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Rearrangements of Rho-family Small GTPases in Cardiac Fibroblasts Caused by Mechanical Stretch

John W. Fuseler, Wayne E. Carver, Purnima Jani and Clarke F. Millette

Department of Cell and Developmental Biology and Anatomy, University of South Carolina School of Medicine, 6439 Garners Ferry Road, Columbia, SC 29209 USA

Rho-family small GTPases are molecular switches activated by GTP. They control polymerization of actin and mediate many cellular events, including changes in shape resultant from mechanical force [1]. During normal functioning of the heart, cardiac fibroblasts undergo mechanical stress due to their attachment to the surrounding endomysium. In addition, alterations in the heart during hypertrophy must engender changes in the mechanical force placed upon cardiac fibroblasts. Recent data have shown that different stimuli applied to cardiac fibroblasts result in differential involvement of Rac and Rho [2,3]. Accordingly, we have investigated the localization of small GTPases in neonatal rat cardiac fibroblasts. Cells were subjected to 5% equi-axial stretch *in vitro* and assayed by confocal microscopy and morphometry to localize the small GTPases. Biochemical determinations of total small GTPase protein as well as total GTP-activated protein were also completed.

Cdc42 undergoes rearrangement soon after application of stretch. Initially localized as small granules seemingly aligned in the cytoplasm, stretch quickly (within 1-2 min) disrupts alignment and yields smaller particles. However, by 5 min, Cdc42 has reorganized into fewer, larger particles. Continued stretch then de-aggregates the larger particles to approximate conditions seen in quiescent cells [Fig 1]. Morphometry confirms changes in average granule size [Fig 2]. Biochemical determinations demonstrate no change in total Cdc42, but activated levels of Cdc42 increase in the initial 5 min of stretch before declining; in excellent correlation with morphological rearrangement of Cdc42 [Fig 3].

RhoA in quiescent cells is localized predominantly in the peri-nuclear cytoplasm, but within 2 min of stretch reorganizes into granules distributed relatively uniformly. Continued force for 10 min results in little further change, but after prolonged stretch (40 min) RhoA granules re-assume a peri-nuclear configuration. Up-regulation of active RhoA occurs within the first 5 min of stretch. Similar results are seen for Rac1, although morphometric measurements reveal significant differences in the average size and number of particles staining for each GTPase protein.

These data suggest that Rho-family GTPases are crucial mediators of cardiac fibroblast function and that both the ratio of GTP/GDP bound protein and the precise cytoplasmic distribution of the proteins are important. Future studies are planned to determine the possible role of direct molecular interactions between the individual Rho-family GTPases in modulating the morphological and physiological responses of cardiac fibroblasts to mechanical and chemical stimulation.

References

[1] K. Kaibuchi et al., Annu. Rev. Biochem 68 (1999) 459.
[2] M abe et al., J. Biol. Chem. 278 (2003) 47707.
[3] DJ Lee et al., Exptl Cell Res. 289 (2003) 86.

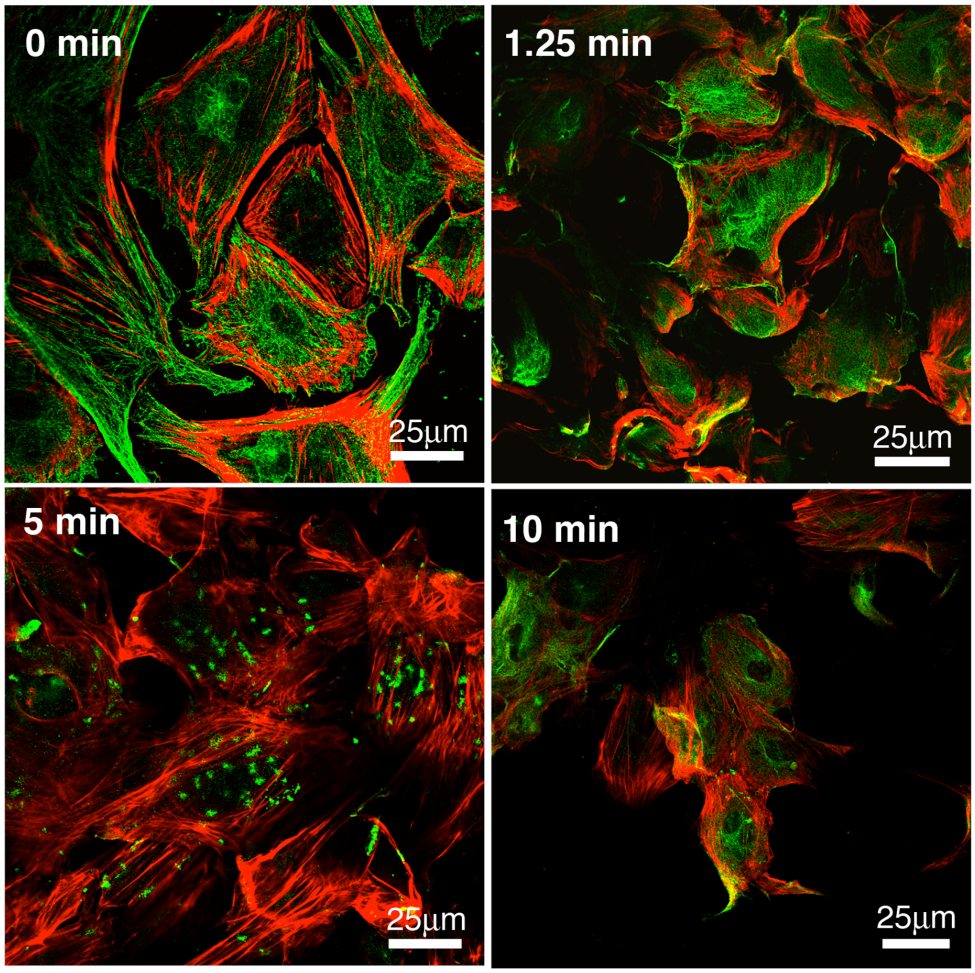

Figure 1: Neonatal rat cardiac fibroblasts under 5% equi-axial stretch, labeled for F-actin (red) and Cdc42 (green). Time of continuous stretch as indicated. Note the aggregation of Cdc42 at 5 min and the subsequent return to initial conditions.

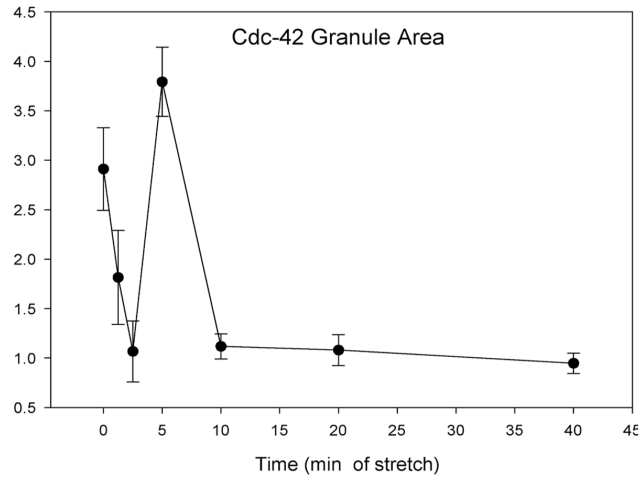

Figure 2: Size of Cdc42 particles as a function of stretch duration.

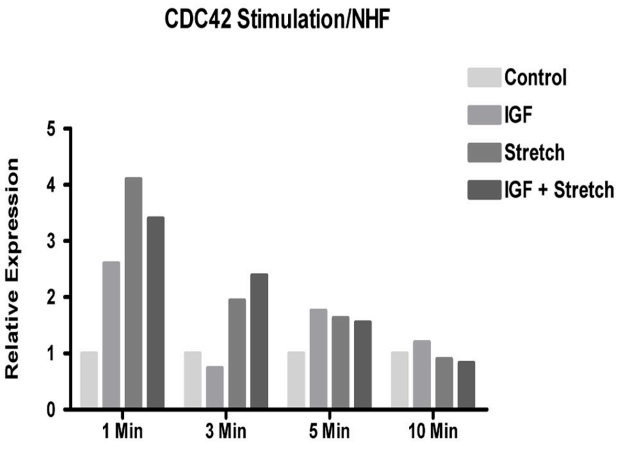

Figure 3: Activation of Cdc42 as a function of stretch duration.

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