Ciliary Beat Frequency Is Controlled by a Dynein Light Chain Phosphorylation

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ABSTRACT cAMP-dependent phosphorylation of a 29-kDa axonemal polypeptide (p29) increases the swimming speed of permeabilized *Paramecium* and in vitro translocation velocity of bovine brain microtubules over 22S dynein extracted from *Paramecium* axonemes. A quantitative relationship between microtubule translocation velocity and beat frequency is developed. We conclude that p29 acts as a regulatory light chain of outer arm dynein in the control of ciliary beat frequency.

In many cilia, beat frequency increases are produced when intracellular cAMP increases. In Triton-treated *Paramecium* swimming speed doubles when >30 μM cAMP is added to the reactivation medium. Hamasaki et al. (1991) have demonstrated that a 29-kDa axonemal polypeptide (p29) that copurifies with 22S outer arm dynein in *Paramecium* becomes phosphorylated (pp29) in a cAMP-dependent manner. When permeabilized *Paramecium* are treated with cAMP, high Ca^{2+} (pCa 4) prevents p29 phosphorylation. cAMP can be diluted out of the reactivation medium and the swimming speed will remain elevated, but only under conditions where phosphorylation of p29 occurs (Fig. 1 *A*).

A high salt extract of 22S dynein that contains p29 has been obtained (Barkalow et al., 1994). This p29 will rebind to 22S dynein specifically. A working hypothesis is that the phosphorylation state of p29 dictates the mechanochemical cycle parameters that translate into speed of sliding and therefore into ciliary beat frequency. This hypothesis has been tested using in vitro microtubule motility assays with three different protocols:

1. Axonemes have been treated with cAMP at pCa 7 versus 4. 22S dynein has then been extracted and used as a substratum for translocation assays. Bovine brain microtubules translocate about 40% faster when 2–10% pp29 is present versus controls (Fig. 1 *B*).
2. Untreated 22S dynein has been isolated and used as a translocation substratum. Then microtubules have been removed and a *Paramecium* protein kinase A (PKA; courtesy C. Walczak and D. Nelson, University of Wisconsin) added. A 50% increase in microtubule translocation rate is measured after treatment of the field of 22S dynein with PKA and cAMP. No increase is seen if PKA is omitted (Fig. 1 *C*).
3. 22S dynein has been isolated under conditions where p29 will rebind to about ⅓ of the molecules. Microtubule translocation rates of 22S dynein reconstituted with p29 versus pp29 have been compared. The latter rates are about 30% faster than the former (Fig. 1 *D*).

If microtubule translocation rate in vitro is considered equivalent to sliding velocity in a beating axoneme, a relationship between translocation rate (v_o) and beat frequency (Hz) can be developed. Where v_o is in μms^{-1}

$$v_o = 0.4 \text{ Hz} \qquad (1)$$

The increase in v_o by experimentally induced cAMP-dependent phosphorylation corresponds to a beat frequency increase for a threefold increase in stimulus. The percent of pp29 might vary over a much wider range in the living axoneme, corresponding to a linear frequency response over many orders of stimulus intensity for the organism.

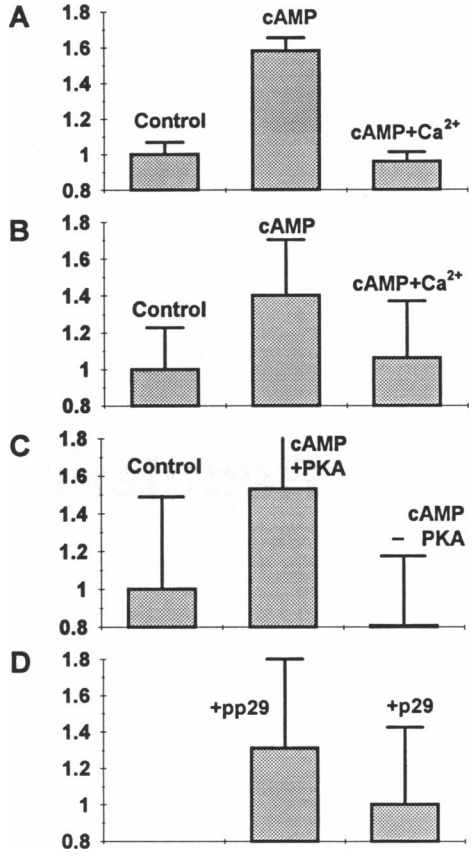

FIGURE 1 Phosphorylation of p29 increases swimming speed of permeabilized *Paramecium* and in vitro microtubule translocation velocity over 22S dynein substrata. (*A*) Average swimming speeds of permeabilized *Paramecium* populations. Normalized to untreated control (=1). cAMP: pretreated with 1 mM ATP-γ-S and 100 μM cAMP at pCa 7. cAMP removed by dilution before reactivation. In all panels increases shown in this column where pp29 is present are statistically significant versus controls. cAMP, Ca^{2+}: pretreated with 100 μM cAMP at pCa 4, removed before reactivation (see Hamasaki et al., 1991 for details). (*B–D*). In vitro translocation velocity over 22S dynein, variously treated (see Barkalow et al., 1994 for details).

REFERENCES

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