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LETTER TO THE EDITOR

In Vitro Movement of Actin Filaments Over Myosin: Role of Actin and Related Problems

In an elegant experimental study, Schwyter *et al.* (1990) have shown that "subtilisin cleavage of actin inhibits *in vitro* sliding movement of actin filaments over myosin". In this study, we were particularly interested by the question put by the authors: "an obvious question is whether these data can be reconciled with cross-bridge theories which invoke conformation change within myosin as the conformational engine driving muscle contraction". Prochniewicz & Yanagida (1990), in a more recent study based on the cross-linking of monomeric and polymeric actin, have drawn conclusions quite similar to those of Schwyter *et al.* (1990). For more than 4 years we have claimed that the *in vitro* movement is not governed by the same molecular mechanisms as muscle contraction (Morel & Bachouchi, 1988 *a*, *b*; Bachouchi & Morel, 1989; Morel, 1991; Morel & Bachouchi, 1991). In particular, Morel & Bachouchi (1991), in a comment on the experimental study of Takiguchi *et al.* (1990), conclude that "the traditional concepts of force generation cannot be considered as valid for explaining the *in vitro* movements. We also confirm that the molecular jet hypothesis is probably the basic process underlying these movements". In the same paper, Morel & Bachouchi (1991) keep their usual position: "the traditional concepts are unsuitable and must be replaced by other concepts". More precisely, Morel (1991) suggests that these concepts can explain only 11–15% of the isometric tension developed by a muscle fibre and that the swelling and electrostatic models are good alternative models for explaining the remaining 85–89% of the contractile force (see also Morel, 1985). In any case, we are now extremely sceptical as concerns the applicability of the usual concepts for the *in vitro* movement, as pointed out in our previous publications. We are encouraged to keep this position in the light of the work by Schwyter *et al.* (1990).

At this stage, it remains to be shown that the molecular jet hypothesis agrees with the experimental results of Schwyter *et al.* (1990). According to Morel & Bachouchi (1988*a*), the equation giving the velocity of an actin filament over myosin is given by:

$$\bar{V}=n^{1/2}\lambda^{1/2}R_0(m/f)(2Ne/m)^{1/2} \tag{1}$$

where m is the mass of the actin filament; e is the energy released from the hydrolysis of one ATP molecule; f is the frictional coefficient of the actin filaments. Since subtilisin cleavage of actin does not change the characteristics of the filaments (Schwyter *et al.*, 1990), these three parameters do not depend upon the fact that actin is cleaved or not. As clearly shown by Morel & Bachouchi (1988*b*), R_0 is not the rate of ATP splitting under steady-state conditions and at infinite actin concentration, but the rate at time zero (including the initial burst), just at the

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moment of fixation of the myosin heads on actin. N is the number of contacts of myosin with actin, which is an extremely important parameter (Takiguchi *et al.*, 1990; Morel & Bachouchi, 1991). n is a parameter accounting for the synchronicity of attachment of the myosin heads on actin: if all the heads attach at exactly the same moment, we have $n=1$; if all the heads attach at different moments, the maximum value of n is N. Finally, λ represents the proportion of energy released from ATP splitting which is converted into mechanical energy for the sliding of F-actin over myosin. In a first approach we can consider that λ would be independent of the integrity of actin. Therefore, in eqn (1), we have only three parameters which could be related to the nature of actin: n, R_0 and N. At this stage, we must recall an important finding by Uyeda *et al.* (1990). These authors have carried out their experiments in the presence of 0·8% methyle cellulose. They have observed that, between 0% and 0·8% methyle cellulose, the viscosity of the buffer increases from about 1 cP to about 100 cP, but that $\bar{V}$ is almost independent of the viscosity. Now, in eqn (1) here, $\bar{V}$ is proportional to $1/f$, i.e. $\bar{V}$ would be reduced by a factor 100 when the proportion of methyle cellulose reaches 0·8%. Such a conclusion is certainly too simplified. *First*, Morel & Bachouchi (1988*a*) and Morel & Bachouchi-Salhi (1991) have strongly suggested that the movement of the myosin heads is very asynchronous and that $n \approx N$, i.e. $\bar{V}$ depends upon N. As clearly suggested by Uyeda *et al.* (1990), the presence of methyle cellulose increases the value of N and we suggest that $N_{0\cdot8\%} \approx (10\text{–}20)\ N_{0\%}$. *Second*, the efficiency λ has been taken to be 1–5% by Morel & Bachouchi (1988*a*). Owing to the "compression" of the heads on F-actin (Uyeda *et al.*, 1990), λ could reach a higher value, say 50–60%, leading to $(\lambda_{0\cdot8\%}/\lambda_{0\%})^{1/2} \approx 3\text{–}8$. *Third*, as pointed out by Uyeda *et al.* (1990), the presence of methyle cellulose could modify the physical chemical properties of the myosin–actin system and we may suggest that $R_0^{0\cdot8\%} \approx 2R_0^{0\%}$. Under these conditions, $(N\lambda^{1/2}R_0)_{0\cdot8\%} \approx (60\text{–}320)(N\lambda^{1/2}R_0)_{\%}$: an increase by a factor 100 in f can be easily counterbalanced by an increase in $(N\lambda^{1/2}R_0)$ and, on the basis of the molecular jet hypothesis, $\bar{V}$ should be independent of the viscosity. Finally, note that Uyeda *et al.* (1990) have found that $\bar{V}$ increases when the density of heads increases, which corroborates the discussion presented by Morel & Bachouchi (1991).

In the experiments by Schwyter *et al.* (1990), it appears that it is not justified to try to correlate $\bar{V}$ to R_∞ (steady state) nor to R_∞^∞ (steady state; infinite actin concentration), since the true parameter is R_0. Therefore, the prerequisite is to study the correlation between R_0 and the percentage of cleaved actin in copolymers, by using, for example, the technique of Lymn & Taylor (1971). Schwyter *et al.* (1990; fig. 5) have shown that, below a proportion of ≈50% of cleaved actin in copolymers, the proportion and velocities of the mobile filaments remain unchanged, as compared to those of intact actin. Thus, the values of n, R_0 and N are probably insensitive to the percentage of cleaved actin under these conditions. Note that R_∞^∞ decreases by around 35% under the same conditions (Schwyter *et al.*, 1990; fig. 4) indicating consequently that R_∞^∞ is not related to the movement. Above 50% of cleaved actin, both the proportion of mobile filaments and the velocities of the filaments drastically decrease (Schwyter *et al.*, 1990; fig. 5). This decrease cannot be predicted by the variation of R_∞^∞ which decreases by only ≈25% for a proportion of cleaved actin increasing from

50 to 100%, while $\bar{V}$ decreases by $\approx$75% under the same conditions. In the absence of data on R_0, we may speculate that R_0 strongly decreases under these conditions. However, n and N may also modulate the dramatic decrease in $\bar{V}$. In fact, as pointed out by Schwyter *et al.* (1990), "intrafilament interactions in the copolymers" could reduce n, R_0 and N. The fact that the number of actin–myosin contacts N would be widely reduced is probably unrealistic, since the arrowhead structures are comparable in all cases (Schwyter *et al.*; 1990). The fact that n could drastically decrease (increase in the synchronicity of attachment of myosin to actin) is also doubtful. Therefore, we think that the major parameter involved is R_0 and that, above 50% of cleaved actin, R_0 is drastically reduced. Finally, it should be noted that, not only does $\bar{V}$ drastically decrease, but also the number of mobile filaments. We suggest that, for a given proportion of cleaved actin, say 70%, there exist 100% polymers, 70% polymers, 20% polymers, etc. In other words, all the copolymers are not strictly made up of 70% cleaved actin and 30% intact actin. For the 100% polymers, $\bar{V}$ is reduced by $\approx$75% and the surface tensions on the heavy meromyosin (HMM)-coated surfaces are probably sufficient to completely stop the movement. Only the copolymers containing, say $\approx$50% of cleaved actin, and which possess a higher $\bar{V}$, can move.

An example of the consequence of choosing R_∞^∞ or R_∞ instead of R_0 is given by a recent work (Uyeda *et al.*, 1990). By using the same notations as these authors (in the following, we always use the notations of the authors) except that V_0 is replaced by V_{max} (maximum speed of sliding of an actin filament over HMM), we have $d = V_{max} t_s$, where t_s is the time the head is strongly bound to actin and d, the displacement of an actin filament per one ATP hydrolysis. By calling R the turnover rate of a head and by using the experimental values of the above authors, we deduce from the formula of the authors $d(\text{nm}) = 50 V_{max}\ (\mu\text{m sec}^{-1})/R(\text{sec}^{-1})$. The authors have found $V_{max} = 7{\cdot}4 \pm 0{\cdot}4\ \mu\text{m sec}^{-1}$. They have taken $R = R_\infty^\infty = 13 \pm 1\ \text{sec}^{-1}$, leading consequently to $d = 28 \pm 4$ nm. Now, Ferenczi *et al.* (1985) have found, *in situ*, $R_\infty = 1{\cdot}9\ \text{sec}^{-1}$, leading to $d = 195 \pm 10$ nm, which is obviously meaningless. On the other hand, we have shown above that it is necessary to take $R = R_0 = 100\ \text{sec}^{-1}$ (Morel & Bachouchi, 1988*b*), leading to $d = 3{\cdot}7 \pm 0{\cdot}2$ nm, which is an extremely low and meaningless value. By using another approach, Toyoshima *et al.* (1990) have found an upper limit of $d = 8 \pm 2$ nm. In their calculations, the authors have taken an average ATPase activity/actin filament $\sum R_\infty = (l/L)V \geq 596\ \text{sec}^{-1}$ (we use the same notations as the authors, i.e. l is the length of an actin filament, L the total length of actin in the flow cell and V the total ATPase activity). Now, Morel (1984) has shown that the spacing between two consecutive binding sites on actin is $\sigma = 6$ nm. Therefore, we have $R_\infty = \sum R_\infty(\sigma/l) = (\sigma/L)V = 24 \pm 4\ \text{sec}^{-1}$. By taking $n_{av} = 1$ (n_{av} is the average number of heads bound to a minimum length actin filament) as the above authors, this means that they have taken $R = R_\infty = 24 \pm 4\ \text{sec}^{-1}$. On the other hand, they have found $V_{max} = 4{\cdot}6 \pm 0{\cdot}4\ \mu\text{m sec}^{-1}$. By once more replacing $24 \pm 4\ \text{sec}^{-1}$ by $1{\cdot}9\ \text{sec}^{-1}$, we obtain $d = 101 \pm 9$ nm, which remains meaningless. By taking $R = 100\ \text{sec}^{-1}$, we get $d = 1{\cdot}9 \pm 0{\cdot}2$ nm, which is close to zero. Another experimental study has been published by Harada *et al.* (1990). By using $\sum R_\infty = 44 \pm 6\ \text{sec}^{-1}$, these authors have found $d > 156 \pm 12$ nm. Their value of $\sum R_\infty$ corresponds to $R_\infty = \sum R_\infty\ (\sigma/l_{min}) = (44 \pm 6)(6/40) = 6{\cdot}6 \pm 0{\cdot}9\ \text{sec}^{-1}$ per myosin head (l_{min} is the

minimum length of moving actin filaments), in agreement with 7·3 sec^{-1} (Harada *et al.*, 1990). By replacing R_∞ by $R_0 = 100\ sec^{-1}$, we find $\sum R_\infty = R_0\ (l_{min}/\sigma) = 667\ sec^{-1}$. By merely keeping the formula and assumptions of Harada *et al.* (1990), we deduce $d = (1/k_1 - 1(k_{ON} - \Delta t)\ v$ (v = sliding velocity = 5·5 μm sec^{-1}; $\Delta t = 0{\cdot}01$ sec; $k_1 = 667/N_{min}\ sec^{-1}$; $k_{ON} = 280/N_{min}\ sec^{-1}$, with $N_{min} > 2$), i.e. $d > 11\,000\ (1/667 - 1/280) - 55 = -78$ nm, which gives no indication on the value of d. By taking $R_\infty = 1{\cdot}9\ sec^{-1}$, we get $\sum R_\infty = 12{\cdot}7\ sec^{-1}$ and $d > 3630$ nm, which remains meaningless. In some extremely elegant experimental work, Ishijima *et al.* (1991) have studied the force fluctuations of actomyosin *in vitro*. By using their notations once more, they have found $k_+ + k_- = 32\ sec^{-1}$ and $(1/k_+ + 1/k_-) = 1/R$, from which we deduce, for example, $k_+ = 16\ [1 - (1 - R/8)^{1/2}]$. By taking $R = 6{\cdot}0\ sec^{-1}$, these authors find suitable values of k_+ and k_-. However, here again $R = 6{\cdot}0\ sec^{-1}$ corresponds, near the isometric conditions, to the steady-state value. The authors analyse fluctuations of the forces and we believe that all the transitory phenomena should be included in the reasoning, especially the initial burst. Under these conditions the value of R to be chosen is 100 sec^{-1}, in which case k_+ and k_- have no physical meaning. At this juncture we shall not discuss the force fluctuation during sliding. In fact, we consider that there is a serious flaw when the usual theory of force generation is used: the results obtained by Ishijima *et al.* (1991) are incompatible with the initial burst in the ATPase activity of actomyosin. In the same issue of *Nature*, Uyeda *et al.* (1991*b*) elegantly develop their abstract (Uyeda *et al.*, 1991*a*). As already pointed out by Morel & Bachouchi-Salhi (1991), the "quantized velocities" they have observed are fully compatible with the molecular jet hypothesis (Morel & Bachouchi, 1988*a*). In this context, the fact that the sliding velocity does not depend upon the F-actin filament length is compatible with the molecular jet hypothesis (Morel, 1990). As concerns the stepsize, the authors have suggested the following formula: $d(\text{nm}) = 500\ V_u(\mu m\ sec^{-1})/R(sec^{-1})$, where V_u is the "unitary velocity" (0·33 μm sec^{-1}). By taking $R = 31\ sec^{-1}$ (i.e. $t_c = 32$ msec) they found $d = 5$ nm. Here again, the transitory phenomena are important, and R should be taken as 100 sec^{-1}, leading consequently to $d = 1{\cdot}6$ nm, which is once more close to zero. An upper limit of $d = 20$ nm has been suggested by the authors, which becomes $d = 6{\cdot}4$ nm ($R = 100\ sec^{-1}$) and which is the only valid value of d of this series, although it is lower than the theoretical value of 12 nm. We conclude that the choice of the value of R is a central point and that, according to this choice, it is possible to obtain contradictory results. Although the experimental work is illuminating, one must be extremely dubious as concerns the quantitative results. Most "traditional" workers have assumed that the ATPase rates determined under conditions of actin mobility is supposed to be identical to those used to determine the velocity of sliding in muscle: this assumption is unwarranted because during sliding *in vivo*, as well as *in vitro*, many transient phenomena must be accounted for. For example V_{max} *in vivo* is ≈4–5 μm sec^{-1}/sarcomere, i.e. 2–2·5 μm sec^{-1}/half sarcomere. Now the amount of shortening *in vivo* is usually ≈10–20% of the muscle length, which means that the duration of shortening is ≈50 msec, which is of the order of magnitude of the duration of the initial burst *in vitro* and also most likely *in vivo*: we confirm that the true ATPase activity is close to R_0 or exactly R_0.

In conclusion, we have shown once more (see Morel & Bachouchi, 1988*b*) that the velocity of the *in vitro* movement of actin over myosin depends upon the rate R_0 of ATP splitting at time zero (moment of attachment of the head on actin), not on the steady-state rate R_∞. In light of the most recent experimental data, we have chosen to comment on the molecular jet hypothesis. However, it would be interesting for the supporters of the traditional models to try to analyse their models by reasoning with the true parameters (for example, R_0, not R_∞). However, a stumbling block would be that the traditional concepts cannot predict the dependence of $\bar{V}$ upon N (see Morel & Bachouchi, 1991). In any case, we do not agree with the opinion of Irving (1991), according to whom "Direct observation of force fluctuations at the piconewton level and of velocity quantization provide dramatic and powerful support for the conventional view of a motor which attaches to actin, executes a power stroke, and detaches". The situation is certainly more complex and we regret that the different authors try to confirm to dogma by performing calculations based, for instance, on incorrect ATPase turnover rates.

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