

Localization of Cytoplasmic Myosin II in Ehrlich Ascites Tumor Cells

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Cells exposed to hypotonic or hypertonic media first swell or shrink, respectively, and then readjust their volume towards the normal steady-state through changes in ion transport mechanisms. It is well established that disruption of the F-actin filament system leads to an inhibition of volume recovery in cells exposed to these conditions. In cells exposed to hypertonic medium it has also been recently demonstrated that inhibition of myosin light chain kinase (MLCK) inhibits volume recovery through an inhibition of the ion transport processes, specifically the $\text{Na}^+, \text{K}^+, 2\text{Cl}^-$ transporter (1). MLCK phosphorylates myosin II and this leads to interaction between actin and myosin. Thus the mechanism involved in stimulation of ion transport in volume regulation appears to involve an active role for actin and myosin.

In order to pursue this question of a role of myosin II in hypertonic volume regulation we first determined the presence and distribution of myosin II in Ehrlich ascites tumor (EAT) cells, a well-established cell model for studies of the mechanism of volume regulation (1). We used a well-characterized monoclonal antibody against cytoplasmic myosin II and employed indirect immunocytochemical methods (2).

Myosin II was localized in two distinct areas within the EAT cell. A discontinuous band of fluorescence was seen closely associated with the cell membrane. This position corresponds to the position of the cortical F-actin seen in these cells. A second, relatively dense accumulation of myosin II was also localized to an area in the cytoplasm that corresponds to the position of the Golgi apparatus.

Treatment of EAT cells with cytochalasin, a fungal metabolite that disrupts F-actin, leads to the formation of membrane blebs. These membrane protrusions can be sheared off the cell and in this state they have an active, but unregulated $\text{Na}^+, \text{K}^+, 2\text{Cl}^-$ transporter. Exposure of the blebs to inhibitors of MLCK does not lead to inhibition of the transporter, as it does in intact cells. Immunocytochemical analysis shows that these blebs are devoid of myosin II.

In summary we have found that in EAT cells myosin II co-localizes with F-actin at a site close to the position of the $\text{Na}^+, \text{K}^+, 2\text{Cl}^-$ transporter. In addition, the lack of myosin in blebs, where the transporter is active and not altered by inhibition of MLCK, indicates that the actin-myosin filament system plays a role in the regulation of the activity of the transporters involved in volume regulation.

References:

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 2. Conrad, A.H., T. Jaffredo and G.W. Conrad. 1995. Differential localization of myosin isoforms A & B in avian interphase and dividing embryonic and immortalized cardiomyocytes and other cell types in vitro. *Cell Motil. Cytoskel.* 31:93-112.
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