||||||| ||||.||||| ..|||||||| ||||||||||
  1 MATSDIQVKE LEKRASGQAF ELILGPRSKE AAPEFPLSPP KKKDLSLEEI
                    63
                    ↓
 51 QKKLEAAEER RKSHEAEVLK QLAEKREHEK EVLQKAIEEN NNFSKMAEEK
    |||||||||| |||||||||| |||||||||| |||||||||| ||||||||||
 51 QKKLEAAEER RKSHEAEVLK QLAEKREHEK EVLQKAIEEN NNFSKMAEEK

101 LTHKMEANKE NREAQMAAKL ERLREKDKHI EEVRKNKESK DPADETEAD   149
    |||||||||| |||||||||| |||||||||. ||||||||.| ||......
101 LTHKMEANKE NREAQMAAKL ERLREKDKHV EEVRKNKEGK DPGEAETN    148
```

Fig. 1. Amino acid sequence alignment of human (upper) and chicken (lower) stathmin. The protein share 93% amino acid identity with only 11 amino acid substitutions (bold). One of the substituted amino acids is the main MAP kinase phosphorylation site in human stathmin, serine 25. The phsophorylation sites in human stathmin are indicated (serines 16, 25, 38 and 63).

80% of the protein phosphorylated, h-stathmin was an efficient substrate for MAP kinase. In contrast, only 8% of c-stathmin was phosphorylated under the same conditions (Fig. 3B) indicating that it is a poor substrate for MAP kinase.

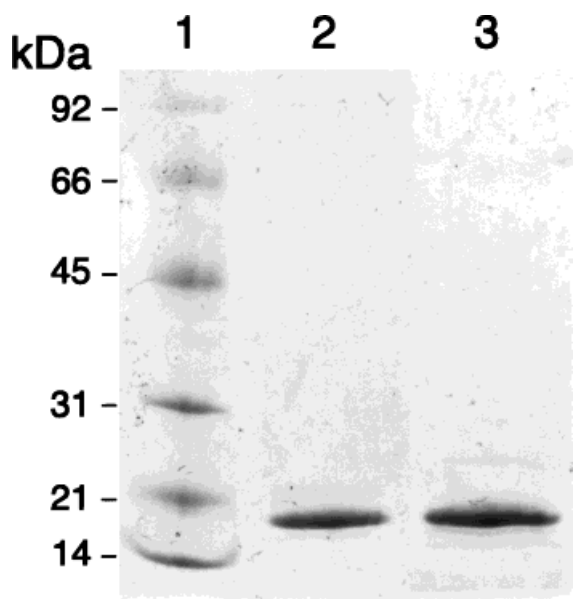

Fig. 2. Purified human and chicken stathmin. The purified proteins were analyzed on 10–15% SDS Phastgels from Pharmacia. Proteins were detected by coomassie blue staining. Lane 1, molecular weight markers; lane 2, chicken stathmin; and lane 3, human stathmin.

Mass spectrometry analysis showed that h-stathmin was mainly mono-phosphorylated by MAP kinase. Only a small amount of the protein was present in the di-phosphorylated form (Fig. 3C). Consistent with previous reports [Leighton et al., 1993], the di-phosphorylated protein was phosphorylated on both serines 25 and 38. The mono-phosphorylated protein was a mixture of protein phosphorylated on either serine 25 (majority) or serine 38 (data not shown). For c-stathmin, only the mono-phosphorylated form could be detected, no di-phosphorylated form was observed (Fig. 3C).

Phosphorylation analysis using $p34^{cdc2}$- and cGMP-dependent protein kinases showed that h-stathmin and c-stathmin were phosphorylated to approximately the same degree (Fig. 3B). While $p34^{cdc2}$ is known to phosphorylate stathmin on serine 38 [Luo et al., 1994], there have been no previous reports on stathmin phosphorylation by cGMP-dependent protein kinase. However, this kinase has been shown to phosphorylate the closely related protein SCG10 [Antonsson et al., 1997].

To map the sites of MAP kinase phosphorylation in c-stathmin, the phosphorylated sample was digested with trypsin, and the peptides were separated on reverse phase-HPLC and analyzed by LC/MS. One phosphorylated peptide was detected after ionization of the pepti-

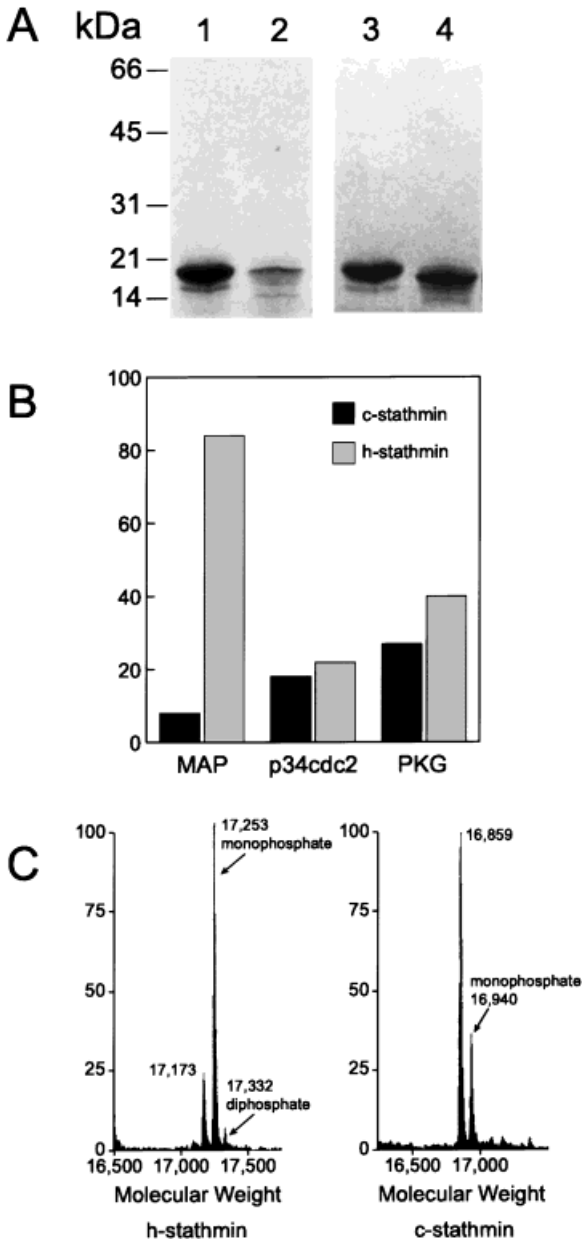

Fig. 3. Phosphorylation of human and chicken stathmin. Panel A. MAK kinase phosphorylated samples of human and chicken stathmin were analyzed on 10–15% SDS Phastgels. The gels were coomassie stained, dried, and autoradiographed. Autoradiograph: lane 1, human stathmin; lane 2, chicken stathmin, coomassie staining of the same gel: lane 3, human stathmin; lane 4, chicken stathmin. **B**: Equimolar amounts of chicken and human stathmin were phosphorylated with MAP kinase, $p34^{cdc2}$ and PKG, the samples were analyzed by mass spectrometry and the percentage phosphorylated protein was determined by peak integration. **C**: Mass spectrometer analysis of MAP kinase phosphorylated human and chicken stathmin. In human stathmin the peaks correspond to unphosphorylated (17,173), monophosphorylated (17,253) and diphosphorylated (17,332) and in chicken stathmin to unphosphorylated (16,859) and monophosphorylated (16,940) protein.

des in the negative ion mode and identification of the PO_3^- ion at m/z 79 (Fig 4A). The phosphorylated peptide had a molecular mass of 1361 Da. When the peptide was sequenced using LC/MS/MS, the sequence was shown to correspond to the tryptic peptide 29–41 and serine 38 was identified as the site of phosphorylation (Fig. 4B). This site corresponds to the minor MAP kinase phosphorylation site in h-stathmin [Leighton et al., 1993]. The absence of any other phosphorylated peptides in c-stathmin indicated that the major MAP kinase phosphorylation site found in stathmin of mammalian species is not replaced by another phosphorylation site in c-stathmin. Our obser-

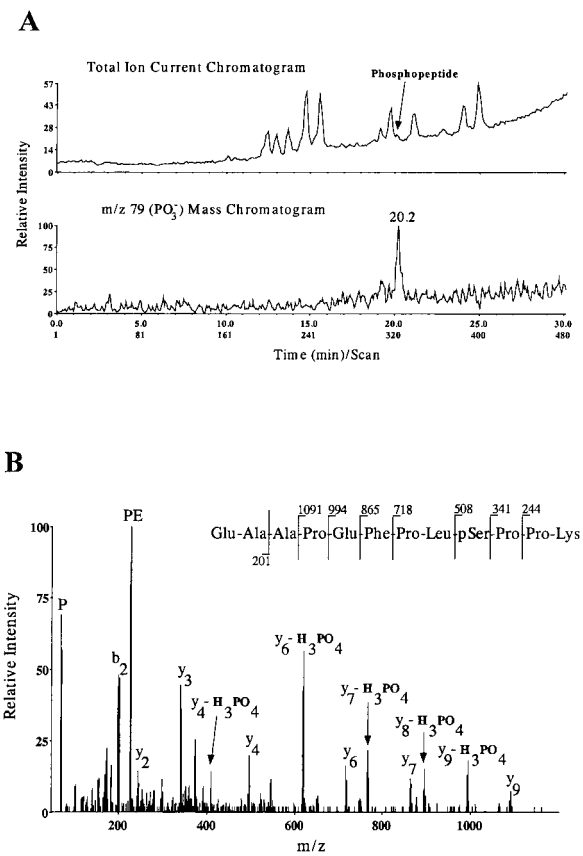

Fig. 4. Determination of the phosphorylation site in MAP kinase phosphorylated chicken stathmin. **A**: Phosphorylated chicken stathmin was digested with trypsin, the peptides separated by RP-HPLC and analyzed by mass spectrometry (top trace). Phosphorylated peptides were identified by negative ion stepped orifice potential analysis (bottom trace). Only one peptide formed the prominent PO_3^- ion indicative of phosphorylation at m/z 79 (20.2 min). **B**: The phosphorylated peptide identified in panel A was submitted to LC/MS/MS analysis. The sequence identified corresponded to the peptide 29–41 in the chicken stathmin sequence. Serine 38 was found to be the site of phosphorylation.

vation that only 8% of c-stathmin was phosphorylated by MAP kinase, compared to 80% of h-stathmin, suggests that phosphorylation of serine 38 is not increased as compared to h-stathmin to compensate for the lack of the main phosphorylation site. These results show a striking difference in the phosphorylation with MAP kinase between the two proteins as a result of the substitution of serine 25.

Although the precise function of MAP kinase regulated phosphorylation of stathmin is not clear, it has been hypothesized that the regulation of microtubule dynamics by stathmin phosphorylation could be fundamental to cytoskeletal rearrangements during neuronal differentiation [Cardinaux et al., 1997; Di Paolo et al., 1996]. Consistent with this idea, phosphorylation of stathmin has been demonstrated to negatively regulate its microtubule-destabilizing activity as well as its interaction with tubulin dimers [Di Paolo et al., 1997; Marklund et al., 1996]. Interestingly, phosphorylation on serine 25 only, had no effect on the microtubule-destabilizing activity of stathmin and only a minor effect on its ability to bind tubulin. In contrast, phosphorylation on serins 16 and 63 had a profound effect on both parameters [Di Paolo et al., 1996]. Thus, it is tempting to speculate that a possible role of MAP kinase regulated stathmin phosphorylation may be to induce a conformational change of the protein that allows phosphorylation by other kinases, and that such a conformational change may not be necessary for chicken stathmin.

ACKNOWLEDGMENTS

We are grateful to Drs. S. Catsicas, T. Wells and A. Prounfoot for helpful discussions. We also thank Dr. G. Di Paolo for making the constructs used in this study, Drs. K. Radford, H. Blasey and A. Bernard for their help with the baculovirus expression system, S. Montessuit for technical assistance, E. Magnenat for amino acid sequencing and C. Hebert and E. Bernardi for art work. This study was in part supported by the National Science Foundation of Switzerland (Grant 31-50948.97) to G.G.

REFERENCES

Antonsson B, Lutjens R, Di Paolo G, Kassel D, Allet B, Bernard A, Catsicas S, Grenningloh G. 1997. Purification, characterization, and in vitro phosphorylation of the neuron-specific membrane-associated protein SCG10. Protein Exp Purif 9:363–371.

Belmont L, Mitchison TJ. 1996. Identification of a protein that interacts with tubulin dimers and increases the catastrophe rate of microtubules. Cell 84:623–631.

Beretta L, Dobransky T, Sobel A. 1993. Multiple phosphorylation of stathmin. Identification of four sites phosphorylated in intact cells and in vitro by cyclic amp-dependent protein kinase and $p34^{cdc2}$. J Biol Chem 268:20076–20084.

Brattsand G, Marklund U, Nylander K, Roos G, Gullberg M. 1994. Cell-cycle-regulated phosphorylation of oncoprotein 18 on ser16, ser25 and ser38. Eur J Biochem 220:359–368.

Cardinaux JR, Magistretti PJ, Martin JL. 1997. Brain-derived neurotrophic factor stimulates phosphorylation of stathmin in cortical neurons. Brain Res Mol Brain Res 51:220–228.

Cheiweiss H, Cordier J, Sobel A. 1992. Stathmin phosphorylation is regulated in striatal neurons by vasoactive intestinal peptide and monoamines via multiple intracellular pathways. J Neurochem 58:282–289.

Cooper HL, Fuldner R, McDuffie E, Braverman R. 1991. T-cell receptor activation induces rapid phosphorylation of prosolin, which mediates down-regulation of DNA synthesis in proliferating peripheral lymphocytes. J Immunol 146:3689–3696.

Di Paolo G, Antonsson B, Kassel D, Riederer BM, Grenningloh G. 1997. Phosphorylation regulates the microtubule-destabilizing activity of stathmin and its interaction with tubulin. FEBS Lett 416:149–152.

Di Paolo G, Pellier V, Catsicas M, Antonsson B, Catsicas S, Grenningloh G. 1996. The phosphoprotein stathmin is essential for nerve growth factor-stimulated differentiation. J Cell Biol 133:1383–1390.

Ding JM, Burkhart W, Kassel DB. 1994. Identification of phosphorylated peptides from complex mixtures using negative-ion orifice-potential stepping and capillary liquid chromatography/electrospray ionization mass spectrometry. Rapid Commun Mass Spectrom 8:94–98.

Doye V, Boutterrin M-V, Sobel A. 1990. Phosphorylation of stathmin and other proteins related to nerve growth factor-induced regulation of pc12 cells. J Biol Chem 265:11650–11655.

Doye V, Soubrier F, Bauw G, Boutterin M-C, Beretta L, Koppel J, Vandekerckhove J, Sobel A. 1989. A single cDNA encodes two isoforms of stathmin, a developmentally regulated neuron-enriched phosphoprotein, J Biol Chem 264:12134–12137.

Godbout R. 1993. Identification and characterization of transcripts present at elevated levels in the undifferentiated chick retina. Exp Eye Res 56:95–106.

Gullberg M, Noreus K, Brattsand G, Friedrich B, Shingler V. 1990. Purification and characterization of a 19-kilodalton intracellular protein. An activation-regulated putative protein kinase C substrate of T lymphocytes. J Biol Chem 265:17499–17505.

Huddleston MJ, Annan R, Bean M, Carr SA. 1993. Selective detection of phosphopeptides in complex mixtures by electrospray liquid chromatography/mass spectrometry. J Am Soc Mass Spectrom 4:710–717.

Koppel J, Boutterin M-C, Doye V, Peyro-Saint-Paul H, Sobel A. 1990. Developmental tissue expression and

phylogenetic conservation of stathmin, a phosphoprotein associated with cell regulations. J Biol Chem 265: 3703–3707.

Larsson N, Melander H, Marklund U, Ostermann O, Gullberg M. 1995. G2/M transition requires multisite phosphorylation of oncoprotein 18 by two protein kinase systems. J Biol Chem 270:14175–14183.

Leighton IA, Curmi P, Campbell DG, Cohen P, Sobel A. 1993. The phosphorylation of stathmin by map kinase. Mol Cell Biochem 127–128:151–156.

Lombardo CR, Consler TG, Kassel DB. 1995. In vitro phosphorylation of the epidermal growth factor receptor autophosphorylation domain by c-src: identification of phosphorylation sites and c-src SH2 domain binding sites. Biochemistry 34:16456–16466.

Luo XN, Mookerjee B, Ferrari A, Mistry S, Atweh GF. 1994. Regulation of phosphoprotein p18 in leukemic cells. Cell cycle regulated phosphorylation by $p34^{cdc2}$ kinase. J Biol Chem 269:10312–10318.

Marklund U, Brattsand G, Osterman O, Ohlsson PI, Gullberg M. 1993a. Multiple signal transduction pathways induce phosphorylation of serines 16, 25, and 38 of oncoprotein 18 in T-lymphocytes. J Biol Chem 268: 25671–25680.

Marklund U, Brattsand G, Shingler V, Gullberg M. 1993. Serine 25 of oncoprotein 18 is a major cytosolic target for the mitogen-activated protein kinase. J Biol Chem 268: 15039–15047.

Marklund U, Larsson N, Gradin HM, Brattsand G, Gullberg M. 1996. Oncoprotein 18 is a phosphorylation-responsive regulator of microtubule dynamics. EMBO J 15:5290–5298.

Pampfer S, Fan W, Schubart UK, Pollard JW. 1992. Differential mRNA expression of the phosphoprotein p19/SCG10 gene family in mouse preimplantation embryos, uterus, and placenta. Reprod Fetil Dev 4: 205–211.

Peschanski M, Hirsch E, Dusart I, Doye V, Marty S, Manceau V, Sobel A. 1993. Stathmin: cellular localization of a major phosphoprotein in the adult rat and human CNS. J Comp Neurol 337:655–668.

Sobel A. 1991. Stathmin: A relay phosphoprotein for multiple signal transduction? TIBS, 16:301–305.

Toutant M, Sobel A. 1987. Protein phosphorylation in response to the tumor promoter TPA is dependent on the state of differentiation of muscle cells. Dev Biol 124: 370–378.