Identification of a 24 kDa Phosphoprotein Associated with an Intermediate Stage of Memory in *Hermissenda*

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A requirement for protein synthesis is a critical feature in dissociating different phases of memory. However, in examples of cellular and synaptic plasticity in which an early or intermediate requirement for protein synthesis has been implicated, specific proteins have not been identified. Here we report the identification of a 24 kDa phosphoprotein (CSP24) associated with an intermediate stage of memory, distinct from short-term memory, detected after one-trial conditioning of *Hermissenda*. CSP24, initially identified from $^{32}PO_4$-labeled proteins resolved by two dimensional (2-D) PAGE, was excised from multiple Coomassie blue-stained 2-D gels and subjected to reverse phase HPLC and automated sequence analysis. The sequenced peptides exhibited a homology to the β-thymosin family of actin-binding protein. Anti-CSP24 antibody recognized CSP24 on 1- and 2-D gels by Western blot analysis. Labeled CSP24 immunoprecipitated with anti-CSP24 antibody revealed that significantly more $^{32}PO_4$ was incorporated in preparations that received one-trial conditioning compared with unpaired controls. In contrast, labeled CSP24 immunoprecipitated with anti-CSP24 from conditioned and unpaired control preparations receiving a procedure that only produced short-term enhanced excitability did not exhibit differences in $^{32}PO_4$ incorporation into the immunoprecipitates. These results show that a specific identified phosphoprotein is associated with an intermediate stage of memory for one-trial conditioning in *Hermissenda*.

Key words: intermediate memory; phosphoprotein; one-trial conditioning; immunoprecipitation; associative learning; Hermissenda

Studies of the time-dependent development of memory have identified components of memory consolidation that can be differentiated based on the contribution of signal transduction pathways, protein synthesis, and gene induction (Otani et al., 1989; Ng and Gibbs, 1991; Rosenzweig et al., 1993; Nguyen et al., 1994; DeZazzo and Tully, 1995; Kang and Schuman, 1996; Kane et al., 1997; McGaugh, 2000). In two different examples of plasticity, 5-HT-induced synaptic facilitation and enhanced excitability produced by one-trial conditioning, an intermediate stage has been shown to depend on translation but not transcription (Ghirardi et al., 1995; Crow et al., 1999). One-trial conditioning in *Hermissenda* results in the biphasic development of enhanced cellular excitability detected in identified sensory neurons of the conditioned stimulus (CS) pathway (Crow and Siddiqi, 1997) and an increase in the phosphorylation of several proteins observed 1–2 hr after conditioning (Crow et al., 1996). One phosphoprotein, a 24 kDa protein (CSP24), exhibited an increase in $^{32}PO_4$ incorporation during an intermediate phase of memory that depended on protein synthesis (Crow et al., 1999). This finding was supported by studies showing that anisomycin, but not the mRNA synthesis inhibitor 5,6-dichloro-1-β-D-ribobenzimidazole, specifically blocked the increased phosphorylation of CSP24 and the accompanying enhanced excitability normally expressed during the intermediate phase of memory (Crow et al., 1999). In this study, we provide evidence identifying CSP24 as a homolog of β-thymosin. We also show that $^{32}PO_4$-labeled CSP24 immunoprecipitated with anti-CSP24 antibody exhibited significantly greater phosphorylation after one-trial *in vitro* conditioning compared with unpaired controls. In addition, we report that increased phosphorylation of CSP24 relative to unpaired controls did not occur after procedures that produced only short-term enhancement.

MATERIALS AND METHODS

Experimental procedures. Three types of preparations were used; an exposed, but otherwise intact nervous system (one-trial conditioning), an isolated circumesophageal nervous system for electrophysiological measurements of excitability at different times after conditioning, and isolated components of the CS pathway consisting of the eye and proximal optic nerve (*in vitro* procedure). Adult *Hermissenda crassicornis* were maintained in artificial seawater (ASW) aquaria at 14 ± 1°C on a 12 hr light/dark cycle. Before conditioning, animals were anesthetized with a 0.25 ml injection of isotonic $MgCl_2$, and a small dorsolateral incision was made to expose the circumesophageal nervous system. Surgically prepared animals were transferred to a chamber containing 50 ml of normal ASW. The one-trial conditioning procedure consisted of a 5 min presentation of light, the CS (10^{-4} W/cm^2) paired with the application of serotonin (5-HT) to the region of the cerebropleural ganglion, where previous immunocytochemistry revealed 5-HT-reactive processes near

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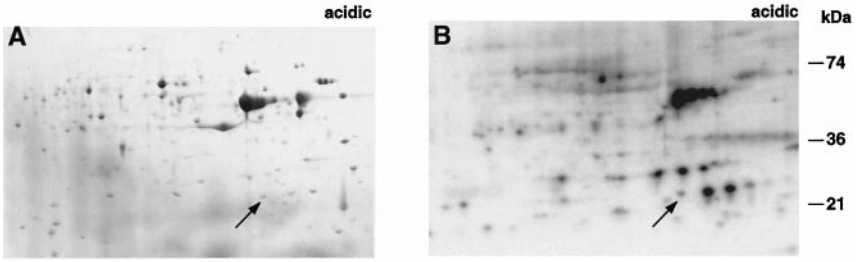

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Peptide	Protein		Amino acid sequence		Identity
1	Thymosin β_{11} (*S. gairdneri*)	1	ESI SGFDK	8	50%
		7	EEVASFDK	14	
2	Thymosin β_{11} (*S. gairdneri*)	1	NPLPTAEAISQER	13	70%
		26	NPLPTKETIEQEK	38	
3	Thymosin β_{4s} (*C.elegans*)	1	VLPSIDDIGQEK	12	83%
		157	VLPSADDIKQEK	168	

Figure 1. The 24 kDa phosphoprotein co-migrates with CSP24 identified from Coomassie blue-stained 2-D gels. In this example, lysates consisted of eyes and proximal optic nerves from five animals. *A*, Print of a Coomassie blue-stained 2-D gel showing the location of CSP24 (*arrow*). CSP24 was excised from multiple 2-D gels, and eluted products were subjected to reverse phase HPLC and automated sequence analysis. *B*, Print from a storage phosphorimaging screen of the same gel as in *A* showing 2-D gel electrophoretic separation of $^{32}PO_4$-labeled phosphoproteins. The *arrow* indicates the location of CSP24 corresponding to the Coomassie blue-labeled protein, indicated by the *arrow* in part *A*. *C*, Comparison of amino acid sequences of three peptides derived from CSP24 with members of the β-thymosin family of actin-binding protein. Identical amino acids are designated by *solid lines*, and similar amino acids are designated by *plus signs*. The percent identities of the *Hermissenda* peptides to the other proteins were generated using the FASTA program.

the optic nerve and photoreceptor terminals in the neuropil. The final concentration of 5-HT in the ASW was 5×10^{-6} M. The *in vitro* procedure consisted of pairing light with 5×10^{-6} or 10^{-4} M 5-HT applied to the isolated components of the CS pathway. Unpaired control groups received the CS and 5-HT (5×10^{-6} or 10^{-4} M) separated by 5 min. For the unpaired control group, the 5-HT was applied in the dark (infrared illumination) and washed out after the 5 min exposure. After the conditioning trial, animals were maintained in the ASW aquaria for the different times before assessing excitability in the isolated nervous system.

Electrophysiology. Intracellular recordings were collected from identified lateral type B photoreceptors at times between 15 and 75 min after conditioning and at 3 and 24 hr after conditioning. Animals were prepared for intracellular recording and stimulation with extrinsic current using previously published standard procedures (Crow and Forrester, 1990; Crow and Forrester, 1993; Crow et al., 1997, 1999). Experiments with the isolated circumesophageal nervous system were conducted in ASW maintained at 15 ± 0.5°C and having the following composition (in mM): 460 NaCl, 10 KCl, 10 $CaCl_2$, and 55 $MgCl_2$, buffered with 10 mM HEPES and brought to pH 7.6 with NaOH. Excitability was assessed with 2 sec 5 and 10 mV depolarizing pulses from a holding potential of −60 to −63 mV. Averages were determined by dividing the total number of action potentials by the duration of the extrinsic current pulses.

Protein phosphorylation and two-dimensional gel electrophoresis. Protein phosphorylation after one-trial conditioning was examined in the components of the CS pathway of *in vitro* preparations incubation for 2 hr in 200 μl of oxygenated ASW containing 11 mM glucose and 0.125 mCi of $^{32}PO_4$ (carrier-free; New England Nuclear, Boston, MA). After the 2 hr incubation the samples were rinsed in an isotonic ice-cold wash solution (in mM: 460 NaCl, 10 KCl, 5 EDTA, and 100 Tris-HCl, pH 7.8) and lysed in a modified lysis solution containing 9.2 M urea, 2% Nonidet P-40, 5% β-mercaptoethanol, 2% carrier ampholytes, 1.6% pH 5–8 and 0.4% pH 3.5–10, 100 mM NaF, 1 mM sodium orthovanadate, and 0.1 mM okadiac acid and stored frozen at −80°C. Samples were analyzed by two-dimensional (2D) gel electrophoresis using a first-dimension isoelectric focusing gel with an immobilized pH gradient (4–7) and a precast SDS polyacrylamide (8–18% linear gradient) second-dimension gel. Western blot analysis of 1- and 2-D gels involved lysates resolved in SDS gels and transferred to polyvinylidene difluoride (PVDF) membranes. Gels containing $^{32}PO_4$-labeled proteins were exposed to storage phosphor screens for 24 hr. Phosphor screens were computer-scanned and analyzed using ImageQuant software (Molecular Dynamics, Sunnyvale, CA) for quantitative analysis.

Peptide sequencing. Coomassie blue-stained protein from whole nervous systems corresponding to CSP24 was excised from multiple 2-D gels. The in-gel digestion protocol was similar to a previously published procedure (Rosenfeld et al., 1992). Samples were subjected to HPLC and automated sequence analysis on Biobrene-treated glass fiber filters using an Applied Biosystems (Foster City, CA) 477A sequencer. Protein databases were searched using the FASTA program (Pearson and Lipman, 1988), through the National Center for Biotechnology Information Network.

Immunoprecipitation. For immunoprecipitation studies, cells incubated in $^{32}PO_4$ were rinsed with PBS and lysed in ice-cold lysis buffer [radioimmunoprecipitation assay (RIPA) buffer-PBS, 1% NP-40, 0.5% deoxycholate, 0.1% SDS, 0.1 mg/ml 4-(2-aminoethyl)-benzenesulfonylfluoride (Calbiochem, La Jolla, CA), 0.6 U/ml aprotinin, and 1 mM sodium orthovanadate]. All steps were conducted at 4°C. Lysates were centrifuged for 20 min, and supernatants were incubated with rabbit polyclonal anti-CSP24 for 1 hr. Protein A/G-agarose (Santa Cruz Biotechnology, Santa Cruz, CA) was then added for overnight incubation with rotation. Immunoprecipitates were collected by centrifugation for 10 min in a

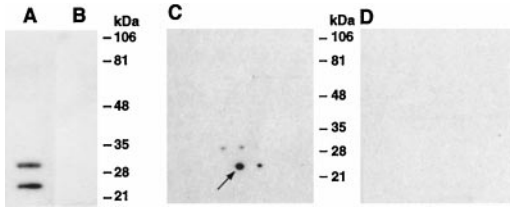

Figure 2. Anti-CSP24 recognition of 24 and 29 kDa proteins on Western blots. Lysates of components of the CS pathway were processed for immunoblotting using an affinity-purified polyclonal antibody raised against a peptide synthesized from a partial sequence of protein CSP24. The lysates were resolved in SDS-polyacrylamide gels and transferred to PVDF membranes. The immunocomplexes were detected with enhanced chemiluminescence reagent following the manufacturer's procedures. *A*, The antibody recognized two protein bands at 29 and 24 kDa. We have obtained a partial sequence of the 29 kDa protein that showed a peptide sequence that was identical to one sequenced peptide generated from CSP24 and used as our antigen. *B*, The detection of the 24 and 29 kDa proteins was completely blocked by preabsorption with excess CSP24 peptide. Identification of CSP24 separated by 2-D gel electrophoresis followed by Western blot analysis is shown. *C*, Proteins were separated by 2-D gel electrophoresis and blotted, and the PVDF membrane was stained with Coomassie blue to identify CSP24. The PVDF membrane was probed with anti-CSP24 antibody, resulting in the recognition of CSP24 (*arrow*) by the antibody. In addition, 29 kDa proteins were detected by anti-CSP24, consistent with the results of the 1-D gel analysis. The additional spots recognized on the 2-D gels may represent different charge states of the same 24 and 29 kDa phosphoproteins. *D*, 2-D gel pretreated with blocking peptide to anti-CSP24. The detection of CSP24 and 29 kDa proteins was completely blocked by preabsorption with excess CSP24 peptide.

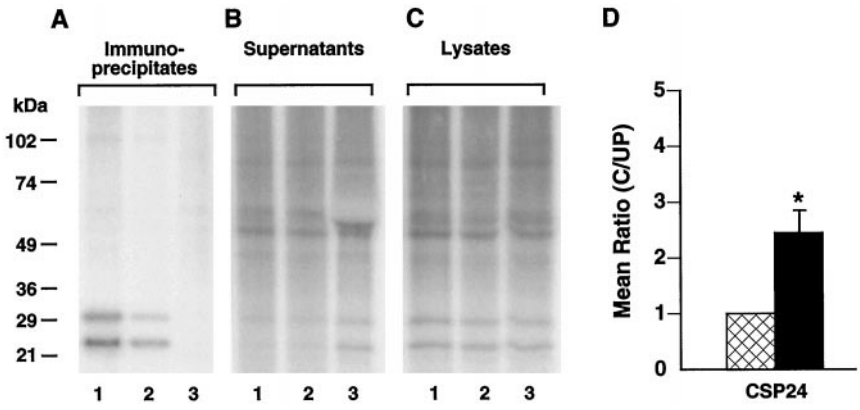

Figure 3. One-trial *in vitro* conditioning results in an increased phosphorylation of CSP24 detected in immunoprecipitates: analysis of anti-CSP24 or preimmune serum immunoprecipitates, supernatants, and lysates. Print from a storage phosphor screen shows 1-D electrophoretic separation of $^{32}PO_4$-labeled proteins. *A*, $^{32}PO_4$ incorporation into anti-CSP24 immunoprecipitates from an *in vitro*-conditioned group (*lane 1*) and unpaired controls (*lane 2*). As a control procedure, we examined $^{32}PO_4$ incorporation into preimmune immunoprecipitates as shown in *lane 3*. Eight eyes were used in each sample. *B*, $^{32}PO_4$ incorporation into proteins of the supernatants from the *in vitro*-conditioned group (*lane 1*) and unpaired controls (*lane 2*). *C*, $^{32}PO_4$ incorporation into proteins in a sample of the lysates from the *in vitro*-conditioned group (*lane 1*) and unpaired controls (*lane 2*). Note that the 24 and 29 kDa bands are labeled for all groups in the lysates. However, immunoprecipitation with the anti-CSP24 antibody dramatically reduced labeling of the 24 and 29 kDa proteins shown in *lanes 1* and *2* of the supernatants, but the same proteins did not exhibit a reduction in labeling after immunoprecipitation with preimmune serum (*lane 3*). *D*, Group data ($n = 6$) showing mean ± SE conditioned/unpaired control ratios of densitometric measurements for the 24 kDa band from immunoprecipitates. One-trial *in vitro* conditioning resulted in a significant increase in $^{32}PO_4$ incorporation in the 24 kDa protein band compared with the unpaired control group. Densitometric analysis of the Coomassie blue-stained 24 kDa bands from immunoprecipitates and lysates did not reveal statistically significant differences between conditioned and unpaired control samples: lysates, $t_{(5)} = 1.3$ (NS); immunoprecipitates, $t_{(5)} = 0.8$ (NS). The results indicate that differences in $^{32}PO_4$ incorporation cannot be accounted for by between-group differences in either protein loading or amount of precipitated protein. The *hatched bar* is used for comparison purposes and represents an E/C ratio of 1, where the conditional group is identical to the unpaired control group. $^*p < 0.01$.

microfuge, and the agarose pellets were carefully resuspended and washed in the RIPA buffer four times. The washed pellets were then rinsed two additional times with the sample buffer described above. After the final wash, 40 μl of SDS sample buffer (0.5 M Tris, 23% SDS, 10% glycerol, and 5% β mercaptoethanol) was added to the agarose pellet and boiled for 3 min. After boiling and centrifugation, samples were loaded for 1-D PAGE followed by phosphorimage analysis of $^{32}PO_4$-labeled proteins.

RESULTS

To identify CSP24 we excised the protein from multiple Coomassie blue-stained 2-D gels and obtained a partial amino acid sequence. Samples were treated to in-gel digestion (Rosenfeld et al., 1992), and eluted products were subjected to reverse-phase HPLC and automated sequence analysis. As shown in Figure 1, *A* and *B*, $^{32}PO_4$-labeled CSP24 co-migrated with CSP24 identified from the Coomassie blue-stained 2-D gel. The sequencing of CSP24 samples yielded several peptides that expressed varying degrees of sequence identity to the β-thymosin family of actin-binding protein (Nachmias, 1993; Carpintero et al., 1995; Safer and Chowrashi, 1997; Stoeva et al., 1997). Figure 1*C* shows a comparison of amino acid sequences of peptides derived from *Hermissenda* protein CSP24 with members or homologs of the β-thymosin family. We selected one peptide to use as an antigen in the development of an antibody to CSP24. As indicated in the Western blots of Figure 2, lysates of components of the CS pathway processed for immunoblotting using affinity-purified polyclonal antibody raised against a peptide from CSP24 showed that the antibody recognized protein bands at 24 and 29 kDa. In addition, proteins separated by 2-D gel electrophoresis, blotted, and probed with anti-CSP24 antibody revealed that the antibody recognized proteins with apparent molecular weights of 29 and 24 kDa. The detection of the 29 and 24 kDa proteins with Western blot analysis was completely blocked by preabsorption with excess CSP24 peptide (Fig. 2*B,D*). To further investigate the recognition of the 29 kDa protein by anti-CSP24, we obtained a partial sequence of the 29 kDa protein excised from multiple 2-D gels. The results of the sequence analysis identified a peptide with an identical amino acid sequence to the CSP24 peptide used as our antigen. The multiple proteins with molecular weights of 29 and 24 kDa recognized by the antibody may be explained by different charge states of the same phosphoproteins (Fig. 2*C*).

To provide evidence that the phosphorylation of CSP24 is increased by one-trial conditioning, we immunoprecipitated $^{32}PO_4$-labeled protein with anti-CSP24 in conditioned groups and unpaired controls. Figure 3 shows that the lysates contained CSP24, as indicated by the labeling in *lanes 1–3*. The samples shown in Figure 3*A* were immunoprecipitated with either the anti-CSP24 antibody or preimmune serum before SDS-PAGE and phosphorimage analysis. Immunoprecipitation with the anti-CSP24 antibody dramatically reduced $^{32}PO_4$ labeling in *lanes 1* and *2* of the supernatant fractions (Fig. 3*B*), but not after immunoprecipitation with preimmune serum, as indicated in *lane 3*. The immunoprecipitates from the *in vitro*-conditioned group (Fig. 3*A*, *lane 1*) showed greater $^{32}PO_4$ incorporation in CSP24 than the unpaired controls (*lane 2*). The group data ($n = 6$) in Figure 3*D* show the mean ± SE conditioned/unpaired control ratios computed from densitometric measurements of CSP24 bands from immunoprecipitates. The statistical analysis of the group data revealed that one-trial *in vitro* conditioning resulted in a significant increase in $^{32}PO_4$ incorporation in CSP24 ($t_{(5)} = 3.36$; $p < 0.01$) compared with the unpaired controls.

Previous research with *Hermissenda* has shown that components of memory formation after Pavlovian conditioning can be dissociated based on the contribution of different signal transduction pathways, protein synthesis, and gene induction (Matzel et al., 1990; Farley and Schuman, 1991; Crow and Forrester, 1993; Crow et al., 1993, 1998). We have now isolated short-term memory from intermediate and long-term memory by using a less-concentrated solution of 5-HT (5×10^{-6} M) than normally used in our one-trial conditioning studies of *Hermissenda*. As shown in the example in Figure 4*A* and the group data in Figure 4*B*, the CS paired with 5×10^{-6} M 5-HT produced only a short-term pairing-specific increase in the excitability of lateral B photoreceptors as measured with 2 sec depolarizing extrinsic current pulses 15 and 30 min after conditioning (Fig. 4*A1*, *A2*). Excitability had decremented to levels comparable with unpaired controls

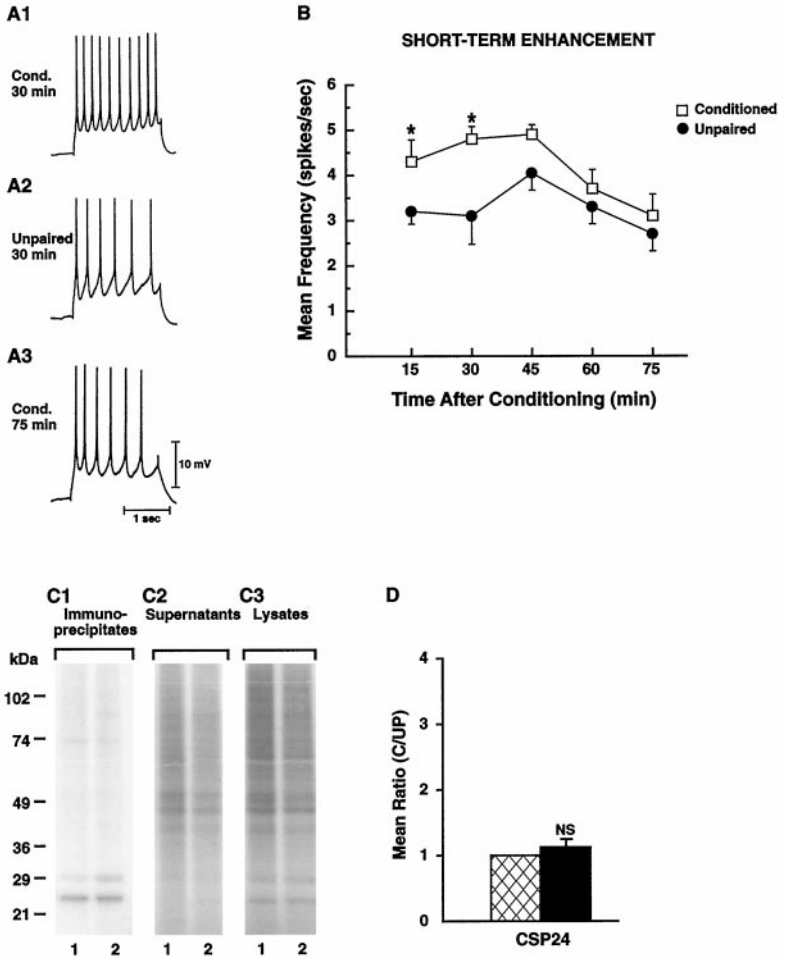

Figure 4. Light paired with 5×10^{-6} M 5-HT results in short-term enhanced excitability. *A*, Examples of enhanced excitability examined in lateral B photoreceptors from conditioned (*Cond.*; *A1*) and unpaired (*A2*) controls 30 min after conditioning and a conditioned group assessed 75 min after conditioning (*A3*). *B*, Group data depicting mean frequency ± SE in spikes per second elicited by 2 sec depolarizing extrinsic current pulses. Excitability was assessed for independent groups at 15, 30, 45, 60, and 75 min after conditioning. Significant differences between conditioned and unpaired controls were only detected at 15 min ($p < 0.05$) and 30 min ($p < 0.001$) after conditioning. *C*, Short-term enhanced excitability produced by one-trial *in vitro* conditioning does not result in an increased phosphorylation of CSP24 based on the analysis of anti-CSP24 immunoprecipitates, supernatants, and lysates. Print from a storage phosphor screen shows 1-D SDS gel electrophoretic separation of $^{32}PO_4$-labeled proteins. *C1*, $^{32}PO_4$ incorporation into anti-CSP24 immunoprecipitates from the *in vitro*-conditioned group (*lane 1*) and unpaired control (*lane 2*). *C2*, Supernatant samples. *Lane 1*, Conditioned group; *lane 2*, unpaired control group. *C3*, Lysate sample for conditioned group (*lane 1*) and unpaired control group (*lane 2*). No significant differences between groups in $^{32}PO_4$ incorporation were found after a conditioning procedure that produced only short-term enhancement. *D*, Group data ($n = 7$) showing mean ± SE conditioned/unpaired (*C/UP*) control ratios of densitometric measurements for the 24 kDa band from immunoprecipitates. The *hatched bar* is used for comparison purposes and represents an experimental/control ratio of 1.

60 and 75 min after conditioning (Fig. 4*A3,B*), indicating that enhancement produced by this procedure is indeed short-term. In addition, excitability assessed at 3 hr ($t_{(8)} = 1.06$; NS) and 24 hr ($t_{(10)} = 0.57$; NS) after conditioning was not significantly different from unpaired controls. The results of the ANOVA revealed a significant overall effect of conditioning ($F_{(1,50)} = 21.5; p < 0.001$) and significant changes in excitability assessed at different times after conditioning ($F_{(4,50)} = 6.9$; $p < 0.001$). Significant differences between the conditioned group ($n = 6$) and unpaired controls ($n = 6$) were observed at 15 min ($t_{(10)} = 2.2; p < 0.05$) and 30 min ($t_{(10)} = 4.3; p < 0.001$) after conditioning. To determine whether short-term enhancement is also accompanied by phosphorylation of CSP24, we examined $^{32}PO_4$ labeling of CSP24 in immunoprecipitates of samples after one-trial *in vitro* conditioning using 5×10^{-6} M 5-HT in conditioned groups ($n = 7$) and unpaired controls ($n = 7$). As shown in the examples of Figure 4*C1*, the $^{32}PO_4$ labeling of CSP24 was similar for the conditioned group (*lane 1*) and the unpaired controls (*lane 2*). The statistical analysis of the group data shown in Figure 4*D* revealed that the conditioned group was not significantly different from the unpaired controls ($t_{(6)} = 1.1$; NS). These results show that increased phosphorylation of CSP24 is not observed after procedures that only result in short-term enhanced excitability.

DISCUSSION

We have shown that peptides derived from a phosphoprotein (CSP24) associated with an intermediate stage of memory after one-trial conditioning exhibit amino acid sequences that are similar to members of the β-thymosin family of actin-binding protein. Western blot analysis of 1- and 2-D gels probed with anti-CSP24 antibody showed a recognition of CSP24. Immunoprecipitation studies using anti-CSP24 revealed significantly greater $^{32}PO_4$ incorporation into immunoprecipitates from conditioned preparations compared with unpaired controls. Finally, a modification of the one-trial conditioning procedure that only produced short-term enhanced excitability did not produce statistically significant differences in $^{32}PO_4$ labeling of CSP24 in immunoprecipitates from conditioned groups compared with unpaired controls.

The requirement for protein synthesis in supporting the intermediate component of memory may be dependent on the learning paradigm, species, and signal transduction pathways responsible for the induction, maintenance, and expression of learning. As an example, the increased excitability of *Aplysia* sensory neurons measured 3 hr after activation of PKC is independent of protein synthesis (Manseau et al., 1998). One form of 5-HT-induced intermediate-term facilitation is reported to be independent of both protein synthesis and persistent PKA activity (Sutton and Carew, 1999). Moreover, there are examples from conditioning of *Drosophila* in which an intermediate phase of memory is not dependent on protein synthesis (DeZazzo and Tully, 1995). In contrast, an intermediate phase of synaptic facilitation produced by a concentration-dependent exposure to 5-HT in co-cultured *Aplysia* sensory neurons is protein synthesis-dependent and mRNA synthesis-independent (Ghirardi et al., 1995). The results from these studies of synaptic facilitation suggest that the transi-

tion from short- to long-term facilitation involves a transient intermediate phase consisting of several distinct mechanisms that are engaged by different concentrations of 5-HT or the number of 5-HT applications. However, the same phosphoproteins have been proposed to account for both short- and long-term facilitation in *Aplysia* (Sweatt and Kandel, 1989). In contrast, using a concentration of 5-HT sufficient to produce long-term enhancement with a single conditioning trial, we have identified an intermediate stage of memory that is protein synthesis-dependent and mRNA synthesis-independent (Crow et al., 1999). We have also shown that long-term enhancement can be expressed after procedures that totally block the induction and expression of short-term enhancement (Crow and Forrester, 1993), indicating that the components of memory involve independent mechanisms. Moreover, our evidence indicates that short-term enhancement is Ca^{2+}- and PKC-dependent (Crow et al., 1991; Falk-Vairant and Crow, 1992) but not dependent on activation of the mitogen-activated protein kinase pathway; a protein kinase that is activated by conditioning of *Hermissenda* (Crow et al., 1998). It is thus likely that the three components of memory after one-trial conditioning of *Hermissenda* may involve distinct signaling pathways and different phosphoproteins.

The assembly and disassembly of actin filaments induced by extracellular signals underlie a number of cellular processes and may play a role in cellular and synaptic plasticity (Fifkova and Morales, 1992; Kim and Lisman, 1999). To perform functions supporting cellular plasticity, the organization of the actin cytoskeleton requires temporal and spatial regulation by proteins. The activity of these proteins is modulated by intracellular signals that recruit actin nucleation and polymerization to specific cellular sites (Schmidt and Hall, 1998). Although all known vertebrate and invertebrate β-thymosins bind actin monomers (Nachmias, 1993; Safer and Chowrashi, 1997), recent evidence suggests that β-thymosins are not just simple actin-buffering proteins (Sun et al., 1996). The regulation of β-thymosin-like proteins by one-trial conditioning may amplify the effect of filament uncapping by creating a reservoir of G-actin that can be desequestered to supply actin to filament ends. In addition, actin-binding proteins such as β-thymosin, which stabilize actin monomers, may facilitate nucleation. Our results would thus support a potential role for β-thymosin-like proteins in plasticity by regulating the turnover of actin filaments in neurons of the CS pathway of conditioned animals during the intermediate-term transition period between short- and long-term memory.

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