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cAMP-stimulated phosphorylation of an axonemal polypeptide that copurifies with the 22S dynein arm regulates microtubule translocation velocity and swimming speed in *Paramecium*

(ciliary motility/ATPase/phosphoproteins/*in vitro* translocation)

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ABSTRACT **In *Paramecium tetraurelia*, cyclic nucleotides are important physiological second messengers that could regulate dynein mechanochemistry by phosphorylation. A 29-kDa polypeptide that is phosphorylated in a cAMP- and Ca^{2+}-sensitive manner in permeabilized cells and isolated axonemes is the only significant phosphorylated moiety that consistently copurifies with 22S dynein from paramecium cilia. It is not a component of 14S dynein. This polypeptide can be thiophosphorylated in a cAMP-sensitive manner, and this form of 22S dynein is stable when stored at −70°C. cAMP-mediated thiophosphorylation of the 29-kDa polypeptide significantly increases the velocity with which 22S dynein causes microtubules to glide *in vitro*. The increase is abolished, together with the thiophosphorylation of the 29-kDa polypeptide, by preincubation with high Ca^{2+}. Pretreatment with high Ca^{2+} does not alter the thiophosphorylation pattern of, or the velocity of microtubule translocation by, 14S dynein. The same preincubation conditions that permit or abolish the increase in velocity of microtubule translocation by 22S dynein permit or fail to permit swimming speed of permeabilized cells to increase on reactivation even after cAMP is removed. The effect of cAMP on swimming speed can therefore be accounted for by changes in the mechanically coupled 22S dynein activity via phosphorylation or thiophosphorylation of the 29-kDa polypeptide, which could act as a regulatory dynein light chain.**

In *Paramecium*, an organism used classically to study the effects of second messengers on ciliary motility, cyclic nucleotides are important physiological second messengers. cAMP and cGMP increase the swimming velocity of Mg^{2+}-ATP-reactivated, Triton X-100-permeabilized cells (1, 2). The responding structure is the ciliary axoneme, since swimming responses are obtained when the ciliary membrane and soluble matrix components are compromised or missing entirely (3). The question then arises as to whether a particular phosphorylation event in the axoneme, or more specifically in ciliary dynein, correlates with these behavioral responses to cyclic nucleotides. Recent investigations (4, 5) have identified a 29-kDa axonemal polypeptide that is phosphorylated in a cAMP- and Ca^{2+}-sensitive manner in permeabilized cells and in isolated axonemes and that copurifies with 22S dynein of paramecia. No label is incorporated into this polypeptide in the absence of cAMP, and phosphorylation occurs under conditions in which permeabilized axonemes will beat and permeabilized cells swim (4). This study will demonstrate that cAMP-stimulatable thiophosphorylation of this 29-kDa polypeptide regulates *in vitro* microtubule translocation velocity by paramecium 22S dynein in a manner that suggests that phosphorylation of this moiety is a major factor in the cAMP control of swimming speed of permeabilized cells.

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MATERIALS AND METHODS

Preparation of Permeabilized Cells and Measurement of Swimming Speed. Axenic *Paramecium tetraurelia* were harvested and treated with 0.01% Triton X-100 for 25 min on ice. See refs. 3 and 4 for details. After washing, permeabilized cells were placed in preincubation buffer (10 mM Tris maleate, pH 7.0/4 mM $MgSO_4$/2 mM EGTA/30 mM potassium acetate) on ice for 20 min. Controls were preincubated with 1 mM ATP or adenosine 5′-[γ-thio]triphosphate ([γ-S]ATP). Experimental (cAMP-pretreated) samples were preincubated with 1 mM ATP or [γ-S]ATP plus 100 μM cAMP. $CaCl_2$ (2 mM) was added to some experimental samples. Buffer was then added to dilute the suspension by 50-fold or more. Aliquots, taken at specified times after dilution, were mixed with an equal volume of preincubation buffer containing 2 mM ATP for reactivation of ciliary motility. In some cases, 2 μM cAMP was present in the reactivation buffer. The reactivated swimming cells were videotaped at room temperature for motion analysis with an Expertvision system (Motion Analysis, Santa Rosa, CA) (3). In all cases, average swimming speeds of matched experimental and control samples from the same initial suspension were compared.

Preparation of Paramecium Axonemes. Axonemes were isolated by Ca^{2+} shock deciliation. After deciliation, cilia were isolated by centrifugation, treated with 1.0% Triton X-100 to remove membranes, washed three times, and resuspended in axoneme buffer [30 mM Hepes, pH 7.6/5 mM $MgSO_4$/0.5 mM EDTA/20 mM KCl/1 mM dithiothreitol with aprotinin (50 kallikrein-inhibitory units/ml) and leupeptin (10 μg/ml)]. See ref. 4 for details.

Preincubation of Axonemes and Extraction of Dynein. Axonemes were separated into control and experimental samples. Control axonemes were preincubated with [γ-^{32}P]ATP (specific activity, 10 mCi/ml; 1 mCi = 37 MBq) or, alternatively, [γ-^{35}S]ATP or their nonradioactive counterparts, in a concentration range of 0.1–1 μM, for 10–20 min on ice. Experimental samples were treated identically except that 10–20 μM cAMP was added to the incubation solution. $CaCl_2$ (0.4 mM)—which lowers pCa from 7 to 4.0–4.5—was simultaneously added to some aliquots. Axonemes were washed to remove nucleotides and Ca^{2+}, then treated with 0.5–0.6 M KCl for 30 min to extract dynein arms. Disappearance of arms was monitored by negative-stain electron microscopy.

Sucrose-Gradient Purification and Assays of Dynein. The high-salt extract was loaded on a 5–30% sucrose density gradient and centrifuged with a Beckman SW41 rotor at 35,000 rpm for 15 hr at 4°C. Fractions were collected. Each fraction was assayed for protein and ATPase activity and

Abbreviation: [γ-S]ATP, adenosine 5′-[γ-thio]triphosphate.

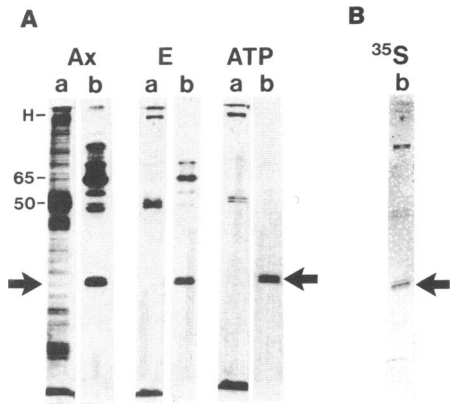

FIG. 1. cAMP-phosphorylated or -thiophosphorylated 29-kDa polypeptide copurifies with the dynein arm by an alloaffinity procedure. (*A*) Each pair of lanes shows a silver-stained gel (10% acrylamide) (lane a) and a corresponding autoradiogram (lane b). Axonemes (Ax) were preincubated with [γ-^{32}P]ATP and 10 μM cAMP, pCa 7 for 10 min. A 29-kDa band (arrow) became phosphorylated. Axonemes were then treated with 0.5 M KCl for 30 min to extract dynein. Major constituents in extract (E) were dynein heavy chains (H) and tubulin (50-kDa bands). Phosphorylated 65- and 29-kDa polypeptides were coextracted. Fresh unlabeled doublet microtubules were added to extract under conditions where arms rebind to doublet microtubules. Then arms were released from the doublet microtubules by addition of 1 mM ATP. The ATP lanes show that dynein heavy chains and phosphorylated 29-kDa polypeptide were released together. No other labeled bands copurified with dynein heavy chains. (*B*) Autoradiogram of ATP-released material from a corresponding experiment where axonemes were preincubated with [γ-^{35}S]ATP and 20 μM cAMP, pCa 7.

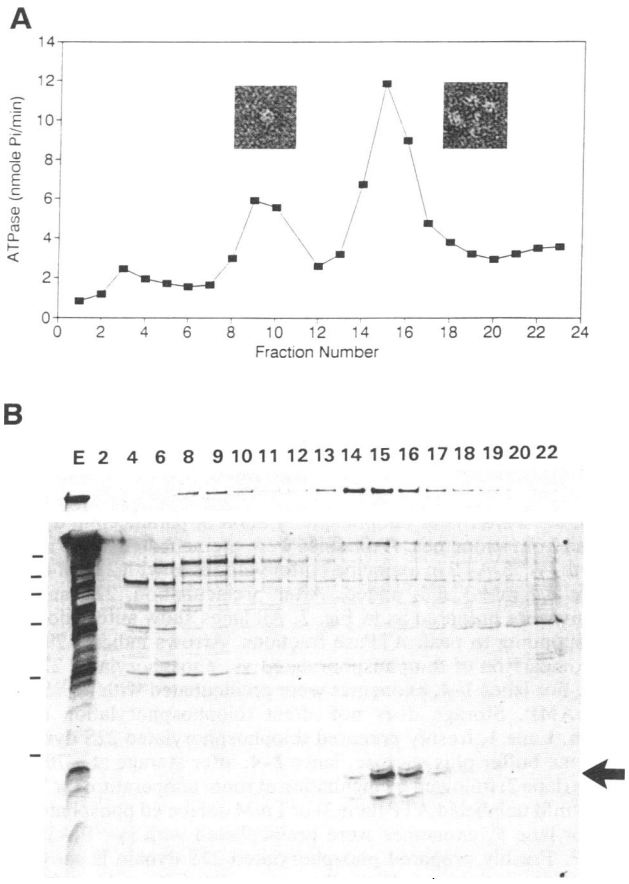

FIG. 2. Sucrose gradient purification of paramecium dynein. Axonemes were preincubated with [γ-^{35}S]ATP and 20 μM cAMP, pCa 7, for 20 min and then extracted with 0.6 M KCl for 30 min. The extract (E) was subjected to sucrose density gradient centrifugation. Fraction 1 was from the top of the gradient. (*A*) Two peaks of ATPase activity are seen at 22 S (fractions 14–17) and 14 S (fractions 8–10), respectively. Typical negative-stain images of dyneins from peak fractions are shown (*Insets*). See ref. 6 for further details. (*B*) The thiophosphorylated 29-kDa band (arrow) copurifies only with 22S dynein fractions. Heavy-chain region of Coomassie blue-stained 10% acrylamide gel is shown at top with autoradiogram of entire gel below. In the autoradiogram, together with the labeled 29-kDa band, a "sky chain" and some minor contaminant bands are also present in the 22S fractions. Several thiophosphorylated bands also copurify with 14S dynein but the 29-kDa band is not present in these fractions. Markers at left indicate (from top) 200, 116, 97, 66, 45, and 32 kDa.

analyzed by NaDodSO$_4$/PAGE followed by autoradiography. Fractions containing the 14S and 22S dyneins were examined by negative-stain electron microscopy and pooled separately. For assay details see refs. 4 and 6. Some dynein fractions were stored at −70°C for later use. Dynein was also purified by a microtubule affinity method (7). Rebinding to microtubules and release were independently monitored by NaDodSO$_4$/PAGE of supernatants and pellets and by corresponding negative-stain microscopy. Phosphorylation and thiophosphorylation of dynein after cAMP pretreatment were determined by measuring cpm of γ-^{32}P or -^{35}S incorporated per mole of dynein, assuming a molecular weight of 2 $\times$ 10^6 for 22S dynein and 6 $\times$ 10^5 for 14S dynein. Moles of label incorporated were determined using radiolabeled nucleotide standard solutions that were then used in the incorporation experiments.

***In Vitro* Translocation.** Motility assays (8) were carried out as adapted for paramecium dyneins (6). Dynein samples, adjusted to about 0.1 mg of protein per ml, were used to coat a small (15-μl) glass assay chamber. Two 20-μl aliquots were applied for 2 min; unabsorbed dynein was removed by washing. Twice cycled, phosphocellulose-purified bovine brain tubulin was used to polymerize microtubules in the presence of 5 μM taxol and 1 mM GTP at 37°C. The microtubules were added to the dynein-coated chamber, and reactivation buffer (preincubation buffer plus 1 mM ATP) was perfused into the chamber. Motility was video-recorded from a darkfield image obtained from an SIT camera (Hamamatsu Photonics, Hamamatsu, Japan). Measurements were made by tracing translocation of individual microtubules for 2–4 sec.

RESULTS

After cAMP-Mediated Phosphorylation, the 29-kDa Polypeptide Is the Only Significant Labeled Band That Consistently Copurifies with the Dynein Arm. We first confirmed that the phosphorylatable 29-kDa axonemal polypeptide would be extracted with paramecium dynein under several conditions of purification. In an alloaffinity procedure (7), the labeled 29-kDa polypeptide binds to microtubules and is released by 1 mM ATP in parallel with arms identified structurally or with dynein heavy chains seen on corresponding gels (Fig. 1).

The Thiophosphorylated 29-kDa Polypeptide Cosediments with Fractions of the 22S Dynein Peak but Does Not Cosediment with 14S Dynein. The labeled dynein fraction has also been examined by sucrose density gradient centrifugation (Fig. 2). Two main peaks of Mg^{2+}-ATPase activity, corresponding to 14S and 22S dynein, respectively, are observed (6, 9). The 22S dynein is a three-headed bouquet structure in negative stain, while the 14S dynein, which is heterogeneous, consists largely of single-headed species. The thiophosphorylated 29-kDa polypeptide cosediments with 22S dynein (Fig. 2). Fractions corresponding to the peak ATPase activities incorporate 5 mmol of phosphate or thiophosphate per mol of 22S dynein and 2 mmol of thiophosphate per mol of 14S dynein.

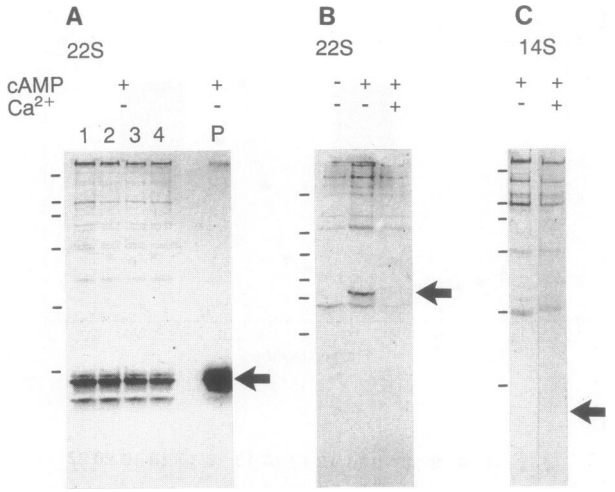

FIG. 3. Patterns of thiophosphorylation of paramecium dyneins derived from axonemes. Axonemes were preincubated for 20 min on ice with [γ-^{35}S]ATP in axoneme buffer without or with 20 μM cAMP and/or 0.4 mM $CaCl_2$ added. After preincubation, 22S and 14S dyneins were prepared as in Fig. 2. All lanes show autoradiograms corresponding to peak ATPase fractions. Arrows indicate 29 kDa. (*A*) Comparison of thiophosphorylated vs. phosphorylated 22S dyneins. For lanes 1–4, axonemes were preincubated with [γ-^{35}S]ATP and cAMP. Storage does not affect thiophosphorylation of the dynein. Lane 1, freshly prepared thiophosphorylated 22S dynein in axoneme buffer plus sucrose; lanes 2–4, after storage at −70°C for 5 days (lane 2) followed by incubation at room temperature for 10 min with 1 mM unlabeled ATP (lane 3) or 1 mM unlabeled phosphate (lane 4). For lane P, axonemes were preincubated with [γ-^{32}P]ATP and cAMP. Freshly prepared phosphorylated 22S dynein is shown for comparison with lane 1. Virtually the only labeled band is at 29 kDa. Electrophoresis was in a 10% acrylamide gel. Markers are as in Fig. 2. (*B*) Comparison of thiophosphorylation patterns of 22S dynein extracted from axonemes subjected to various preincubation conditions. Thiophosphorylation of 29-kDa band (arrow) is prominent after preincubation with cAMP in the absence of Ca^{2+}. Only the 29-kDa band is consistently labeled after +cAMP, $-Ca^{2+}$ preincubation but is absent after +cAMP, $+Ca^{2+}$ preincubation; other bands show much more minor changes, if any. Electrophoresis was in a 15% acrylamide gel. Markers indicate 66, 45, 36, 32, 24, and 20 kDa. (*C*) Thiophosphorylated 14S dynein extracted from axonemes subjected to preincubation with cAMP in the presence or absence of Ca^{2+}. No thiophosphorylated band is seen at 29 kDa (arrow) under either condition. Control (no cAMP, data not shown) was not detectably different from sample incubated with cAMP. Electrophoresis was in a 10% acrylamide gel. Markers are as in Fig. 2.

22S Dynein That Has Been Thiophosphorylated in the Presence of cAMP Can Be Stored at −70°C for Several Days with No Significant Loss or Turnover of Label. When 22S dynein is extracted from axonemes that have been phosphorylated in a cAMP-mediated reaction, virtually the only labeled species is the 29-kDa band (Fig. 3*A*). Sometimes a small amount of label is seen in a high molecular weight "sky chain" (10). In contrast, eight or so bands, including the sky chain, are weakly labeled after thiophosphorylation, although the major labeled species is still the 29-kDa band. No significant labeling of 22S dynein heavy chains is seen. The thiophosphorylated 22S dynein has been stored at −70°C for 5 days, thawed, incubated at room temperature with buffer, nonradioactive ATP, or phosphate for 10 min, and rerun. No changes in the thiophosphorylation pattern are observed (Fig. 3*A*). In general, stored dynein prepared from axonemes that have been preincubated in different ways is used for the microtubule translocation assays.

cAMP-Mediated Thiophosphorylation Increases Velocity of Microtubule Translocation by 22S Dynein. Under standard conditions, the translocation rate of microtubule-associated protein (MAP)-free taxol-stabilized microtubules by paramecium 22S control dynein ([γ-S]ATP pretreatment in the absence of cAMP) is 1.82 ± 0.04 μm/sec (mean ± SEM; five preparations comprising 296 measurements; Fig. 4). For 22S dynein prepared under conditions where cAMP-mediated thiophosphorylation of the 29-kDa polypeptide is present (Fig. 3*B*), the translocation rate increases by about 40% (Fig. 4). Differences in the translocation velocities ± cAMP pretreatment are highly significant ($P < 0.001$). After pretreatment with cAMP, some microtubules translocate at speeds greater than those measured for any control microtubule. This stable increase occurs independently of microtubule length (in the range of 1–20 μm). Except for the dramatic

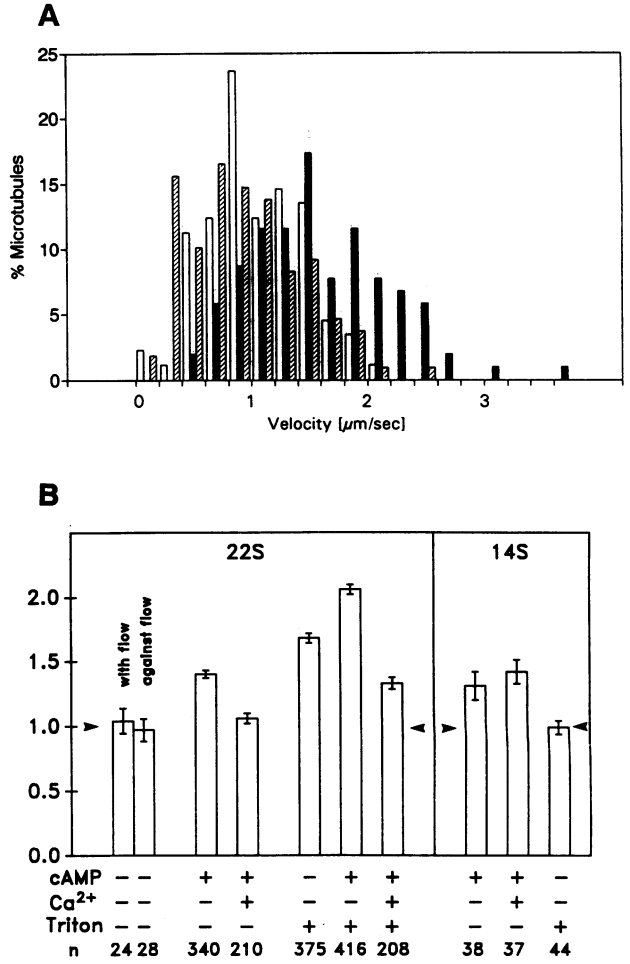

FIG. 4. *In vitro* microtubule translocation velocity of paramecium dyneins in 1 mM Mg^{2+}-ATP, pCa 7 at room temp. See Fig. 3 for different axonemal preincubation conditions. Dynein fractions were isolated as in Fig. 2 and stored frozen at −70°C until further use. Where indicated, 0.1% Triton X-100 was added to reactivation buffer with 1 mM ATP. (*A*) Velocity profile of microtubule translocation from one experiment using 22S dynein comparing control (open bars, $n = 89$); +cAMP, $-Ca^{2+}$ (black bars, $n = 104$); and +cAMP, $+Ca^{2+}$ (hatched bars, $n = 109$) axonemal preincubations. (*B*) Matched experimental/control velocity ratios for different conditions of axonemal preincubation. Values are means ± SEM; n = no. of microtubules measured. (*Left*) Translocation over 22S dynein preparations. Control ratio = 1.00 (arrowhead). Control samples: "with flow," microtubules moving in the direction of buffer flow; "against flow," microtubules moving opposite to the direction of flow. Significance (t tests): with vs. against flow, not significant (ns); cAMP vs. control, $P < 0.001$; cAMP vs. cAMP/Ca^{2+}, $P < 0.001$; cAMP/Ca^{2+} vs. control, ns; cAMP/Triton vs. Triton, $P < 0.001$; Triton vs. control, $P < 0.001$; cAMP/Triton vs. cAMP, $P < 0.001$. (*Right*) Translocation over 14S dynein preparations. Control ratio = 1.00 (arrowhead). Significance (t tests): cAMP vs. control, $P < 0.01$; cAMP vs. Ca^{2+}, ns; Triton vs. control, ns.

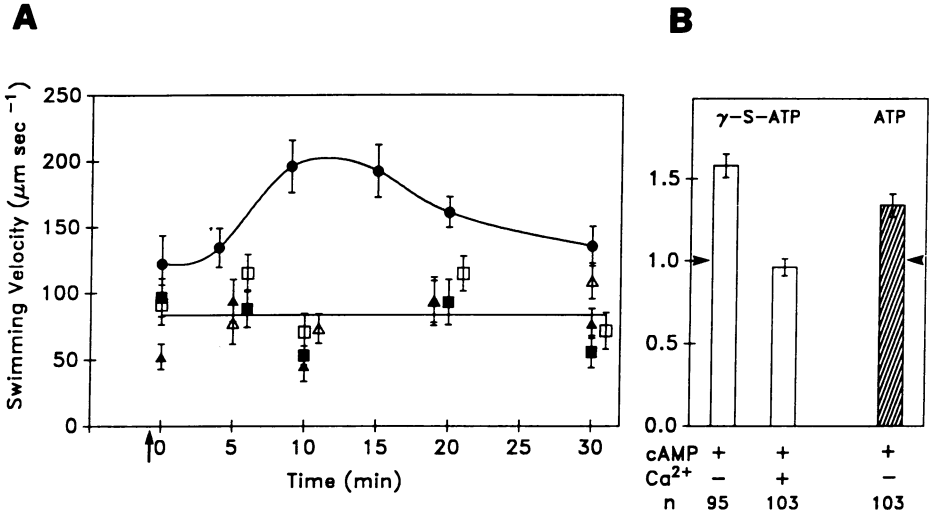

FIG. 5. Swimming speed of permeabilized paramecia reactivated under standard conditions after preincubations with 1 mM ATP (▲, △), 1 mM [γ-S]ATP (□, ■), or 1 mM [γ-S]ATP plus 100 μM cAMP (●). All reactivation was in 1 mM Mg^{2+}-ATP, pCa 7; open symbols indicate the addition of 2 μM cAMP to the reactivation medium. (*A*) Time course from one experiment comparing cells preincubated in [γ-S]ATP plus 100 μM cAMP with controls. Acceleration of the experimental population at 10–15 min is not seen with ATP-plus-cAMP preincubation. Sample sizes for each determination were 10–20 cells. (*B*) Matched experimental/control velocity ratios for different conditions of preincubation. Control ratio = 1.00 (arrowhead). Where indicated, preincubation buffer contained 2.0 mM $CaCl_2$. Values are means ± SEM; *n* = no. of measurements. Significance (*t* tests): cAMP vs. control, $P < 0.001$; cAMP/Ca^{2+} vs. control, not significant.

increase in label of the 29-kDa band, other bands show minor changes in the amount of thiophosphorylation with or without cAMP pretreatment.

cAMP Preincubation Also Increases Translocation Velocity of 22S Dynein Reactivated in the Presence of Triton. Addition of 0.1% Triton X-100 to isolated control 22S dynein produces an increase in microtubule translocation velocity, as expected (8). However, the velocity of microtubule translocation by 22S dynein preincubated with cAMP increases further after Triton addition (Fig. 4*B*), so that the difference is still significant. The effects of cAMP preincubation and Triton addition on velocity are additive. Addition of Triton to 14S dynein has no effect on microtubule translocation velocity.

The Increase in Microtubule Translocation Velocity Is Abolished Together with Thiophosphorylation by Preincubation in High Ca^{2+}. Preincubation in 20 μM cAMP with high Ca^{2+} (pCa 4.0–4.5) results in virtually complete inhibition of cAMP-mediated thiophosphorylation of the 29-kDa polypeptide in 22S dynein (Fig. 3*B*). Except for this, little or no change is seen in the thiophosphorylation pattern of other constituents (Fig. 3*B*) (4). Further, if thiophosphorylation is carried out in the presence of cAMP but in high Ca^{2+}, the 22S dynein translocates microtubules at speeds nearly identical to matched controls preincubated in [γ-S]ATP without cAMP at pCa 7; that is, in parallel to the absence of cAMP-stimulatable thiophosphorylation of the 29-kDa polypeptide, the cAMP-mediated microtubule translocation velocity increase is abolished (Fig. 4*A*). The stimulatory effect of cAMP pretreatment on translocation velocity in the presence of Triton is also abolished (Fig. 4*B*).

Pretreatment with High Ca^{2+} Does Not Change Thiophosphorylation Pattern or Velocity of Microtubule Translocation by 14S Dynein. Under standard conditions, 14S dynein from control preparations translocates microtubules with a velocity of 1.04 ± 0.06 μm/sec (two preparations, 49 measurements). Pretreatment with [γ-S]ATP in the presence of cAMP also increases the translocation rate of 14S dynein significantly (Fig. 4*B*). The velocity of microtubule translocation by 14S dynein pretreated with cAMP in high Ca^{2+} remains at the stimulated level (Fig. 4*B*), while the pattern of thiophosphorylation with or without Ca^{2+} remains virtually unchanged (Fig. 3*C*).

The Swimming Speed of Permeabilized Paramecia Increases After Removal of cAMP Only Under Conditions Where the 29-kDa Polypeptide Is Phosphorylated or Thiophosphorylated. To test the physiological significance of these findings, we have measured the swimming speed of permeabilized paramecia preincubated with 1 mM [γ-S]ATP under different conditions. After preincubation, the preparations are diluted >50-fold with buffer, drastically reducing cAMP and [γ-S]ATP concentrations. In controls (no cAMP preincubation), swimming velocity upon reactivation with ATP is always low, averaging 52.2 ± 0.07 μm/sec for the 35-min measurement period (two experiments, 59 measurements). Addition of 2 μM cAMP (the maximum concentration after dilution) to the ATP reactivation medium is insufficient to accelerate the population. However, preincubation in 100 μM cAMP with [γ-S]ATP (and low Ca^{2+}) permits the swimming velocity of the reactivated population to increase by 40–135% compared with controls (Fig. 5*A*). Comparable increases are seen when ATP is substituted for [γ-S]ATP in the preincubation step (Fig. 5*B*). In contrast, when the preparations are preincubated with cAMP and [γ-S]ATP in high Ca^{2+}, where 29-kDa thiophosphorylation is prevented, an increase in swimming speed of the reactivated population is not observed (Fig. 5*B*). All cells swim forward after reactivation.

DISCUSSION

Preincubation of paramecium axonemes with cAMP under conditions where a 29-kDa polypeptide that copurifies with 22S dynein becomes phosphorylated or thiophosphorylated is necessary and sufficient to permit the 22S dynein to translocate bovine brain microtubules about 40% faster than controls. The same conditions permit permeabilized paramecia to swim faster than controls. The average percent increase in swimming speed of permeabilized cells in control vs. cAMP-pretreated preparations (Fig. 5*B*) and the percent increase in translocation rate by control vs. cAMP-pretreated 22S dyneins (Fig. 4*B*) are similar. Although the average increases in microtubule translocation velocity and swimming speed are modest but significant, individual measurements sometimes indicate that much greater increases are possible, so that the doubling or more of swimming speed reported elsewhere (2) is not inconsistent with these values.

Thiophosphorylation is a useful tool in these experiments, since thiophosphorylated dynein is stable and thiophosphorylation functionally substitutes for phosphorylation in determining both microtubule translocation velocity and swimming speed. Several polypeptides that copurify with 22S dynein are thiophosphorylated to some extent in these preparations, but these show, at most, very minor changes in dyneins preincubated under conditions where velocity does not increase. Except for a "sky chain," which is generally not an integral part of the dynein arm (10), corresponding polypeptides are absent from phosphorylated preparations. Therefore, it is unlikely that these play a significant role in the increases in velocity observed.

One would expect that one 29-kDa polypeptide represents 1.5% of 22S dynein by weight. From Fig. 1, the 29-kDa polypeptide is substoichiometric with respect to 22S dynein (see also ref. 9, table 3). This may mean that (*i*) some 29-kDa polypeptide dissociates from 22S dynein and is lost during preparation or, alternatively, (*ii*) the 29-kDa polypeptide is not originally associated with every 22S dynein arm. A simple assumption might be that the 29-kDa polypeptide is associated with only one in four outer arms. We estimate that about 30% of the isolated 22S dynein molecules contain a 29-kDa polypeptide in preparative gels.

Quantitation of thiophosphorylation of 22S dynein using radiolabeled [γ-S]ATP for standardization indicates that about 5 mmol of thiophosphate are incorporated per mol of dynein; that is, about one thiophosphorylated 29-kDa polypeptide is present for every 200 22S dynein molecules. Therefore, about 2% of the 29-kDa polypeptides in the isolated 22S dynein used for the *in vitro* translocation assays are thiophosphorylated by cAMP stimulation.

How could so few thiophosphorylated dyneins result in a significantly faster rate of *in vitro* microtubule translocation? The translocation assays occur on saturated fields of dynein such that there are many dyneins acting even on a 1-μm-long microtubule. It could be that dyneins where the 29-kDa polypeptide remains associated with the arm are, in general, more native than other molecules in the translocation assays. After cAMP preincubation and thiophosphorylation of the dynein, some microtubules in the population would be translocating on an additional phosphorylated 22S dynein. If the phosphorylated molecules acted as pacemakers, the translocation velocity of the population would be shifted upward and some microtubules would translocate at faster speeds than the controls, as in Fig. 4*A*. This interpretation is consistent with present hypotheses on how myosin light-chain phosphorylation may control smooth muscle contraction (11). Tests of this suggestion will rely on a more complete description of dynein–microtubule interactions in our translocation assay system.

We do not understand why microtubule translocation velocity by 14S dynein is also increased after cAMP-stimulated thiophosphorylation. However, phosphorylation of 14S dynein is unlikely to be a critical event in the physiological regulation of swimming speed, since an identical pattern of thiophosphorylation is found after preincubation with cAMP and Ca^{2+}, where microtubule translocation velocity remains high but swimming speed does not increase.

An increase in microtubule translocation velocity is likely to depend on parameters of the mechanochemical cycle of the dynein arm. Different treatments such as Triton and phosphorylation might alter the cycling parameters differently to produce the additive effects observed. It is likely that the relationship between microtubule translocation velocity by 22S dynein and axonemal beat frequency is direct, so that if no hydrodynamic complications exist, one would predict that swimming speed of paramecia would be directly related to microtubule translocation velocity. It seems probable that *in vivo*, 29-kDa phosphorylation is a major factor in the cAMP control of paramecium swimming speed. In essence, the effect of cAMP on swimming speed can be accounted for by a change in the mechanically coupled 22S dynein activity of the cilia via phosphorylation of the 29-kDa polypeptide. In this regard, the 29-kDa polypeptide could function as a regulatory light chain of 22S dynein. This defines one important factor in the complicated regulation of ciliary behavior in paramecium (12).

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1. Bonini, N. M., Gustin, M. C. & Nelson, D. L. (1986) *Cell Motil. Cytoskeleton* **6**, 256–272.
2. Bonini, N. M. & Nelson, D. L. (1988) *J. Cell Biol.* **106**, 1615–1623.
3. Lieberman, S. J., Hamasaki, T. & Satir, P. (1988) *Cell Motil. Cytoskeleton* **9**, 73–84.
4. Hamasaki, T., Murtaugh, T. J., Satir, B. H. & Satir, P. (1989) *Cell Motil. Cytoskeleton* **12**, 1–11.
5. Bonini, N. M. & Nelson, D. L. (1990) *J. Cell Sci.* **95**, 219–230.
6. Larsen, J., Barkalow, K., Hamasaki, T. & Satir, P. (1991) *J. Protozool.* **38**, 55–61.
7. Nasr, A. & Satir, P. (1985) *Anal. Biochem.* **151**, 97–108.
8. Vale, R. D. & Toyoshima, Y. Y. (1989) *J. Cell Biol.* **108**, 2327–2334.
9. Travis, S. M. & Nelson, D. L. (1988) *Biochim. Biophys. Acta* **966**, 73–83.
10. Tang, W.-J. Y., Bell, C. W., Sale, W. S. & Gibbons, I. R. (1982) *J. Biol. Chem.* **257**, 508–515.
11. Butler, T. M., Siegman, M. J., Vyas, T., Mooers, S. U., Narayan, S. R. & Owen, C. S. (1991) *Biophys. J.* **59**, 426a (abstr.).
12. Bonini, N. M., Evan, T. C., Miglietta, L. A. P. & Nelson, D. L. (1991) *Adv. Second Messenger Phosphoprotein Res.* **23**, 227–272.