In focus in HCB

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In this second Editorial of 2020, we provide a brief synopsis of three Original Papers nicely illustrating the breadth and variety of histochemical and cell biological investigations. We begin by highlighting a new one-step method for staining semithin sections from tissues prepared for transmission electron microscopy, and then cover detailed cell biological and high-resolution imaging approaches to investigate mechanisms involved in the trafficking of a cell surface receptor to the nucleus, and the organellar origin of the membrane of phagophores responsible for selective autophagy of insoluble cytoplasmic protein aggregates. We hope you enjoy reading about these highlighted studies, as well as the other interesting offerings in this issue.

One-step polychromatic staining of semithin sections of epoxy-embedded tissues

Stained semithin sections prepared from double aldehyde- and osmium-fixed and resin-embedded tissues and cells provide high structural resolution for light microscopy, and importantly the same specimens can be subsequently analyzed by transmission electron microscopy (Loussert Fonta and Humbel 2015). Among the various mono- and polychromatic staining protocols, relatively few do not require prior removal of the resin and osmium (Horobin 1983). Recently, Morikawa et al. (2018) reported the use of an alkaline alcoholic solution of azure B and basic fuchsin as a polychromatic stain for semithin sections of double aldehyde-fixed and epoxy-embedded tissues. Manskikh and Sheval (2020) report now a simple, one-step polychromatic staining

protocol using an aqueous alcoholic mixture of neutral red and fast green FCF, which is based on the histological stain introduced by Twort (1924). The adapted Twort's staining protocol was performed on semithin sections (0.25–0.5 µm thin) of glutaraldehyde- and osmium-fixed and epoxy resin-embedded tissues, and does not require a pretreatment to remove the epoxy resin and osmium. When directly compared with methylene blue-stained semithin sections, the neutral red and fast green FCF provided very high contrast due to the polychromatic staining of the different tissue elements (Fig. 1). The various cellular structures could be clearly distinguished since they were stained at greatly different intensities by neutral red. Of note, collagen and elastic fibers were solely stained by fast green FCF. The authors emphasize two aspects crucial for the proper functioning of the polychromatic stain: optimal osmium post-fixation and carefully chosen incubation time. As shown by the authors, the requirement for osmium fixation provided the means for

Fig. 1 Semithin section from glutaraldehyde- and osmium-fixed and epoxy-embedded mouse kidney. A glomerulus sectioned through the vascular and urinary pole together with adjacent proximal and distal tubules is shown. The high contrast provided by the polychromatic staining permits easy identification of the various tissue and cell components (from Manskikh and Sheval 2020)

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subsequent analysis by transmission electron microscopy, which may be an important aspect in surgical pathology.

Travel stories on a CD

The translocation of plasma membrane proteins and receptors to the cell nucleus to activate transcription factors is a well-known occurrence in multiple signaling cascades (see Dubovy et al. 2018 for a recent example). In this light, the hyaluron-binding surface receptor CD44 has also been shown to exhibit nuclear translocation via two different mechanisms. In the first, intact surface CD44 is proteolytically cleaved and processed to yield a surface ecto-domain and an intracellular domain fragment which then undergoes nuclear translocation (Nagano and Saya 2004). In the second mechanism, intact whole surface CD44 has been postulated to be internalized into the cytoplasm, followed by nuclear translocation of the unprocessed protein (Lee et al. 2009; Janiszewska et al. 2010). Thorne et al. (2020) have now performed experiments on several cultured cell lines aimed at determining whether they can provide supporting evidence for the nuclear translocation of intact surface CD44. They used multiple antibodies recognizing various domains of the CD44 protein in conjunction with siRNA gene editing techniques, western blotting, subcellular fractionation, and semi-quantitative high-resolution confocal microscopy. The results of these detailed experiments failed to find support for the translocation of intact CD44 protein from the cell surface to the nucleus. Rather, clear support for nuclear transport of the cleaved intracellular domain fragment of CD44 was provided. Moreover, the confocal microscopy imaging experiments combined with co-localization and line-scan intensity analyses demonstrated that nuclear localization of intact surface CD44 protein could be detected when a low-resolution 20× objective lens (0.75 NA) was used versus a high-resolution 63× (1.4 NA) objective. The authors caution that limitations in experimental imaging techniques may lead to erroneous conclusions concerning immunofluorescence localization of cellular molecules.

Waste removal in a not so rough neighborhood

Cells have developed multiple well-choreographed mechanisms, all falling under the general term “autophagy”, for removing unwanted cytoplasmic debris and organelles during normal cellular physiology, development and disease (Deng et al. 2018; Mizushima 2018; Offei et al. 2018). The materials for disposal may be selectively targeted to a unique internally formed cytoplasmic structure referred to as the “phagophore” via a variety of autophagy receptors. The origin and formation of the phagophore itself have been the subject of intense investigation, with various possibilities posited depending upon cell type and conditions examined. Damaged and misfolded insoluble cytoplasmic protein aggregates are removed from the cell by a special form of selective autophagy called “aggrephagy” (Metcalf et al. 2012). In earlier work, Roth and colleagues showed that the protein EDEM1, a component of the endoplasmic reticulum-associated protein quality control (ERAD), and RER-associated fibrinogen $\alpha\gamma$ upon exit from the rough endoplasmic reticulum (RER) form insoluble cytoplasmic aggregates which undergo subsequent autophagy (Zuber et al. 2007; LeFourn et al. 2013). This same group has now extended these earlier studies using multifuorescence confocal microscopy combined with serial section immunoelectron microscopy of fed HepG2 cells to determine which cytoplasmic organelle is involved in providing the membrane component of the phagophores utilized in selective autophagy of insoluble protein aggregates (Park et al. 2020). Their careful high-resolution imaging investigation revealed that in fed HepG2 cells, EDEM1 and fibrinogen $\alpha\gamma$ insoluble cytoplasmic protein aggregates were engulfed by smooth, ribosome-free subdomains of the RER. Importantly, using fed HepG2 cells, the authors were able to demonstrate that these “smooth” RER domains were structurally different from the RER-formed “omegasomes”, thought to provide the membrane component of phagophores in a starvation-induced autophagy model (Lamb et al. 2013). Their results add further structural proof to the contribution of the RER as the origin of the phagophore membrane in selective autophagy.

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News from the Society for Histochemistry

16TH INTERNATIONAL CONGRESS OF HISTOCHEMISTRY AND CYTOCHEMISTRY

Dear Colleagues,

On behalf of the board of the Society for Histochemistry, it is our great pleasure to invite you to the world 16th International Congress of Histochemistry and Cytochemistry (ICHC), to be held on August 30 – September 2, 2020, in Prague, Czech Republic.

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.

Looking forward to meeting you in Prague,

Klara Weipoltshammer, President of the SfH
Pavel Hozak, Chair of the Local Organizing Committee, Secretary of the SfH

Contacts

Home: www.ichc2020.com

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If you have any questions about abstracts, please contact: abstracts@ichc2020.com

Other inquiries and comments about the conference, please contact: info@ichc2020.com

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

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