

GENE INDUCTION BY CYCLIC SURFACE STRAIN: ROLE OF ACTIN CONTRACTILITY

A. Sarasa-Renedo, V. Tunç-Civelek, M. Chiquet

ITI Research Institute, University of Bern, Bern, Switzerland

INTRODUCTION: When cells are attached to an elastic surface, strain is transmitted to the cells via their extracellular matrix (ECM) adhesion sites. The resulting mechanical stress triggers intracellular signals which change the pattern of gene expression. We have shown previously^{1,2} that in chick fibroblasts cyclic tensile strain results in a twofold increase in the mRNA level for the ECM protein, tenascin-C, within six hours. This response is not affected by inhibitors for mitogen activated protein kinase (MAPK) pathways, but strongly attenuated by Y27632, a specific inhibitor for Rho-dependent kinase (ROCK)². Here we explored the function of the actin cytoskeleton and of RhoA/ROCK-controlled contractility in the induction of tenascin-C by cyclic tensile strain.

METHODS: Chick embryo fibroblasts were cultured on fibronectin coated silicone membranes and subjected to equibiaxial cyclic strain (10%, 0.3Hz) by means of a custom made device². Activators (lysophosphatidic acid [LPA], thrombin, colchicin) or inhibitors (Y27632, latrunculin A) of RhoA/ROCK mediated actin contraction were added 30 min prior to the application of cyclic strain for 6 hours. Tenascin-C and glyceraldehyde phosphate dehydrogenase (GAPDH) mRNAs were quantified from Northern blots.

RESULTS: We found that chemical activation of RhoA/ROCK by thrombin³ (Fig.1) or LPA is sufficient to double the amount of tenascin-C mRNA in resting fibroblasts within 6 hours. When cells were pretreated with these drugs, cyclic strain (6h) caused a super-induction (3.5-fold) of the tenascin-C mRNA level (Fig.1); the additional increase was suppressed by ROCK inhibitor Y27632. Microtubule disruption, which is known to trigger ROCK-dependent actin contraction, also induced tenascin-C mRNA¹. Conversely, disorganization of the actin cytoskeleton with latrunculin-A completely abolished induction of tenascin-C mRNA by either chemical RhoA/ROCK activators, mechanical stress, or both (Fig.1). Cyclic strain itself increased the amount of active RhoA in fibroblasts after 5 min as measured by a pulldown assay, and within 30 min triggered ROCK-dependent contraction of a collagen gel layer by the cells. Moreover, myosin II activity

was shown to be required for tenascin-C induction by mechanical stress.



Fig. 1: Northern blot with RNA from chick embryo fibroblasts seeded on a silicone membrane and either left at rest (R) or subjected to cyclic strain (10%, 0.3 Hz) for six hours (S). The blot was hybridized with radioactively labeled chick cDNA probes complementary to tenascin-C (TN-C) mRNA, and to GAPDH mRNA for control. Note that thrombin (Thr, 1U/ml) increased the tenascin-C mRNA in cells at rest, and synergistically enhanced its induction by cyclic strain. Latrunculin-A (LatrA) abolished the cyclic strain-dependent up-regulation of tenascin-C mRNA, as well as its thrombin-mediated super-induction.

DISCUSSION & CONCLUSIONS: From our results, we conclude that RhoA/ROCK-mediated contractility of the actin cytoskeleton has a mechanosensory function in fibroblasts that relates directly to the expression level of the tenascin-C gene. Furthermore, we suggest that prior activation of actin contraction, by either chemical or mechanical signals, renders fibroblasts more sensitive to further external mechanical stress. This principle might be important in connective tissue regeneration and wound healing.

REFERENCES: ¹ M. Chiquet, A. Sarasa-Renedo, F. Huber, M. Flück (2003) *Matrix Biol* **22**:73-80. ² M. Chiquet, A. Sarasa-Renedo, V. Tunç-Civelek (2004) *Biochim Biophys Acta* **1693**:193-204. ³ A. Sarasa-Renedo, M. Chiquet (2005) *Scand J Med Sci Sports* **15**:223-230.

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