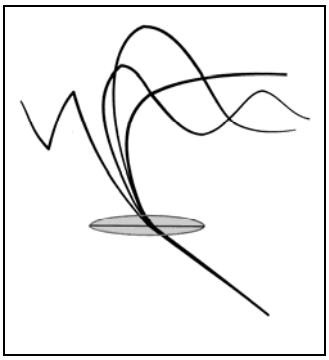


HRVATSKO MIKROSKOPIJSKO DRUŠTVO

POZIV NA 233. SASTANAK

Hrvatskog mikroskopijskog društva, koji će se održati u prostorijama
Instituta „Ruđer Bošković“, Bijenička cesta 54,
predavaonica **I. krila** (krilo Ivana Supeka)**,** u

utorak, 18. srpnja 2017. u 16:00 sati
u organizaciji Igora Webera s Instituta „Ruđer Bošković“

uz sljedeći

D n e v n i r e d:

1. **Izvještaj o skupu ELMI2017**

2. **Izlaganja stipendista HMD-a:**

Nikola Bijelić, MEF Osijek: Dinamika pojavnosti TFF1 i TFF3 peptida u probavnom sustavu mišjih zametaka od 14. do 18. dana intrauterinog razvoja

Edi Rođak, MEF Osijek: Nova i ekonomična metoda bojenja za brzi pregled parafinskih rezova u histološkom laboratoriju

Marko Šoštar, IRB: Visualization and analysis of small GTPases dynamics in highly motile cells

Kruno Vukušić, IRB: Mechanism of chromosome segregation in human cells revealed by laser microsurgery and photoactivation

3. **Kratak izvještaj o organizaciji MCM2017**

4. **Razno**

Tajnica:
Jelena Macan

Predsjednica:
Andreja Gajović

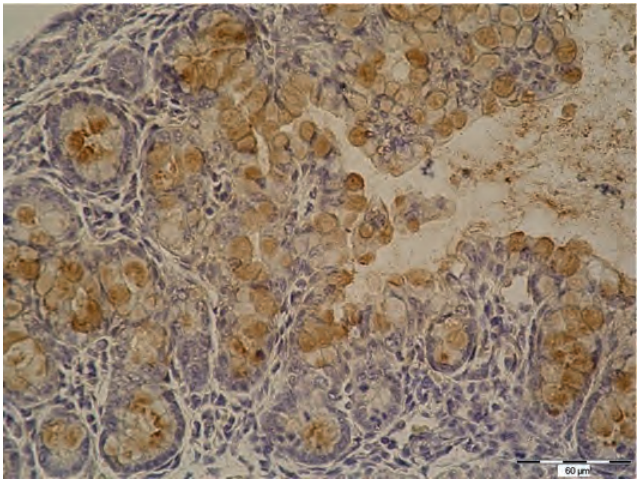
P2 The dynamic of Tff1 and Tff3 peptide presence in mouse embryonic gastrointestinal system from day 14 to 18 of intrauterine development

***Nikola Bijelić (1)**, Tatjana Belovari (1), Ivana Lovrić (1), Mirela Baus Lončar (1)*

1) Department of Histology and Embryology, Faculty of Medicine, University of Osijek, J. Huttlera 4, 31000 Osijek, Croatia

Keywords: trefoil factor, mouse, embryonic development, immunohistochemistry

Abstract: INTRODUCTION: Trefoil factor family (Tff) peptides are important for protection and restitution of gastrointestinal (GI) mucosa. They are found in a large part of the GI tract, from the oral cavity to the rectum, as well as in some glands associated with the GI tract. Endodermal to gastrointestinal epithelium differentiation in mice occurs during 15th or 16th day of intrauterine development. Tff peptides have been researched in this context to an extent, but no systemic immunohistochemical studies have been performed in order to describe the presence of these peptides depending on the developmental stage. The aim of this research was to describe the presence of Tff1 and Tff3 peptide in the GI mucosa from day 14 to 18 of mouse intrauterine development. MATERIALS AND METHODS: 4% paraformaldehyde fixed and paraffin-embedded CD1 mouse embryos at gestational days 14 to 18 were used. Sagittal sections 6µm thick were cut and transferred to adhesive slides. The embryos were stained with purified proprietary, self-made anti-Tff1 and anti-Tff3 rabbit primary polyclonal antibodies. Labeled Streptavidin Biotin (LSAB) method was used, with PBS as a negative control. Mayer's hematoxylin was used for counterstaining. RESULTS: The localization of Tff1 and Tff3 peptide was similar, but somewhat different from that of adult mice, with specific localization patterns for both peptides. Immunohistochemistry showed weak or no signal at 14-day stage and strenghthening of the



Caption: Strong TFF3 immunohistochemical staining in the mucosa of mouse colon at day 18 of embryonic development. Scale bar: 60 µm.

signal until day 18. Both peptides were found in stomach, small intestine, colon, liver and pancreas. Their localization and distribution depended on the developmental stage. CONCLUSION: Tff1 and Tff3 peptides can be viewed as markers of the GI mucosa maturation during embryonic development. They also might have an influence on the differentiation of endoderm into mature epithelium. Further research might confirm or deny that, as well as show the extent of their influence on embryonic development of GI system.

References: 1. W. Hoffmann et al. Histol. Histopathol. 16 (2001) 319-334. 2. G. Regalo et al. Cell. Mol. Life Sci. 62 (2005) 2910-2915. 3. T. Belovari et al. Bosn J Basic Med Sci. 15 (2015) 33-37.

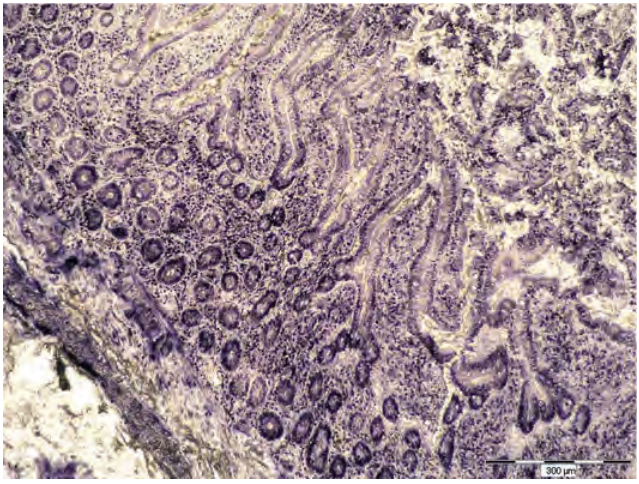
P3 A novel and economical staining method for quick identification and evaluation of FFPE sections in a histology laboratory

Edi Rođak (1), Ivana Lovrić (1), Nikola Bijelić (1), Tatjana Belovari (1)

1) Department of Histology and Embryology, Faculty of Medicine, University of Osijek, J Huttlera 4, 31000 Osijek, Croatia

Keywords: histology, staining and labeling, paraffin, haematoxylin

Abstract: Histological procedures can be a complex and lengthy process. Staining of formalin-fixed, paraffin-embedded (FFPE) sections requires a lot of intermediate steps in which the consumption of chemicals can be extensive and time-consuming. Furthermore, an unknown tissue sample may occur, or a quick look at the tissue structure may be required for further planning. In order to avoid the numerous intermediate steps, we developed a quick method for staining of FFPE sections that takes about 10 to 15 minutes overall. Human liver, skin and small intestine FFPE samples from our department's histological archive were used. 5µm sections were cut, placed on slides and dried for an hour on a thermal plate at 50 °C. Dry slides were left to cool down and 1.5 ml 3% (w/v) haematoxylin in 100% ethanol was added on slides using a micropipette. After 5 minutes, 1 ml of 2.3% iron (III) chloride solution was added to the already present haematoxylin solution. Mixed solution was left on slides for another 5 min, then slides were washed with dH2O. Slides were then immersed in same iron chloride solution to remove excess stain and rinsed with dH2O. The slides were examined and photographed within an hour after the staining was completed; no cover slip was placed on the tissue. Blue nuances of haematoxylin were predominant, and paraffin crystals were visible. Characteristic details could be identified in all three examined tissues (e.g. central veins, hepatocytes, portal triads; epidermis, hair



Caption: Strong TFF3 immunohistochemical staining in the mucosa of mouse colon at day 18 of embryonic development. Scale bar: 60 µm.

follicles, sebaceous glands; intestinal villi, crypts, goblet cells). Although the paraffin crystals distorted the image to an extent, the tissues could be easily identified, with characteristic tissue details visible enough for quick analysis. This method can be useful for quick identification or screening of FFPE samples. Apart from being fast, it is also valuable for economical reasons, since the usage of xylene, ethanol and other chemicals is reduced to minimum.

References: The authors wish to thank Ms Danica Matić for her invaluable help in the histology lab.

P50 Visualization and analysis of small GTPases dynamics in highly motile cells

Marko Šoštar (1), Maja Marinović (1), Vedrana Filić (1), Vlatka Antolović (1), Igor Weber (1)

1) Division of Molecular Biology, Ruđer Bošković Institute, Bijenička cesta 54, 10000 Zagreb, Croatia

Keywords: Rho GTPases, live cell imaging, FLIM, Rac1 dynamics, cell motility

Abstract: Small Rho GTPases play various important roles in the regulation of actin cytoskeleton dynamics in eukaryotic cells, including rapidly moving *Dictyostelium* amoebae. In contrast to other cell types, appropriate probes for monitoring the activity of many Rho GTPases in *Dictyostelium* are still lacking. Here we describe the use of a newly developed fluorescent probe for visualization of the active form of Rac1 GTPases in live *Dictyostelium* cells. The probe is based on the GTPase-binding domain (GBD) from DPAKa kinase fused with the yellow fluorescent protein (DYFP). The probe was selected on the basis of the yeast two-hybrid screen, GST pull-down assay, and FRET measurements in live cells by fluorescence lifetime imaging microscopy (FLIM). Images of cells expressing the DPAKa(GBD)-DYFP probe obtained with the laser scanning confocal microscope exhibit a high membrane-to-cytoplasm intensity ratio, which enables discerning fine details of Rac1 activity within the cell membrane. Importantly, due to its high contrast, the probe enables prolonged imaging of live cells with low-intensity excitation light, thus contributing to low levels of photobleaching and phototoxicity. Additionally, moderate expression of the DPAKa(GBD)-DYFP probe doesn't have any adverse effects on cell motility, cytokinesis and cell growth. The detailed information about the active Rac1 membrane distribution obtained by imaging of DPAKa(GBD)-DYFP will facilitate the quantitative analysis of the mechanisms that regulate the Rac1 dynamics in *Dictyostelium*.

Mechanism of chromosome segregation in human cells revealed by laser microsurgery and photoactivation

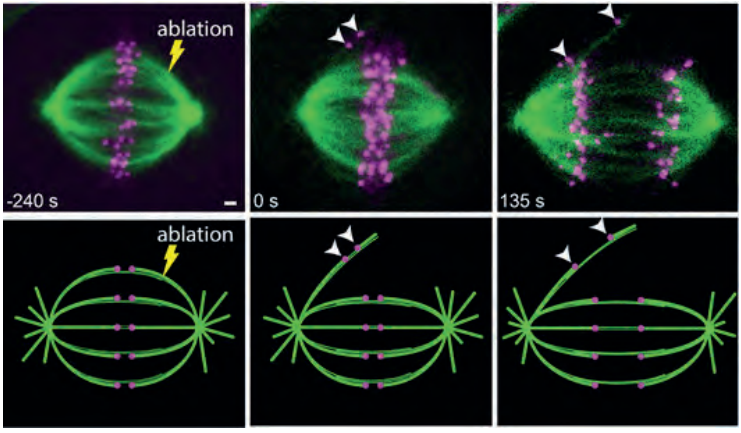
Kruno Vukušić (1), Renata Buđa (1), Agneza Bosilj (2), Ana Milas (1), Nenad Pavin (2), Iva M. Tolić (1)

1) Division of Molecular Biology, Ruđer Bošković Institute, Bijenička cesta 54, 10000 Zagreb, Croatia

2) Department of Physics, Faculty of Science, University of Zagreb, Bijenička cesta 32, 10000 Zagreb, Croatia

Keywords: mitotic spindle, anaphase, bridging microtubules, laser microsurgery, photoactivation

Abstract: During cell division, microtubules of the mitotic spindle segregate chromosomes by exerting forces on kinetochores, protein complexes on the chromosomes. The central question is what forces drive chromosome segregation. The current model for anaphase in human cells includes shortening of kinetochore fibers and separation of spindle poles. Both processes require kinetochores to be linked with the poles. Here we show, by combining live cell imaging, targeted laser microsurgery and photoactivation of human spindles (methods reviewed in Buđa et al., 2017) with theoretical modeling that kinetochores can separate without any attachment to one spindle pole. This separation requires the bridging fiber, which connects sister kinetochore fibers (Kajtez et al., 2016; Milas and Tolic, 2016). Bridging microtubules in intact spindles slide apart together with kinetochore fibers, indicating strong



Caption: Kinetochore segregation after laser ablation of kinetochore fiber. Kinetochores (arrowheads) displaced from spindle pole by laser ablation (lightning sign) separate during anaphase in manner similar to unperturbed kinetochores. Scale bar, 1μm.

crosslinks between them. Kinetochore segregation and pole separation are slower after depletion of MKLP1/KIF23 (kinesin-6), faster after depletion of KIF4A (kinesin-4), and unaffected by reduction of Eg5/KIF11 (kinesin-5) or KIF15/Hklp2 (kinesin-12). We conclude that motor-generated sliding in the bridging fibers drives pole separation and pushes kinetochore fibers poleward by the friction of passive crosslinks between these fibers. Thus, sliding in the bridging fiber works together with the shortening of kinetochore fibers to segregate chromosomes.

References: 1. R. Buđa et al., Methods Cell Biol. 139 (2017) 81-101. 2. J. Kajtez et al., Nat. Commun. 7 (2016) 10298. 3. A. Milas & I.M. Tolić Matters (Zür.) (2016) doi:10.19185/matters.201603000025. 4. This work is funded by the European Research Council (ERC).