cytoskeleton. Cortical filaments, which are linked to the substrate (for example via integrins or other adhesion molecules) might be the most stable. Further, such filaments would not be expected to move with respect to the substrate.

More work remains to be done to determine whether there are different populations of actin filaments distinguishable by their stability or their mobility relative to the cell or substrate. The existence of these hypothetical subpopulations of actin filaments is clearly compatible with functions of myosin in addition to those that are considered by Theriot and Mitchison, and with models of cell motility other than the nucleation-release model.

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THERIOT AND MITCHISON REPLY — The possibility that two (or more) populations of actin exist in the lamellipodium with very different dynamics was certainly of concern to us. We decided against discussing that possibility mainly because of lack of space, and because we saw nothing in our results that required the existence of a second population of filaments. Specifically, we never saw a broadening of the activated bar, even at 5- or 10-second time points, which would be strongly predicted if a moving population of filaments coexisted with the stationary population. In addition, we saw no reproducible deviation of the intensity versus time turnover curves from exponential, even at early time points. If a large population of filaments existed with shorter average lifetimes, we should have seen biphasic decays. But of course these arguments are certainly far from conclusive.

In our experiments[1], the cells were cooled to 15 °C, in part because preliminary experiments at 23–25 °C had indicated very short bar half-lives, probably closer to 5 or 10 seconds than the 25-second half-lives we saw at 15 °C. This temperature difference might in part explain the quantitative difference in our results.

It seems, though, that even if a second population of filaments exist, whose average lifetime is of the order of 5 seconds rather than 25 seconds, the basic requirements of our nucleation-release model that the filaments be short relative to the dimensions of the lamellipodium and that they be both polymerizing and depolymerizing throughout the entire structure are indicated even more strongly. The spatial movements of this hypothetical second network are of course a separate and more interesting issue.

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Channel control

SIR — Hartzell *et al.*[1] concluded that a membrane-delimited pathway for modulation of calcium channels by the stimulatory guanine nucleotide-binding protein G_s does not function in amphibian and mammalian cardiomyocytes. Because their conclusion regarding mammalian cardiomyocytes differs from ours[2] and others[3,4], we believe that a pivotal experiment presented by Hartzell *et al.*[1] in their Fig. 2*d* may have been misinterpreted.

Isoprenaline (ISO, 1 μM) quickly increased rat myocyte calcium current (I_{Ca}) from about 1.5 to about 2.85 nA, and dialysis of 5 mM adenosine 3′,5′-(Rp)-phosphothioate (Rp-cAMP-S, an inhibitor of protein kinase A (PKA)) abolished the stimulation over the next 20 min. The authors drew a straight line between the pre-ISO values (about 1.5 nA) and the late Rp-cAMP-S values (about 0.9 nA), and attributed the difference to I_{Ca} rundown. They said: "Assuming that I_{Ca} rundown occured at a constant rate during an experiment, 1 μM ISO increased I_{Ca} in rat 127% ± 13% and 5 mM Rp-cAMP-S decreased the ISO-stimulated current 117% ± 9% (slightly below the control level, n=7)".

The wording is ambiguous, but Fig. 2*d* shows that Rp-cAMP-S after isoprenaline restores I_{Ca} to (rundown-compensated) control amplitude. With regard to rundown compensation, we feel that the linear interpolation between pre-isoprenaline and late Rp-cAMP-S I_{Ca} amplitudes (20 min elapsed time; Fig. 2*d* in ref. 1) is an unsupported assumption. However, giving the authors the benefit of the doubt, the reduction of isoprenaline-stimulated I_{Ca} to control level by Rp-cAMP-S actually supports our earlier conclusion.

Our reasoning is based on two series of unpublished experiments related to our studies[2] on guinea pig ventricular cardiomyocytes. In the first of these (dual-pipette method), pressure-assisted test dialysis of control solution containing 1 mM Rp-cAMP-S was preceded by dialysis with control solution (5 mM ATP, 1 mM GTP, and so on; see ref. 2 for technical details). Rp-cAMP-S reduced basal (control) I_{Ca} by 27 and 32% within 2 min (n=2). In the second series (single-pipette method), I_{Ca} after 10 min dialysis with control solution containing Rp-cAMP-S was 25–40% (n=4) smaller than after 10-min control dialysis (n=8).

Thus, it appears that Rp-cAMP-S, like heat-stable inhibitor protein and regulatory subunit of PKA[5], blocks both isoprenaline-induced and basal phosphorylation and therefore should reduce isoprenaline-enhanced I_{Ca} below its basal pre-isoprenaline level. Because an analogous reduction was not observed by Hartzell *et al.*[1], it must have been offset by concomitant stimulation. With PKA blocked, the latter stimulation can be attributed to a non-cytoplasmic, GTP-dependent component of stimulatory isoprenaline action mediated by G_s (refs 2, 6).

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SIR — Because they did not observe a fast response to β-adrenergic stimulation in adult guinea-pig cardiac myocytes as we reported[7], Hartzell *et al.*[1] claim that the response does not exist. In our view, this is a version of the 'all swans are white' argument; the figure (over page) shows that isoproterenol (Iso) applied as a concentration jump during the slowly declining phase of the voltage-sensitive Ca^{2+} channel current produced a rapid increase in current (126 ± 11%, mean ± s.d., n=14). Both rate and magnitude of the increase were concentration-dependent[1]. The fast response has been observed in at least 53 experiments, 23 of which we described in ref. 7, and we are certain of its existence. The fast response was not observed under two conditions: when cells were leaky as the outward tail currents in Fig. 2*f* of ref. 1 indicate; and when the block of Ca^{2+} channel current by Cd^{+} was delayed (see figure), unlike the rapid block that we required as a control[1]. Under these same two conditions the slow response, although attenuated, persisted. Thus, the fast response is not as robust as the slow response and perhaps this is why Hartzell *et al.* missed it. Another factor may be methodological differences. The perfusion system of Hartzell *et al.* is different from ours and the delay in response to acetylcholine is much longer