



## In focus in HCB

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As we provide this monthly Editorial for May, we would like to begin by wishing all of our readers good health and well-being during this most unsettling time. The global reach of the SARS-CoV-2 pandemic is staggering, with positive tests found on all continents except Antarctica. Our fervent hope is that science will unlock the key to combatting this virus through the development of efficient pharmaceutical treatments and vaccines. In the past, microscopy certainly has provided much needed morphological and ultrastructural correlates of potential disease-causing organisms (see Rand and Taatjes 2018 for a couple of brief historical examples). Perhaps today's high resolution microscopy techniques, such as cryoelectron microscopy together with sophisticated image analysis algorithms, will play a role through providing clues towards understanding the virus structure at the molecular level, and its interaction with cell membranes.

As always, we hope that you enjoy this issue of Histochemistry and Cell Biology, and that reading through the articles may help provide a sense of normalcy to your day.

### SAF(B) interactions with a hormone receptor

Steroid hormones play many roles in brain function, including influencing health along a brain–body axis (McEwen 2020). The estrogen receptor  $\alpha$  (ER  $\alpha$ ), with a particularly strong presence in the hypothalamic area, is a nuclear transcription factor involved in regulating genomic functions. Scaffold attachment factor B (SAFB) serves as a corepressor for ER $\alpha$ , inhibiting its transcriptional activity. Since little is currently known concerning the functional significance of corepressors in the brain, Hashimoto and colleagues (2020)

have now provided a detailed description of the immunolocalization of SAFB1 and its paralog SAFB2 in rat brain, as well as its interaction with ER $\alpha$ . Their results showed (1) SAFB1 and SAFB2 were widely distributed throughout the brain regions in a very similar pattern; (2) both were also expressed in the nucleus by a variety of brain cell types including neurons, glial cells, astrocytes, and oligodendrocytes; (3) immunostaining on primary cultures of a variety of brain cell types confirmed the results obtained from rat brain with the exception that SAFB2 immunoreactivity was detected in both the nucleus and cytoplasm of the cultured cells; (4) double immunofluorescence staining demonstrated that both SAFB1 and SAFB2 were co-expressed with ER $\alpha$  in the nucleus of cells throughout the hypothalamus; and (5) co-precipitation experiments revealed that both SAFB1 and SAFB2 interact with ER $\alpha$  in the medial preoptic area of the hypothalamus. Taken together, their results provide new information regarding the distribution and function of the SAFB corepressors in regions of the brain containing steroid receptors.

### Keratin phosphorylation keeps mother and daughter centrioles together

Keratins represent a large and diverse family of intermediate filaments that are expressed in epithelia in a cell- and tissue-specific manner (Moll et al. 2008). Like other cytosolic proteins, they receive different post-translational modifications such as phosphorylation and *O*-GlcNAcylation at serine which are of functional importance (Hart et al. 2011; Yi et al. 2018). Previous studies on the functional implications of serine-52 and serine-33 phosphorylation of keratin 18 have demonstrated a role in cell cycle regulation and cell proliferation (Ku and Omary 1994; Ku et al. 2014; Schatten and Sun 2018). Now, Yu et al. (2020) have analyzed the subcellular distribution of phospho-Ser52 keratin 18 through the cell cycle of multiple cell types by applying various combinations of

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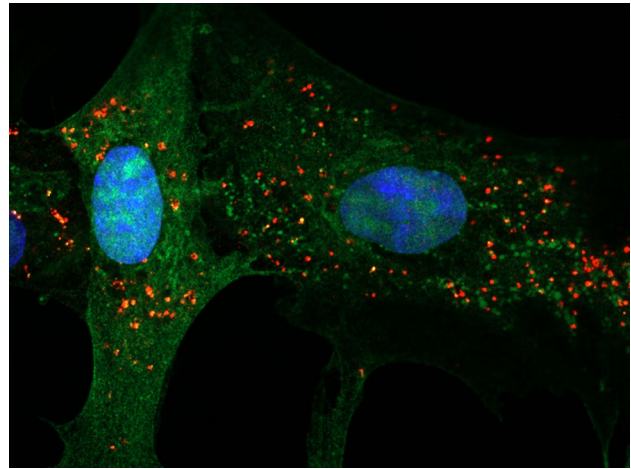
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double immunofluorescence, Western blotting and co-immunoprecipitation combined with Western blotting, and site directed mutagenesis of keratin 18 serine-52 by alanine. They found that phospho-Ser52 keratin 18 accumulated at mitosis and was associated with the spindle poles of centrosomes during metaphase. Replacing serine-52 by alanine prevented this interaction. Their additional detailed analyses demonstrated that phospho-Ser52 keratin 18 was located at the proximal end of mother centrioles and that it was involved in the regulation of the engagement of mother and daughter centrioles. In addition, evidence was presented that phospho-Ser52 keratin 18 is involved in microtubule nucleation.

### Illuminating peroxisomes in health and disease

Peroxisomes are ubiquitous and key metabolic organelles, and their biogenesis is complex (Islinger et al. 2018). Peroxisomal matrix proteins synthesized on cytosolic polysomes contain peroxisomal targeting signals (PTS) which are recognized by peroxins (PEX) that target them to the peroxisomal matrix. PTS1 consists of a conserved three amino acid sequence (ser-lys-leu) at the extreme C-terminus of most peroxisomal matrix proteins and is the target of PEX5. Mutations in the PEX genes result in peroxisome biogenesis disorders collectively named Zellweger spectrum disorders (Argyriou et al. 2016; Pavelka and Roth 2015). To evaluate peroxisome biogenesis *in vitro*, immunofluorescence for the detection of peroxisomal membrane and matrix proteins and transient peroxisomal fluorescent reporter transfections are used. To overcome various drawbacks inherent to these approaches, Demaret et al. (2020) have developed a third-generation lentiviral transfer plasmid expressing a fusion protein consisting of PTS1 and enhanced green fluorescent protein (eGFP-PTS1). This vector permitted the stable expression of the eGFP-PTS1 fusion protein in normal and Zellweger spectrum disorder mouse and human fibroblasts. By evaluating the eGFP-PTS1 signal alone, the authors demonstrated that the number of eGFP-positive particles and their average size was significantly lower in Zellweger spectrum disorder human fibroblasts as compared with normal fibroblasts. Through time-lapse live cell imaging analyzed and quantified by an automated tracking tool, the motility of peroxisomes in Zellweger spectrum disorder human fibroblasts was shown to be significantly slower than in normal fibroblasts. The authors also performed immunofluorescence for the peroxisomal membrane protein PMP70 in formaldehyde-fixed eGFP-PTS1 transduced cells (Fig. 1).



**Fig. 1** Immunofluorescence for PMP70 (red) in Zellweger spectrum disorder human fibroblasts stably expressing the eGFP-PTS1 fusion protein (green). As compared to normal human fibroblasts, the degree of colocalization is much lower. Nuclei stained with DAPI (blue). From Demaret et al. (2020)

The low degree of colocalization between the eGFP-PTS1 fluorescence and the PMP70 immunofluorescence confirmed the peroxisomal biogenesis disorder in the Zellweger spectrum disorder fibroblasts. By calculating Manders' overlap coefficient and Pearson's correlation coefficient, the authors demonstrated that this approach was as sensitive as the conventional double immunofluorescence detection of PMP70 and the matrix protein catalase. It was concluded that the developed plasmid represents a useful tool for the *in vitro* evaluation of peroxisome biogenesis and for drug screening for Zellweger spectrum disorders.

### Method to detect anti-tumor agents in paraffin-embedded cells and tissues

To assess the potential chemotherapeutic effect of anti-cancer treatments, it is important to determine with what efficiency the targeting agent reaches the intended tumor cells, since it is well known that the tumor microenvironment restricts access of many agents to the solid tumor (Nandigama et al. 2018). Traditionally, this assessment has been performed on fresh-frozen tissue samples, or on methanol-fixed cultured tumor cell lines. It would be most useful, however, if such evaluations could be made on the enormous cache of formalin-fixed paraffin-embedded (FFPE) blocks archived in hospitals and laboratories the world over. Böckleemann and colleagues (2020) now describe the detection of the chemotherapeutics cisplatin,

doxorubicin, and therapeutic antibodies in sections from FFPE blocks of multiple human tumor cell lines and tumor xenografts from drug-treated mice. Cell lines were treated either with cisplatin, doxorubicin, or therapeutic monoclonal antibodies, followed by standard FFPE. Human tumors grown in mice were likewise treated with chemotherapeutic agents and then subjected to FFPE. Immunohistochemistry was performed to evaluate the localization of cisplatin (with anti-Pt-[GpG] monoclonal antibody) and the therapeutic monoclonal antibodies, while the anthracycline doxorubicin was detected via its natural autofluorescence emission in the red wavelength range. The results on the cell lines demonstrated that the tested agents could be imaged by either conventional wide-field light or fluorescence (doxorubicin) microscopy in routine FFPE-processed samples. These results were validated by positive immunostaining observed in sections from the archival drug-treated tumor-bearing mice. The ability to detect chemotherapeutic agents in sections from FFPE tissues in addition to frozen sections, provides a whole new range of possibilities for re-examining and analyzing archived tumor blocks.

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## News from the Society for Histochemistry

# 16<sup>TH</sup> INTERNATIONAL CONGRESS OF HISTOCHEMISTRY AND CYTOCHEMISTRY

5 - 8 September  
**PRAGUE 2021**

Dear Colleagues,

In the light of the COVID-19 pandemic, the ICHC 2020 organizers and IFSHC Executive Council decided to postpone the ICHC 2020 to **5 - 8 September 2021**. The ICHC 2021 will take place as originally planned in the Cubex Centre, Prague, Czech Republic. The safety of all participants is our top priority. We are sorry for any inconvenience the postponement might have caused you.

The ICHC is held every four years under the auspices of the International Federation of Societies for Histochemistry and Cytochemistry (IFSHC), which continually strives to provide grounds for communication and cooperation among scientists all over the world in the areas of cyto- and histochemistry, cell and tissue biology, microscopy, pathology and other relevant fields.

The city of Prague, also known as the heart of Europe, provides easy access for scientists from all over the world. The congress venue, Cubex Centre Prague which offers technologically and visually unique space, promises to leave everyone with an unforgettable experience. Of course, Prague prides itself with its beautiful historical architecture, technical monuments, celebrated cafés, great food, and beer. This will be underlined by the ICHC gala dinner in the famous Art Nouveau Municipal House, and a free beer party organized in the premises of the Staropramen brewery.

We hope that you will join us in Prague to discuss together your latest achievements and that the venue will provide great opportunities for specialists at all levels of their career, bringing lots of opportunities for strengthening international collaborations. Special attention will be therefore given to the presentations of students. We also expect a rich commercial exhibition where new and emerging technologies will be presented.

We are delighted to inform you that the following speakers will present a lecture at the congress:

**Stefan Hell**, a Nobel Prize laureate, Max Planck Institute for Biophysical Chemistry, Germany (keynote speaker)

**Alev Erisir**, Department of Psychology, University of Virginia, USA

**Toyoshi Fujimoto**, Juntendo University, Nagoya, Japan

**Hans-Joachim Gabius**, Institute of Physiological Chemistry, Ludwig Maximilians University of Munich, Germany

**Bozena Kamińska**, Nencki Institute of Experimental Biology PAS Warszawa, Poland

**Takehiko Koji**, Department of Histology and Cell Biology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

**Ohad Medalia**, Department of Biochemistry, University of Zurich, Switzerland

See you all in Prague, September 2021!

Hinke Mulhaupt, President of the IFSHC  
Klara Weipoltshammer, President of the Society for Histochemistry  
Pavel Hozak, Chair of the Local Organizing Committee

### Contacts

We will keep the current domain:

[www.ichc2020.com](http://www.ichc2020.com)

If you have any questions about registration, please contact: [registration@ichc2020.com](mailto:registration@ichc2020.com)

If you have any questions about abstracts, please contact: [abstracts@ichc2020.com](mailto:abstracts@ichc2020.com)

Other inquiries and comments about the conference, please contact: [info@ichc2020.com](mailto:info@ichc2020.com)

## ANNOUNCEMENT

**The Society for Histochemistry**

Invites scientists to apply for the 2021 Robert Feulgen Prize. The prize is awarded for an outstanding achievement in the field of histochemistry.

The contributions may be either towards the development of new histochemical and cytochemical techniques or in the application of existing technology towards solving important problems in biology and/or medicine. Addressed are scientists working in microscopical sciences (in the widest sense) as well as in biochemistry, cell biology, endocrinology, in situ molecular techniques, and neurosciences. Scientists in their mid-career (assistant or associate professor, priv. doz.) are encouraged to apply. The prize is not intended for lifetime contributions.

The Prize consists of a monetary prize of €2,000

*All applications should be submitted before January 31, 2021 via the electronic submission system at: <https://www.greception.com/form-login-window/191a281d/>*

The application should contain a short curriculum vitae, a 1,000 word summary of the contributions of the applicant and PDF reprints of the pertinent publications. Full description of conditions is available on the Society website: [http://histochemistry.eu/description\\_of\\_conditions\\_.html](http://histochemistry.eu/description_of_conditions_.html)

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