ACTIN ORGANIZATION AND GENE EXPRESSION IN *BETA VULGARIS* SEEDLINGS UNDER CLINOROTATION

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ABSTRACT

Actin microfilaments (MFs) as highly dynamic structure respond by rapid reorganization to different external influences, including gravity. The object of our experiments was to examine both the actin organization and actin gene expression during growth and differentiation of root cells under clinorotation. It was shown that MFs acted as the indicator of changes caused by altered gravity in distal elongation zone (DEZ) cells, particularly actin cytoskeleton is enhanced in cortex cells. The data testify stable actin expression under altered gravity. The F-actin MFs enhancement in cortex cells of the DEZ occurred under clinorotation at the same level of the total actin content as in the stationary conditions is suggested to be caused by transformation of G-actin into F-actin.

1. INTRODUCTION

The cytoskeleton has been supposed to play a significant role in plant cell gravisensitivity [1]. A participation of MFs in gravirelated processes was shown both in cells specialized and non-specialized to gravity perception. Actin is characterized by multiplicity of molecular forms [2]. They express varying levels of proteins in different temporal and spatial patterns. The object of our experiments was to examine the organization of actin cytoskeleton during growth and differentiation of graviperceiving (root cap statocytes) and gravireacting (DEZ) cells as well as actin gene expression in seedling roots in the stationary conditions and under clinorotation.

2. MATERIAL AND METHODS

Beta vulgaris fruits were germinated and seedlings grew for 3 days in the stationary growth conditions and under slow horizontal clinorotation (2 rpm). Actin pattern was investigated by using a confocal microscope LSM 5 PASCAL after falloidin-FITC treatment, hybridization *in situ*, ELISA and Western-blot analysis after 1- and 2-dimentional denaturing polyacrylamide gel electrophoresis of total soluble proteins.

3. RESULTS AND DISCUSSION

The meristematic cells of a cap and a root proper of *B. vulgaris* seedlings possess the same organization of actin MFs in the control and under clinorotation. In cap meristematic cells, actin was found to surround the nucleus and radiate in a form of bundles to the cell periphery. The meristematic cells of a root proper are characterized by intensive actin bundles in the control and under clinorotation. We observed the fine endoplasmic actin network, but did not observe any cortical MFs in statocytes. Both in the control and under clinorotation, diffuse actin labeling was found to surround the statoliths and nucleus. There were less MFs radiating from the nuclear surface to the cell periphery under clinorotation than in control.

The actin cytoskeleton in epidermis and cortex cells of the DEZ consisted of radial MFs, connected with the cell wall and separate fragments with nonpreferential alignments in the control as it was determined in other species [3; 4]. Under clinorotation, the transverse network of cortical MFs was also observed in cortex cells of the DEZ. The presence of cortical actin is a distinctive feature of cells in the DEZ, which makes them different from statocytes. In the clinorotated roots, the more prominent network of cytoplasmic actin in the cortex cells of DEZ was shown (Fig. 1).

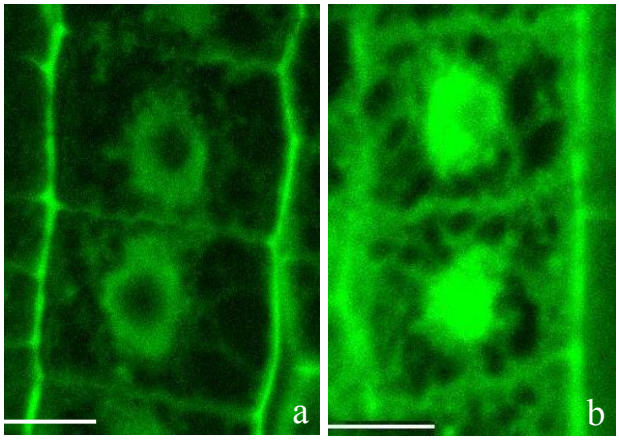

Fig. 1. Cortex cells of the DEZ of *Beta vulgaris* seedling roots grown in the stationary conditions (a) and under clinorotation (b). Bar 10 μm.

In addition to the thick bundles, thin, more fragile bundles of either longitudinally or irregularly oriented MFs were observed. There were an increased abundance and bundling of the perinuclear F-actin, bundles of different density radiate from nuclear surface to the cell periphery. So, actin MFs in cells of the DEZ act as an indicator of changes in the cytoskeleton caused by clinorotation: F-actin cytoskeleton is enhanced in cortex cells.

The data on actin gene expression in the control and clinorotated *B. vulgaris* seedling roots by using the method of hybridization *in situ* did not show differences in the level and localization of actin expression on the RNA transcription rate. The most intensive signal was in the cells of the first and second layers of the root cortex.

ELISA and Western-blot analysis after 1-dimentional electrophoresis did not reveal statistically trustworthy differences in the actin quantity between the variants. Western-blot analysis of actin after 2-dimentional electrophoresis revealed three isoforms (45 kD, pI 5,65, 5,80 and 5,95) (Fig. 2).

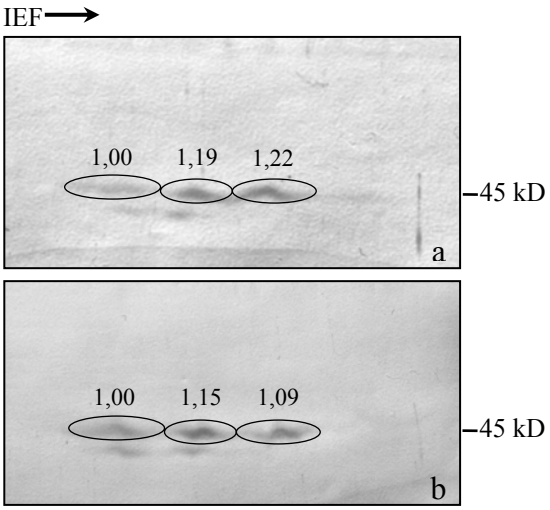

Fig. 2. Actin-specific immunoblots of 2-dimentional electrophoretically separated total protein of the roots of the control (a) and clinorotated (b) *B. vulgaris* seedlings. Relative protein quantity of the each isoform (to the first one) is printed in the brackets.

Expression of three isoforms in the control and experimental *B. vulgaris* seedlings might argue their functional necessity under the both normal conditions and clinorotation. Relative intensity of protein spots shows relative protein quantity in the each spot differs a little between the variants. This may indicate some changes in intensity of synthesis and destruction of the isoforms under the clinorotation and therefore their different functional loads. However, this assumption is needed further investigations.

It is known coexpression of multiple molecular forms of actin can take place in one organ, tissue or cell type [2, 5]. Differences in physicochemical properties of protein molecular forms result in different binding a substrate or cofactor and different interactions with other proteins etc. It has been suggested that coexpression of isoforms leads to extraordinary flexibility in the dynamic behavior of the cytoskeleton via polymerization of various actin monomers into heteropolymers and during the interaction of actin with numerous actin-binding proteins [2]. Apparently, such coexpression and interaction of isoforms lead to more robust and highly buffered responses of cells to an external influence.

The results obtained in the framework of this communication indicate that the visual F-actin cytoskeleton enhancement in cortex cells of the distal elongation zone occurred under clinorotation at the same level of the total actin content as in the stationary conditions is suggested to be caused by transformation of G-actin into F-actin. It is supposed these changes provide the cell growth stability in altered gravity and may be considered as an adaptive feature.

4. ACKNOWLEDGEMENTS

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5. REFERENCES

1. Kordyum E.L. A role for the cytoskeleton in plant cell gravisensitivity and Ca^{2+} - signaling in microgravity, *Cell Biol Int*, Vol. 27, 219-221, 2003.

2. Meagher R.B., et al. Isovariant dynamics expand and buffer the responses of complex systems: the diverse plant actin gene family, *Plant Cell*, Vol. 11, 995-1005, 1999.

3. Balushka F., et al. Rearrangements of F-actin arrays in growing cells of intact maize root apex tissues: a major developmental switch occurs in the postmitotic transition region, *Eur J Cell Biol*, Vol. 72, 113-121, 1997.

4. Blancaflor E.B. The cytoskeleton and gravitropism in higher plants. *J Plant Growth Regul,* Vol. 21, 120-136, 2002.

5. Schnabl H., et al. Effects of fast clinostat treatment and microgravity of *Vicia faba* L. mesophyll cell protoplast ubiquitin pools and actin isoforms, *Microgravity sci. technol*., IX/4, 275-280, 1996.