Electronic Supplementary Material (ESI) for Integrative Biology

Supplementary Material

Intracellular photoactivation of caged-cGMP induces myosin II and actin responses in motile cells

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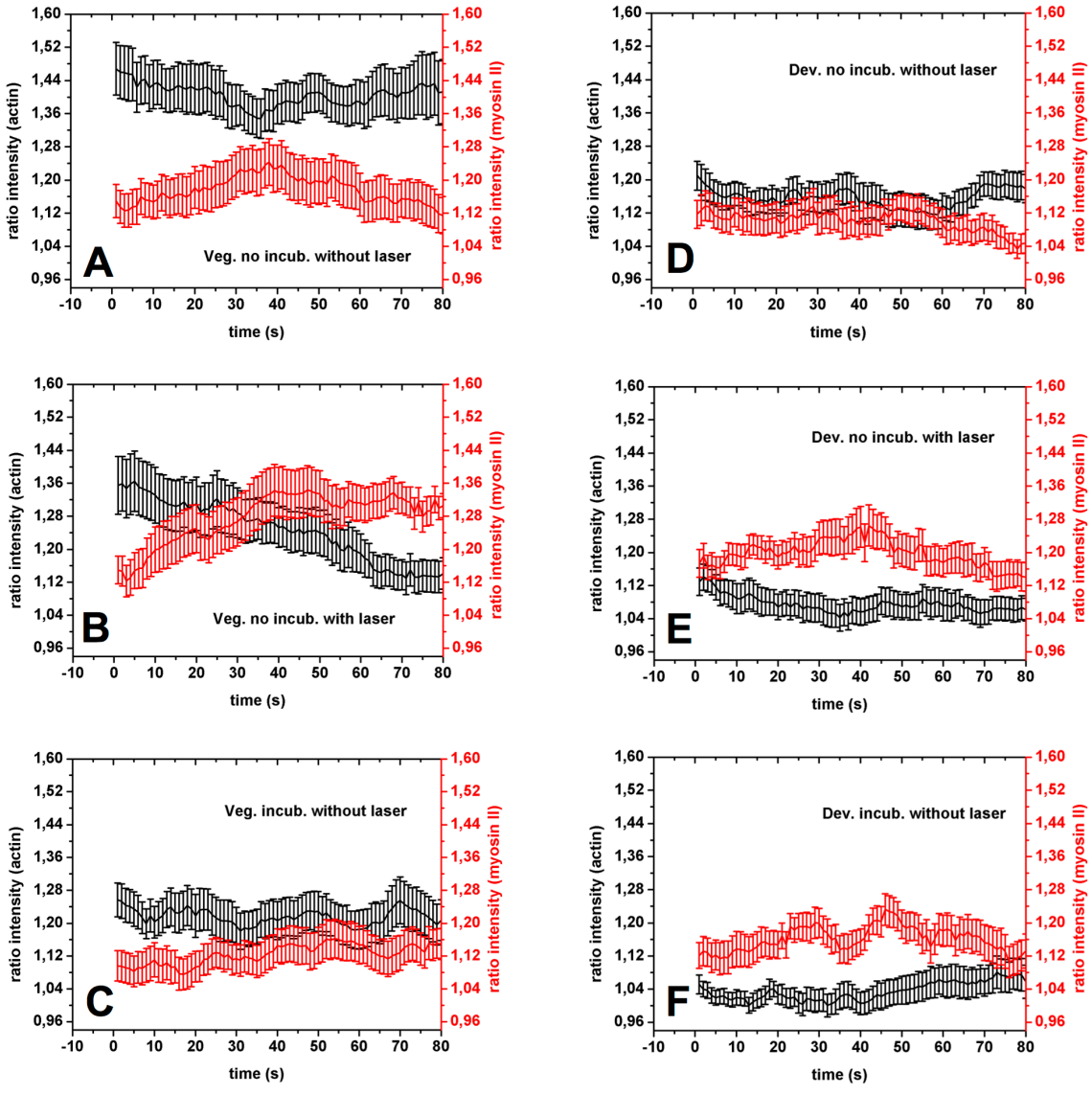
Photouncaging controls

To test whether the actin and myosin II responses observed were due to cellular photodamage caused by the uncaging laser, or due to the imaging process, several control experiments were performed. When cells were imaged in the absence of caged-cGMP and without exposure to the uncaging laser, neither actin nor myosin II responses were detected (Suppl. Figures 1A and D). Also, when cells were exposed to the 405nm laser, without incubation in caged-cGMP, we did not see a clear actin or myosin II response either (Suppl. Figures 1B and E). Finally, no response was observed in control cells that were incubated in caged-cGMP but not exposed to the photouncaging laser (Suppl. Figures 1C and F).



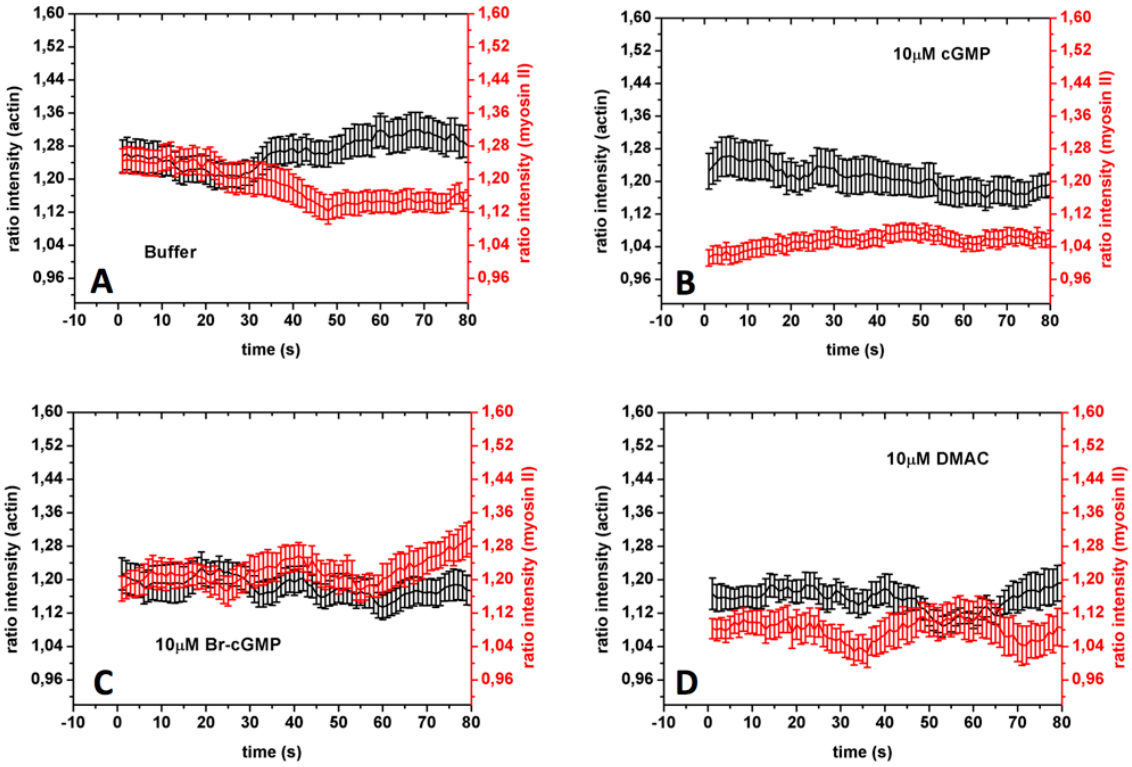
Supplementary Figure 1. Actin and myosin II control measurements of vegetative (A, B, and C), and starvation developed cells (D, E, and F). In A, vegetative cells were measured without caged-cGMP incubation and without the laser stimulation (n=23). In B, vegetative cells, not incubated in caged-cGMP were stimulated with the photouncaging laser pulse (n=25). In C, the cortical actin and myosin II localization in vegetative cells (n=22) incubated in 10µM caged-cGMP were quantified, without the photouncaging stimulation. In D, the cortical actin and myosin II localization of starvation developed cells (n=23 cells) not incubated in caged-cGMP and without photouncaging laser pulse were quantified. In E, cells were not incubated in caged-cGMP, but exposed to the photouncaging laser pulse (n=29). In F, cells were incubated in 10µM DMACM-caged-cGMP, but not exposed to the photouncaging laser (n=19). In all plots, the y axis on the left (black) shows the values for the actin curve, the y axis on the right (red) the values for myosin II. Also for all graphics, the error bars indicate the standard error.

Micropipette controls

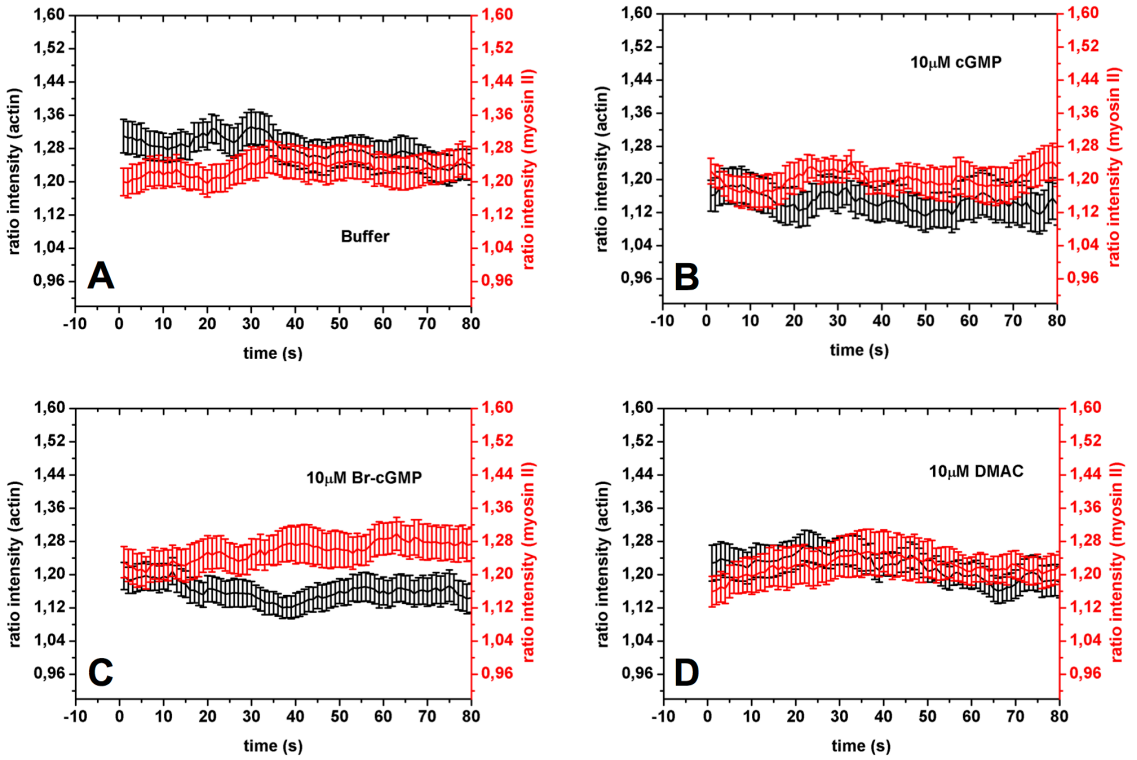
During the uncaging experiment, not only the cell but also the neighboring liquid is exposed to the light of the uncaging laser. Thus, Br-cGMP as well as the cage is released also in the medium outside the cell. Can extracellular cGMP or the extracellular DMACM-cage affect actin and myosin II dynamics inside the cells? This question is particularly relevant, keeping in mind, that pronounced responses could be observed also in cases where the cells are not directly hit by the uncaging laser. To test for extracellular effects of cGMP and the DMACM-cage, we exposed cells to either cGMP (Sigma-Aldrich, Taufkirchen, Germany), the membrane permeable equivalent Br-cGMP (Sigma-Aldrich, Taufkirchen, Germany), the caging molecule DMAC (7-Dimethylamino-4-methyl-coumarin) (Sigma-Aldrich, Taufkirchen, Germany), or Sørensen phosphate buffer (control), using the Femtojet micropipette aspiration device (Eppendorf, Hamburg, Germany). For this assay, a micropippete (Femtotip) was filled with a 10µM solution of either DMAC, cGMP, or Br-cGMP or with Sørensen buffer. Cells were plated, the micropipette was positioned next to a chosen cell, and imaging started, using an LSM 710 microscope with a PlnApo 63x/1.4 Oil DicIII objective. Images were acquired at a rate of one frame per second, and after 20s, the liquid was released from the pipette next to the cell for 10s, while imaging. Image analysis was performed as stated in the materials and methods section of the main text. Experiments were executed both with vegetative and developed cells.

When starvation developed cells were exposed to Sørensen buffer, no actin or myosin II response was observed (Supplemental figure 2A). Cells were as well exposed to cGMP and Br-cGMP (suppl. Figure 2B and C, respectively). No significant response was observed either. Note, however, that the increase in the myosin II response after 55s in figure 2C could be due to the slow entry of Br-cGMP (which is membrane permeable) into the cell. The same experiments were repeated for vegetative cells, with no actin or myosin II responses (supplemental figure 3A, B and C). At last, we checked whether the DMACM-cage itself is able to trigger actin or myosin II responses. The analog of the cage, DMAC was used, and the results are shown in supplementary figure 2D (developed cells) and 3D (vegetative cells). No significant actin or myosin II responses could be detected in these cases either.

Together, the results of the control experiments show that neither cGMP nor the DMACM-cage are responsible for the initiation of actin and myosin II responses from outside the cell. We can thus conclude that the actin and myosin II responses seen in the case of uncaging outside the cell (Fig. 3 in the main text) were indeed due to intracellular uncaging via stray light and the subsequent intracellular action of cGMP.



Supplementary Figure 2. To verify that the uncaging effect is due to photoactivation and action of cGMP inside and not outside of the cell, starvation developed cells were stimulated with buffer, cGMP, Br-cGMP or DMAC (analogue of the cage used in the uncaging experiments) using a micropipette (Femtojet, from Eppendorf, Hamburg, Germany). Following the stimulus, the actin and myosin II translocation was quantified. In A, cells were exposed for 10s to Sørensen Buffer flowing out of a Femtotip (Eppendorf, Hamburg, Germany). No evident actin or myosin II responses were detected (n=35). In B, cells were exposed for 10s to a 10µM solution of cGMP. No evident actin or myosin II responses were detected for this case either (n=24). In C, cells were exposed for 10s to a solution of 10µM Br-cGMP (membrane permeable cGMP). The increase starting around 55s could be due to Br-cGMP that entered the cell (n=20). In D, cells were exposed for 10s to a 10µM solution of DMAC. No evident actin or myosin II responses could be observed (n=15). In all plots, the y axis on the left (black) shows the values for the actin curve, the y axis on the right (red) the values for myosin II. Also for all graphics, the error bars indicate the standard error.



Supplementary Figure 3. Actin and myosin II control curves of vegetative cells, stimulated with buffer, cGMP, Br-cGMP, or DMAC (cage analogue). In A, cells (n=25) were exposed for 10s to Sørensen buffer only, flowing out of a Femtotip (Eppendor, Hamburg, Germany). No evident actin or myosin II response was detected. In B, cells (n=24) were exposed for 10s to a 10µM solution of cGMP. No significative actin or myosin II response could be observed either. In C, cells (n=22) were exposed for 10s to a solution containing 10µM Br-cGMP. No actin or myosin II responses were observed. In D, cells were exposed for 10s to 10µM DMAC. No significant actin or myosin II responses were observed in this case either. In all plots, the y axis on the left (black) shows the values for the actin curve, the y axis on the right (red) the values for myosin II. Also for all graphics, the error bars indicate the standard error.