## 1. SUPPORTING DISCUSSION

### Regulation of myosin Va basal ATPase by $Ca^{2+}$

It appears that myosin Va basal ATPase rate is less regulated by $Ca^{2+}$ than the actin-activated ATPase rate. Single turnover experiments showed that the actin-activated ATPase rate is activated by $Ca^{2+}$ for ~100 times, whereas the basal ATPase rate is activated by $Ca^{2+}$ for ~10 times (1, 2). The major effect of the GTD binding is the 1000 times decrease in the Pi off (phosphate release) rate of acto-myosin Va ATPase reaction (1). On the other hand, the actin dependence of the Pi off step revealed that the affinity for actin is not decreased in EGTA (1). Therefore, a large decrease in Pi release rate in EGTA is not due to the change in the affinity for actin but the change in the communication between the actin binding and the Pi release from the active site. In other word, the actin binding does not effectively accelerate Pi release in the inhibited conformation.

The extent of regulation by $Ca^{2+}$ on the basal ATPase activity is less than that of the actin-activated ATPase activity, and one possibility to account for this result is that the head can go through all the required nucleotide states in the folded state. However, it is likely that the precise positions of converter/lever arm in various nucleotides of the triangular shape are not the same. Indeed, averaged images of negatively stained myosin Va molecules in various nucleotides are different [comparing Fig1a-e, h, and i, in ref. (3)]. We think that the GTD binding to the motor domain prevents the converter/ever-arm movement thus inhibiting the conformational changes of the active site and the product release (Pi off) even in the absence of actin. In the presence of $Ca^{2+}$, the actin binding effectively accelerates Pi release in the active form while it does not much accelerate the Pi release in the inhibited form (1). Thus, the extent of regulation is thus higher in the presence of actin than its absence.

## 2. SUPPORTING MATERIALS AND METHODS

### Calculation of the dissociation constant ($K_d$) of GST-GTD and M5HMM.

If concentration of M5HMM is much less than $K_d$, the concentration of free GST-GTD would be equal to the total concentration of GST-GTD, and $K_d$ can be obtained by a simple hyperbolic fit. If the concentration of M5HMM is similar in magnitude to the apparent $K_d$, significant portion of GST-GTD binds to M5HMM, and the concentration of free GST-GTD is significantly less than the total concentration of GST-GTD. In this case, $K_d$ can be obtained by a quadratic fit.

The interaction between M5HMM (R) and GST-GTD (L) is defined by scheme 1, where RL represents M5HMM/GST-GTD complex.

Scheme 1: $R + L \leftrightarrow RL$

The dissociation constant of M5HMM and GST-GTD ($K_d$) is obtained by eq 1

$$K_d = [R_{free}][L_{free}]/[RL] \quad (1)$$

Substituting $[R_{free}] = [R] - [RL]$ and $[L_{free}] = [L] - [RL]$ into eq 1 yields

$$K_d = ([R] - [RL])\,([L] - [RL])/[RL] \quad (2)$$

Eq 2 is rearranged to

$$[RL]^2 - ([R] + [L] + K_d)\,[RL] + [R][L] = 0 \quad (3)$$

[RL] is obtained by solving eq 3.

$$[RL] = ([R] + [L] + K_d - (([R] + [L] + K_d)^2 - 4\,[R][L])^{1/2})/2 \quad (4)$$

The ATPase activity of M5HMM in the presence of GST-GTD (V) is the sum of the activity of free M5HMM and M5HMM/GST-GTD complex and can be calculated by eq 5.

$$V = [R_{free}]\,k_{cat1} + [RL]\,k_{cat2} \quad (5)$$

The rate of free M5HMM ($k_{cat1}$) and the rate of M5HMM/GST-GTD complex ($k_{cat2}$) is defined by $V_{max}$, the activity of M5HMM in the absence of GST-GTD and $V_{min}$, the activity of M5HMM in the presence of saturated concentration of GST-GTD.

$$k_{cat1} = V_{max}/[R] \quad (6)$$

$$k_{cat2} = V_{min}/[R] \quad (7)$$

Substituting eqs 6 and 7 into eq 5 yields

$$V = V_{max}\,[R_{free}]/[R] + V_{min}\,[RL]/[R] \quad (8)$$

Substituting $[R_{free}] = [R] - [RL]$ into eq 8 yields

$$V = V_{max}\,([R] - [RL])/[R] + V_{min}\,[RL]/[R] \quad (9)$$

and rearranged to

$$V = V_{max} - (V_{max} - V_{min})[RL]/[R] \quad (10)$$

Substituting eq 4 into eq 10 yields

$$V = V_{max} - (V_{max} - V_{min})\,([R] + [L] + K_d - (([R] + [L] + K_d)^2 - 4\,[R][L])^{1/2})/2/[R] \quad (11)$$

and rearranged to

$$V = (V_{max} + V_{min})/2 - (V_{max} - V_{min})([L] + K_d - (([R] + [L] + K_d)^2 - 4[R][L])^{1/2})/[R]/2 \quad (12)$$

The ATPase activities (V) of M5HMM (R) in the presence of GST-GTD (L) were plotted and fit to eq 12 by non-linear least squares regression using the Kaleidagraph graphics program (Synergy Software, Reading, PA).

**Modeling the interaction between the GTD and the motor domain of myosin Va in the pre-power stroke conformation.**

This model was created by docking the GTD of Myo2p (2f6h.pdb) (4), a yeast myosin V, with scallop myosin II in the "pre-power stroke" conformation with an ATP analogue in the active site (1dfl.pdb) (5). The first 21 residues of scallop myosin II were deleted from the structures, since the homologous sequence is missing in myosin Va. P149 of scallop myosin, homolog of D136 of myosin Va, was mutated into D by using Swiss PDB viewer. The distances between the oxygen atom (Oδ) of D136 in the motor domain and nitrogen atom (Nη) of R272 and nitrogen atom (Nζ) of K343 in Myo2p-GTD (homolog residues of K1706 and K1779 in mouse myosin Va respectively) are ca 4 Å.

## 3. **SUPPORTING REFERENCES**


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