

The *Histochem Cell Biol* conspectus: the year 2013 in review

Douglas J. Taatjes · Jürgen Roth

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Abstract Herein, we provide a brief synopsis of all manuscripts published in *Histochem Cell Biol* in the year 2013. For ease of reference, we have divided the manuscripts into the following categories: Advances in Methodologies; Molecules in Health and Disease; Organelles, Subcellular Structures and Compartments; Golgi Apparatus; Intermediate Filaments and Cytoskeleton; Connective Tissue and Extracellular Matrix; Autophagy; Stem Cells; Musculoskeletal System; Respiratory and Cardiovascular Systems; Gastrointestinal Tract; Central Nervous System; Peripheral Nervous System; Excretory Glands; Kidney and Urinary Bladder; and Male and Female Reproductive Systems. We hope that the readership will find this annual journal synopsis of value and serve as a quick, categorized reference guide for “state-of-the-art” manuscripts in the areas of histochemistry, immunohistochemistry, and cell biology.

Keywords Histochemistry · Cell biology · Light microscopy · Electron microscopy · Immunocytochemistry

Introduction

Histochem Cell Biol enjoyed a successful year in 2013, publishing manuscripts on a myriad of topics utilizing a variety of histochemical, immunohistochemical, and imaging techniques. Included in the year were three “In Focus”

special issues: In the July issue, several review manuscripts were published highlighting the role of intermediate filaments in health and disease, while the September and October issues were dedicated to reviews on the Golgi apparatus, signifying 115 years since the first description of this organelle by Camillo Golgi. Moreover, 2013 represented the 155th anniversary of Rudolph Virchow’s famous quote “omnis cellula e cellula” (cells come only from pre-existing cells), which expounded upon the original cell theory developed by Theodor Schwann and Matthias Schleiden in 1837–1838, stating that all living organisms consist of cells (Otis 2007). These two events represent bellweather moments in the development of the field of cell biology as we know it today.

In this conspectus, we provide a brief synopsis of each article published in *Histochem Cell Biol* for 2013. By sorting the manuscripts into broad topic areas from methods, to molecules, to organelles, to organ systems, we hope that this review will provide a “go-to” guide, serving as a quick reference for up-to-date literature in a given area of histochemistry and cell biology.

Advances in methodologies

Since its inception, *Histochem Cell Biol* has served at the forefront of publishing new and enhanced methods in cell biological research, and 2013 was no exception. Characterization of antibody specificity has emerged as an area of concern for immunohistochemistry over the past several years. Fan et al. (2013) investigated the specificity of a series of antibodies directed against resistin-like molecules (RELM), either purchased commercially or laboratory produced. Since the RELM family consists of four members in the mouse, and two in humans, it is of importance to

D. J. Taatjes (✉)
Department of Pathology and Microscopy Imaging Center,
College of Medicine, University of Vermont, 89 Beaumont
Avenue, Burlington, VT 05405, USA
e-mail: douglas.taatjes@uvm.edu

J. Roth
University of Zurich, 8091 Zurich, Switzerland

differentiate among the various isoforms. To test the specificity of the anti-RELM antibodies, they transfected HEK 293 cells with the various RELM isoforms and then performed Western blot analysis and immunocytochemistry. Not surprisingly, they found a degree of cross-reactivity among the antibodies for the various RELM isoforms. Moreover, not all antibodies that worked well for Western blotting could also be used for immunocytochemistry. The manuscript of Fan et al. (2013) serves once more as a cautionary tale regarding antibody characterization, showing that it is the responsibility of the investigator to provide details concerning the specificity of the antibody for the antigen in question.

Similarly, Kremser et al. (2013) developed antibodies specifically against the non-glycosylated and glycosylated forms of ceramide synthase 2 (CerS2) to investigate the expression of this enzyme in various cell types. Testing of the rabbit antibodies showed that they recognized the CerS2 protein in wild-type mouse tissues, but were unreactive with tissues from CerS2-deficient animals. In developing and adult mouse brain, the antibodies recognized CerS2 protein in oligodendrocytes, but not in neurons. These results contrast with earlier studies suggesting that CerS2 is expressed in brain neurons in addition to oligodendrocytes. In mouse liver, the antibodies stained hepatocytes, but not Kupfer or Ito cells. By immunoblot analysis, the authors also found that their new antibodies recognized CerS2 in mouse lung, spleen, and kidney, with much smaller amounts detected in skin, heart, and skeletal muscle. With these specific anti-CerS2 antibodies now available, studies to investigate correlation of phenotypes of CerS2-deficient mice with the loss of the protein are possible. This study once again demonstrates the requirement of employing well-characterized antibodies to posit unequivocal conclusions from antibody-based assays.

The isolation and purification of specific cell types from a tissue sample often requires antibody-based techniques to recognize and sort the targeted cell type. These methods can be costly and require the availability of specific antibodies. Grondona et al. (2013) have developed a method for the isolation and purification of ciliated ependymal cells from rodent brain. Starting with explants from the striatal and septal walls of the lateral ventricles, they developed an isolation procedure employing low incubation temperature in tandem with gentle enzymatic digestion. After 6 h of treatment, most of the ependymal cells have been removed from the ventricular wall, together with a small proportion (approximately 6 %) of contaminating cells; however, these contaminating non-ependymal cells can be removed by culturing the cells in a simple culture medium consisting of α -MEM with glucose (but no further supplements) at very low density. Following culture under these conditions for 48 h, only ependymal cells remained, which could then be

maintained for up to 7–10 days. By 7 days in culture, the ependymal cells begin to lose their characteristic ciliated feature, acquiring features of glial cells. Thus, this simplified purification procedure will allow *in vitro* investigations of adult ependymal cells.

Chemical fixation protocols employing aldehydes for cells and tissues were established in the 1960s, with many modifications introduced in the intervening years. In many instances, protocols have been established solely on an empirical basis, without rigorous experimentation. Such an instance occurs with choosing the lowest aldehyde concentration required for the complete chemical fixation of various intracellular structures. Zeng et al. (2013a) have now performed a rigorous evaluation of the lowest concentrations of glutaraldehyde alone, or in combination with paraformaldehyde required for complete fixation of the following human umbilical vein endothelial cell structures within 20 min of exposure: focal adhesions, cell surface particles, stress fibers, cell cortex, and inner structures. They used live-cell imaging with differential interference contrast microscopy on a confocal microscope, as well as the MTT assay to determine the effects of the different aldehyde concentrations on the various cellular compartments. They also investigated the effect of aldehyde concentration on the production of cell blebs. Although differing concentrations of aldehydes were required to fix the individual cellular components examined, the authors speculate that for complete fixation of a whole cell within 20 min, concentrations of at least 0.1 % glutaraldehyde and 1.5 % paraformaldehyde would be required; if longer fixation times are employed, these concentrations could likely be decreased.

Small-molecule fluorescent probes are commonly used in microscopy to visualize uptake and trafficking of specific molecules within live cells. Horobin et al. (2013) presented a timely review of various cellular uptake methods for fluorescent DNA and RNA probes. They reviewed the ability of quantitative structure activity relations (QSAR) models to predict processes of uptake and intracellular transport of fluorescent probes. The QSAR methods take into account the physicochemical properties of the probes via a numerical assessment together with localization data. Using QSAR, they analyzed various fluorescent probe uptake models, including: (1) adsorptive to lipid or protein domains, (2) endocytic phagocytosis and pinocytosis, and (3) passive diffusion. In the realm of intracellular trafficking of the probes, they discussed localization models for: (1) cytosol, (2) endoplasmic reticulum, (3) Golgi apparatus, (4) lipid droplets, (5) lysosomes, (6) mitochondria, (7) nucleus, and (8) plasma membrane. After discussing the QSAR modeling parameters, they presented a case study for the ability of the model to predict intracellular localization of eukaryotic chromatin DNA and ribosomal RNA in living cells, as well as for prokaryotic chromosomes. Their

most interesting review highlighted the fact that QSAR models can be most informative for predicting the behavior of fluorescent probes in live cells when the physicochemical features of the probe are known. The QSAR model may be employed to further the understanding of existing fluorescent probes and additionally to offer insights for the development of new probes for live-cell fluorescence imaging.

A common approach in multicolor fluorescence microscopy involves the colocalization analysis of two fluorophores. Such analysis has progressed from a simple qualitative assessment of color overlap, to a quantitative evaluation using mathematical parameters such as the Manders and Pearson coefficients. Obara et al. (2013) have expanded upon these analysis methods by introducing a bioimage informatics approach to perform quantitative colocalization analysis on blob-like structures in high-resolution three-dimensional fluorescence microscopy datasets from sparsely labeled molecules. This new technique involved two steps: (1) detection of bright isotropic structures representing immunostained human leukocyte antigen receptors in three dimensions in separate channels using confocal microscopy and (2) “pruning” of the data, followed by application of a matching procedure based upon centroid detection to reveal the colocalization distance between the fluorescence object in the different channels. Three-dimensional distance maps are constructed to determine how closely centroids from different spots colocalize, and histograms are then used to analyze colocalization distances. This new method provides a means for the creation of super-resolved object-based colocalization maps of isotropic particles, especially in instances of sparse colocalization.

DNA-binding dyes are often used in immunofluorescence experiments as a specific marker for nuclei, thereby providing a point of reference for cell architecture and the pattern of immunostaining. Among the many available fluorescent probes for DNA, 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) has found widespread use in immunofluorescence protocols due to its excitation by UV light, which can then be used in conjunction with other visible light-excited fluorophores for immunolabeling procedures. Indeed, some cell/tissue mounting media are available with DAPI as a component, providing ease of nuclear staining in the final slide mounting step. Jez et al. (2013) now report on a potential problem with DAPI staining resulting from photoconversion of DAPI yielding specific detection with a standard microscope fluorescence filter set for blue excitation and green emission. They found that initial imaging of a DAPI-stained sample with a standard green filter set (FITC/GFP) resulted in a weak cytoplasmic autofluorescence signal. Subsequent imaging with a DAPI filter set produced the expected intense nuclear staining;

however, re-imaging with the green filter set then resulted in a strong nuclear signal in the green channel. The authors propose that excitation of the DAPI stain with UV light results in a photoconversion of the dye, leading to excitation and emission with a standard green filter set. This phenomenon could pose a significant interpretation issue if the DAPI staining is combined with the immunofluorescence localization of a nuclear antigen with a blue excitation/green emission fluorophore. To avoid this issue, the authors propose: (1) using low concentrations of DAPI for staining; (2) acquire the DAPI images after all other fluorophores have been imaged; and (3) avoid mounting medium with high glycerol concentrations.

The technique of ribopuromylation (RPM) can be used to quantitatively analyze and visualize translating ribosomes in permeabilized and fixed cells using immunofluorescence. The RPM technique involves the puromylation of nascent chains bound to ribosomes undergoing translation, with subsequent identification of the chains with an anti-puromycin monoclonal antibody. David et al. (2013) now show that pretreatment of cells with the irreversible translation elongation inhibitor emetine results in increased nascent chain puromylation and thus an increase in immunofluorescence signal. Moreover, they developed a modified RPM protocol providing a maximum RPM fluorescence signal on inert cells permeabilized with digitonin.

Since their widespread introduction in the mid-2000s, super-resolution fluorescence microscopy techniques have initiated new avenues of research for the study of macromolecules. Malkusch et al. (2013) combined single-molecule super-resolution imaging (dSTORM) with post-acquisition coordinate-based cluster analysis to investigate the distribution and morphology of HIV-1 Gag polyprotein clusters at the plasma membrane in fixed cells. Using these methodologies, they determined three different states of Gag polypeptide cluster formation: (1) small Gag clusters; (2) Gag clusters measuring approximately 140 nm in size; and (3) a “patchy” aggregation of Gag proteins. Moreover, using coordinate-based algorithms, they determined the size for single viral assembly sites, which was in good accordance with data obtained by electron microscopy. This coordinate-based cluster analysis of super-resolution images can also be used for assessment of other cellular proteins, making it a broadly applicable image analysis method.

The incorporation of cells into a pellet for embedding in a diagnostic setting is referred to as the “cell block technique.” A limitation to the usefulness of this technique is the tight packing of cells into a small volume, making it problematic for the creation of tissue microarrays (TMA), as well as potentially masking antigenic sites due to the overlap of cells. Zanini and Forni (2013) have modified the

cell block technique for cells cultured under different conditions to be subsequently used in a TMA. They introduced a mechanical stirring step during cell clot formation, resulting in a homogeneous dispersion of cells throughout the cell block. The creation of an evenly dispersed cell block throughout a TMA will allow for the long-term storage of cells embedded in a paraffin block, which can then be used in clinical diagnostics for comparison between tissue sections of a tumor with cultured cells derived from a corresponding tumor.

Photodynamic therapy (PT) has been employed in recent years to treat both neoplastic and non-neoplastic diseases. In PT, tissues are treated with photosensitive compounds (PS), which enter the cell, absorb applied light, and then through photochemical processes dissipate energy, leading to the production of a variety of oxidizing chemical molecules which damage intracellular processes resulting in cell death. PS can be modified with a variety of chemicals, especially acetate, to alter their plasma membrane permeability properties, resulting also in a modification of their fluorescence properties. Pellicciari et al. (2013) sought to determine the intracellular sites of PS activity by performing a diaminobenzidine (DAB) photoconversion of fluorescence reactivity to an electron dense enzymatic product visible by transmission electron microscopy. They treated cultured HeLa cells with two different fluorescent PS, Rose Bengal acetate (RB-Ac) or Hypocrellin B acetate (HypB-Ac), photoconverted their fluorescence properties into an electron dense reaction with DAB, and imaged the final product by electron microscopy. They found that both RB-Ac and HypB-Ac were internalized into the cell through the conventional endocytotic route, starting at the plasma membrane and proceeding through endosomes, and then into lysosomes and multivesicular bodies; this pathway is consistent with the multiorganelle damage associated with cell exposure to both PS compounds. Therefore, photoconversion of modified PS by DAB in the presence of a light source can be used to investigate at high-resolution intracellular sites and modes of oxidative damage inherent in PT.

In another interesting investigation of the mechanisms responsible for PT, Soriano et al. (2013) followed the internalization of the PS Zn(II)-phthalocyanine (ZnPc) dissolved in either dimethylformamide (ZnPc-DMF) or included in liposomes into human A-549 cells. Coincubation of cells with ZnPc-liposomes and the endocytic pathway inhibitor dynasore resulted in a greatly diminished photodamage response. In contrast, cells incubated with ZnPc-DMF in the presence of dynasore, genistein, or cytochalasin D showed a marked decrease in ZnPc uptake and a concomitant decrease in cellular photodamage. These endocytic inhibitor-based coincubations with PS revealed that: (1) ZnPc-DMF enters cells via a caveolin-mediated

endocytotic process, while (2) ZnPc-liposomes enter via a clathrin-mediated process. Thus, by modifying the vesiculation mechanism of the PS, it is possible to alter the cellular uptake mechanism which may be important in drug therapy regimes.

Development of biomaterials for implanted medical devices has been an area of intense research in the past few years. Introduction of such new materials for medical purposes requires much testing and analysis prior to approval for use in humans. Central to such testing is histological examination of tissue samples from animals containing the inserted medical device. Such histological analyses pose technical issues given the difficulty in infiltrating and embedding both soft and hard tissue samples with the medical implant. Willbold et al. (2013) have presented a solution to this problem by developing a new protocol involving embedding the samples in Technovit 9100 New resin, followed by removal of the metallic implants by electrochemical dissolution. Electrochemical dissolution is a known method for removing and machining metals, based upon dissolution of a positively charged metallic sample which acts as an anode in an electrically conducting liquid solution. After the dissolution step, the tissue is re-embedded and 5- μm -thick sections are cut with a tungsten blade, followed by staining with histochemical dyes or immunohistochemical reagents. This methodological improvement resulted in fine tissue preservation, both at the implant–tissue interface, and in the surrounding tissue itself. Importantly, the electrochemical dissolution procedure did not adversely affect subsequent staining procedures, making this technique universally applicable for research into potential tissue effects resulting from medical implants.

Peripheral nerve damage can be an unintended consequence of some surgical procedures, and thereby label-free imaging techniques have been sought as an adjunct technology for nerve-sparing surgeries. Minamikawa et al. (2013) report on their investigations of spontaneous Raman spectroscopy as a selective label-free detection method for various tissue types. Raman spectroscopy uses visible to near-infrared light to measure spectra that reflect the molecular vibrations of tissue molecules. Since the molecular spectra are indicative of the types of atoms and chemical bonding of molecules, they can be used to differentiate between different tissue profiles. The authors performed Raman spectroscopic analysis on unfixed sections from various rat tissues and were able to differentiate between myelinated nerve, unmyelinated nerve, and adjacent tissues. Moreover, by applying the multivariate image analysis technique of ordinary least squares regression (OLSR) on obtained Raman spectra, they were able to detect unmyelinated and myelinated nerves in sectioned tissues from rat and human periprostatic tissue. A specific Raman spectrum resulting from axons and myelin sheath allowed for

the differentiation of nerve tissue from non-nerve tissues. Further, the lipid and protein content contributing to the Raman spectra provided for the ability to distinguish myelinated from unmyelinated nerve fibers. Due to the ability of Raman spectroscopy to differentiate between nerve and other tissues, it may provide a unique method to assist surgeons in nerve-sparing surgeries.

Mass spectrometry (MS) imaging represents another label-free method for analyzing tissue or cellular components. Specifically, MS can determine the spatial distribution of analytes, including differentiation of various molecular modifications without resorting to labeling of the compound. Sun and Walch (2013) provide a timely and thorough review of MS imaging, with a specific focus on drug research and discovery. In particular, they describe efforts to use MS imaging for the quantitative assessment of various drugs at therapeutic levels, with a detailed table highlighting studies published to date. This review is a “must read” for those wishing to come quickly up to speed on the current literature describing MS imaging in drug therapy and evaluation.

Likewise, Römpp and Spengler (2013) present a very detailed overview of their newly introduced MS imaging method that provides high resolution and mass accuracy at the dimension of the individual cell. They provide a number of examples of their work utilizing matrix-assisted laser desorption/ionization (MALDI) imaging of phospholipids, peptides, and various drug compounds in tissue samples at a spatial resolution of 5–10 μm . Moreover, they present additional examples of MALDI imaging of single cells, human lung carcinoma tissue, and the first demonstration of measurements attainable at a 3- μm pixel size. They compared their MS imaging with conventional histological staining, demonstrating excellent correlation. Overall, this most compelling review demonstrates that their new method advances the MS imaging field by providing the means to combine very high-quality mass analysis with a spatial resolution at the single cell level, certain to augment and advance conventional histochemical techniques for the identification of molecular cellular constituents.

Molecules in health and disease

Outer hair cells (OHCs) in the ear are responsible for amplifying cochlear vibrations, and then converting them into electrical signals. These cells respond to changes in transmembrane potential by modifying their length: membrane depolarization results in cell contraction, whereas hyperpolarization results in cell elongation. The plasma membrane protein prestin, its membrane distribution regulated by thyroid hormone, is believed to account for the electromotility of OHCs. Using a multitechnique approach, Cimerman

et al. (2013) have examined the correlation between the subcellular localization and function of prestin, searching for factors responsible for this membrane sublocalization. They detected cochlear expression of a novel prestin splice isoform called prestin 9b. Employing a yeast two-hybrid assay, they identified a calcium/calmodulin-dependent serine protein kinase (CASK) interacting with prestin. Moreover, they found via immunoprecipitation assays that CASK and prestin 9b interact with full-length prestin and that CASK colocalized with prestin in a specific subdomain of the plasma membrane where prestin-expressing OHC membrane contacts a prestin-free zone. Their results corroborate the premise that the distribution of prestin solely in the basolateral plasma membrane in mature OHCs is likely regulated by the thyroid hormone-dependent CASK protein and that this interaction is required for the generation of electromechanical force resulting in cochlear amplification.

Phosphorylation of transcription factors plays a major role in their activation. Klinz et al. (2013) used immunohistochemistry to investigate the expression of both CREB (cAMP-responsive element-binding protein) and phosphorylated CREB in adult tooth samples. They found expression of CREB in odontoblasts, cementoblasts, and epithelial rests of Malassez in human molars, as well as in dental pulp stromal cells and periodontal ligament fibroblasts. Moreover, they showed that CREB is phosphorylated at Ser-133 in the nucleus of adult human molar odontoblasts and cementoblasts. Their results suggest that the constitutively phosphorylated CREB transcription factor may be involved in the biomineralization process found in adult molar odontoblasts and cementoblasts.

During dental tissue development, the extracellular matrix is remodeled under the control of proteases. One such protease, urokinase-type plasminogen activator (uPA), which promotes cell surface activation of plasminogen to plasmin, is controlled by the uPA receptor (uPAR). Given the role of uPAR in proteolysis, cell migration, proliferation, and adhesion, von Germar et al. (2013) investigated the immunocytochemical distribution of uPAR in cells of rat enamel organ and dental papilla, and its potential association with lipid rafts. Using immunohistochemistry, they detected uPAR immunoreactivity in epithelial cells of the enamel organ, and in ameloblasts and odontoblasts. Moreover, RT-PCR and Western blotting experiments with a phorbol 12-myristate 13-acetate (PMA)-stimulated dental epithelial cell line showed that uPAR was partially localized with a lipid raft cellular fraction, but that uPAR and caveolin-1 do not associate directly with each other. These results indicate that the expression of uPAR by epithelial cells in the enamel organ is regulated to some extent by the protein kinase C-mediated cell signaling pathway.

Many growth factors are known to directly influence tooth development, yet little is known regarding the process of odontoblast differentiation. Such information would be useful in the realm of pulp regenerative therapy and pulp capping. Hosoya et al. (2013) performed an immunohistochemical and immunoprecipitation evaluation of factors that may be involved in the regulation of odontoblast differentiation. They found that the small ubiquitin-related modifier (SUMO) proteins 1 and 2/3 as well as the zinc finger-containing transcription factor Osterix were colocalized in odontoblast lineage cells in the differentiation stages of dentin formation and regeneration. Moreover, using immunoprecipitation, they found that in a mouse myoblast cell culture (C2C12 cells) system, the Osterix protein could be SUMOylated by SUMO-1. Taken together, their results indicate that SUMOylation of proteins such as Osterix affects dentinogenesis via transcriptional regulation.

Ookuma et al. (2013) examined the role of the actin-sequestering peptide T β 4, which is involved in the inhibition of actin polymerization, and as such affects cell motility, in the development of the tooth germ in the murine lower first molar. Growth inhibition of the tooth germ in cultured embryonic day 11 (E11) was detected in an assay using T β 4 antisense sulfur-substituted oligodeoxynucleotide (AS S-ODN); in contrast, no growth arrest was detected in E15 tooth germ. Moreover, T β 4 knockdown resulted in decreased levels of type II/III runt-related transcription factor 2 (*Runx2*) and *nucleolin* (*Ncl*) in the E11 tissues, as well as decreased secretion of matrix metalloproteinase-2, reduced cell motility, and an up-regulation of *E-cadherin* in cultured dental epithelial cells. These results indicate that T β 4 is involved in multiple functions during the development of mouse lower first molar, likely via the regulation of the expression of type II/III *Runx2* and *Ncl*, inhibited secretion of matrix metalloproteinase-2, inhibition of cell motility, and up-regulation of *E-cadherin*.

Retinol-binding protein 4 (Rbp4) is mainly synthesized in the liver and secreted into the blood where it serves as the chaperone for retinol. Since retinol metabolites are known to play a role in osteogenesis, and since Rbp4 has been shown to also be produced in non-hepatic tissues such as limbs, Hatfield et al. (2013) performed an immunohistochemical investigation of the localization of this protein in embryonic and postnatal mouse hindlimbs. They found that Rbp4 was localized to a restricted population of epiphyseal chondrocytes and perichondral cells in a region responsible for subsequent secondary ossification. Moreover, following secondary ossification processes, Rbp4 was demonstrated in chondrocytes both from the resting zone and those bordering invading cartilage canals; less immunostaining for Rbp4 was detected in proliferating chondrocytes from primary ossification. These results suggest that Rbp4 may be

involved in primary bone ossification, as well as formation of the secondary ossification center.

The WASP homolog associated with actin, membranes, and microtubules (WHAMM) is a type 1 nucleation promoting factor involved in linking actin and microtubules in the cytoskeleton and affecting rough endoplasmic reticulum to Golgi apparatus vesicular transport. Given the role of cytoskeletal components in mitosis, and since WHAMM expression had to date only been investigated in interphase cells, Huang et al. (2013a) investigated the localization and function of this nucleation promoting factor in mouse oocytes during meiosis. They used confocal microscopy and specific inhibitors of cytoskeletal components to demonstrate that WHAMM was undetectable in the germinal vesicle stage, but was associated with the spindle during subsequent meiosis stages. Microinjection of the oocyte cytoplasm with specific (si)RNAs resulted in (1) failure of spindle migration, (2) disruption of asymmetric cytokinesis, (3) a decrease in the rate of extrusion of the first polar body during meiotic maturation, and (4) disruption of actin cap formation. These results thus indicate that WHAMM is a required molecule for peripheral spindle migration and asymmetric cytokinetic processes during maturation of murine oocytes.

Aldehyde dehydrogenases (ALDHs) are a family of enzymes involved in detoxification and shown to also be involved in stem cell regulation. Due to this stem cell regulation feature, Kato et al. (2013) investigated the expression patterns of the major ALDH isozymes in oral keratinocytes in reference to tissue-engineered oral mucosal grafts. They used immunostaining, in situ hybridization, and flow cytometric techniques to show that ALDH1A3 and ALDH3A1 were expressed in the upper suprabasal layer of the keratinocytes, and cells with high ALDH activity displayed a proliferative phenotype. By inhibiting ALDH activity with diethylaminobenzaldehyde (DEAB), the keratinocytes displayed a more quiescent, non-proliferative state. Regenerated oral epithelium from ALDH1A3 siRNA knockdown showed a phenotype similar to that from those treated with DEAB, and ALDH3A1 siRNA knockdown inhibited the epithelial regenerative capacity of the keratinocytes. These results suggest that the phenotypic changes expressed by keratinocytes following treatment with DEAB were likely due to inhibition of isozyme ALDH1A3 activity. Moreover, they show that genetic inhibition of ALDH3A1 affected the regenerative ability of oral keratinocytes, suggesting that the inhibitory effect of DEAB was restricted to the differentiated cells in the suprabasal layer rather than on the undifferentiated cells in the basal layer, with implications for oral mucosal regeneration strategies.

In normal arteries, nitric oxide is the main mediator of endothelium-dependent relaxation, whereas in a diet-induced obese rodent model it has been shown that

vasodilation of the saphenous artery transitions from a nitric oxide-dependent mechanism to a calcium-activated potassium channel and myoendothelial gap junction-dependent mechanism. Grayson et al. (2013) expanded upon these studies to investigate whether obesity results in changes in overall eNOS expression and endothelial caveolin isoforms, as well as the number of caveolae in saphenous artery endothelial cells. Using pressure myography, Western blotting, confocal microscopy, and quantitative transmission electron microscopy, they showed that diet-induced obesity in a rodent model leads to: (1) impaired nitric oxide-mediated endothelium-dependent vessel relaxation; (2) no change in the overall expression level of vessel eNOS; (3) the accumulation of oligomeric complexes of caveolins 1 and 2; and (4) an increased expression of the number of caveolae in vessel smooth muscle and endothelial cells. These results indicate that vessel caveolae and caveolin isozymes represent a potential therapeutic target in obesity-dependent vascular disease.

Food intake in animals is affected by release of peptide hormones; ghrelin stimulates food intake, while nucleobindin2 (NUCB2)/nesfatin-1 has been shown to suppress food intake. Due to the key role these peptide hormones may play in regulating food intake and thus potentially affect obesity in humans, Stengel et al. (2013) investigated the gastric cell type responsible for the production of NUCB2/nesfatin-1 and ghrelin. Using immunofluorescence and confocal microscopy, they found that ghrelin and NUCB2/nesfatin-1 were colocalized in human gastric X/A-like cells, suggesting that this cell type may play a dual role in regulating food intake depending upon which peptide hormone is released. Moreover, they found that both peptide hormone expression levels were differentially regulated under obese conditions, with increased NUCB2/nesfatin-1, decreased ghrelin, and decreased ghrelin-*O*-acyltransferase in patients with a higher BMI. These results may indicate that these two peptide enzymes undergo an adaptive change in expression to potentially prohibit a further gain in avoirdupois.

Acyl-CoA thioesterases (Acots) catalyze the hydrolysis of fatty acyl-CoAs to free fatty acids and coenzyme A and thus can regulate the cellular levels of these molecules. In brown fat adipocytes, Acot1 has been proposed to regulate the levels of cytosolic acyl-CoAs during cell maturation. Ohtomo et al. (2013) investigated whether similar regulation may occur in other cell types that utilize fatty acids, specifically white fat adipocytes. Thus, they examined the expression and distribution of Acot1 in white adipose tissue from rats, and following cellular differentiation in culture. They found that Acot1 was detected in the cytoplasm of immature adipocytes located in the perivascular of white adipose tissue. By immunohistochemical analysis, they detected Acot1 in layered structures surrounding blood

vessel adventitia in the subcutaneous white adipose tissue. Digestion of tissue with collagenase followed by centrifugation resulted in Acot1 in the stromal vascular fraction, but not in mature large adipocytes. After adipocyte induction to differentiation by a 15-day pretreatment without insulin, dedifferentiated cells expressed increased Acot1 in the cytoplasm. These results suggest that since Acot1 expression is up-regulated during early adipocyte maturation, but not detectable in mature adipocytes, it may be involved in acyl-CoA metabolism in immature white adipocytes.

Vascular endothelial growth factor C (VEGF-C) has been linked to the growth of lymphatic vessels (lymphangiogenesis), contributing to the pathology of several human diseases, including tumor formation. Fukuhara et al. (2013) investigated the expression of VEGF-C and its receptor VEGFR-3 in the human pterygium (“an elevated, superficial, external ocular mass that usually forms over the perilimbal conjunctiva, and extends onto the corneal surface”, Fukuhara et al. 2013) and normal conjunctival tissues using both Western blotting and immunohistochemistry. Additionally, they analyzed the density of both lymphatic and blood vessels using specific antibody markers. Their results showed that lymphatic vessel density, as well as expression of both VEGF-C and VEGFR-3 was increased in pterygium versus normal conjunctival tissue. VEGF-C immunoreactivity was detected in the cytoplasm of both normal and pterygial epithelial cells, with an increased number of positive stained cells observed in the pterygial samples. These results suggest that VEGF-C may be involved in the pathology of pterygium via lymphangiogenesis, offering a potential target for therapeutic intervention.

The ciliary body, consisting of two epithelial cell layers [the non-pigmented epithelium (NPE) and the pigmented epithelium (PE)], is proposed to be the main site of secretion of solution maintaining the aqueous humor chamber of the eye. Li et al. (2013a) investigated the contribution of the ciliary body to the maintenance of glutathione (GSH) levels in the aqueous humor; glutathione is an important antioxidant, which can detoxify oxidants in the aqueous humor. They localized GSH metabolism pathways to the epithelial layers of the ciliary body. Using immunohistochemical and RT-PCR techniques, they showed that (1) both the PE and NPE epithelial layers are capable of GSH synthesis; (2) only cells of the NPE appear to have a role in uptake of GSH precursor amino acids from the stroma; (3) NPE cells contain transporters responsible for the uptake of GSH precursor amino acids, which are then available to both NPE and PE cells for GSH synthesis; and (4) the location of GSH efflux transporters to the basolateral membrane of NPE cells suggests that these cells can mediate GSH secretion into the aqueous humor. Since the GSH secreted into the aqueous humor is not degraded, it may potentially be available for use by other tissues for antioxidant activities.

The trefoil factor family (TFF) consists of three peptide members, involved with affecting cell proliferation and apoptosis in both normal and tumor cell lines. Weise and Dunker (2013) have investigated the expression and function of TFF peptides in healthy human retina and in eight cell lines derived from human retinoblastoma tumors. By immunoblot analysis, TFF1 was the only family member detected in the retinoblastoma cell lines, expressing a negative correlation with cell growth curves. Moreover, retinoblastoma cell lines expressing high levels of TFF1 showed a down-regulation of cyclin-dependent kinase (CDK) 6, while CDK4 and CDK2 were largely unaffected. By immunofluorescence analysis, TFF1 and CDK2 were found to colocalize in nuclear Cajal bodies (CBs); the number of CBs was found to be higher in cells also expressing high levels of TFF1, though the size of the CBs was smaller compared to cells with low TFF1 expression. These results suggest the premise that TFF1 functions as a tumor suppressor in retinoblastoma cells, and that its localization to nuclear CBs may indicate an effect on cell cycle progression.

Components of the extracellular matrix are believed to play a prominent role in the progression of some cancers. Indeed, the extracellular matrix protein decorin has been shown to possess potent anti-tumor properties. However, in breast cancer, it is unclear whether decorin expression is limited to extracellular matrix stroma, or if it is also expressed by tumor cells themselves. Bostrom et al. (2013) undertook a detailed study to determine the level of expression of decorin in healthy and cancerous breast tissue. They performed gene expression using the GeneSapiens databank, localized decorin mRNA expression with in situ hybridization, and examined the effect of decorin transduction on the subsequent behavior of a human cancer cell line. Their results showed that although significant decorin gene expression was found in human breast cancers by GeneSapiens databank, the cellular resolution of in situ hybridization clearly demonstrated that decorin was not expressed by cancer cells, but only by stromal cells. Moreover, they found that transduced expression of decorin into a cancer cell line altered the proliferation and cohesion properties of these cells. The authors propose that targeted expression of transduced decorin into breast cancer cells could be utilized as a potential adjuvant therapy for breast cancer treatment.

Members of the fibroblast growth factor (FGFs) family are involved in a myriad of developmental processes, including cell proliferation, differentiation, and migration. Receptors for FGFs include a family of receptor tyrosine kinases, one member of which, FGFR1 is implicated in promoting tumor growth in a number of cancer types, including glioblastoma. Irschick et al. (2013) investigated internalization and intracellular trafficking of FGFR1 conjugated to eGFP in a human glioblastoma cell line. They

showed with exquisite immunofluorescence colocalization analysis that upon stimulation of the glioma cells with FGF-2, the overexpressed FGFR1 was endocytosed into the early endosome pathway via a clathrin- and caveolin-dependent manner. Subsequently, the FGFR1 receptor was found to travel into both the recycling and lysosomal degradation pathways. This cell model may prove useful for further investigations into blocking the recycling receptor pathway as a therapeutic approach to glioblastoma treatment.

The runt-related transcription factor 3 (Runx3), a member of the runt domain family, is involved in the regulation of various developmental pathways. It is strongly expressed in the liver, and loss of Runx3 in humans can result in hepatocellular carcinoma. Lee et al. (2013) investigated the role of Runx3 in development of the murine liver using immunohistochemistry and RT-PCR on both wild-type and Runx3 knockout animals. In Runx3 knockout animals, the liver architecture was abnormal (including excessive angiogenesis), and expression of Ki-67 positive proliferating cells, as well as the liver differentiation markers VEGF, vWF, CD31, pSmad2, NF- κ B, WT-1, and Thy-1 were all up-regulated compared with those in the wild-type animals. Due to these liver developmental abnormalities present in Runx3 knockout animals, the authors suggest that Runx3 plays a crucial role in proper mouse liver development.

Recent investigations suggest that erythropoietin (EPO) functions not only in the hematopoietic system, but also exerts effects on many non-hematopoietic tissues and organs. Moriconi et al. (2013) studied the gene expression of EPO and its receptor (EpoR) in a rat model incorporating thioacetamide-induced damage and tumor generation, and in a human cholangiocarcinoma (CC) cell line. Both EPO and EpoR mRNA increased during fibrosis to cirrhotic progression, peaking during tumor formation. In the rat model system, EPO was mainly produced in regenerative cirrhotic hepatic nodules and dysplastic bile duct cells. Likewise, these cells along with vessel endothelial cells displayed the highest expression of EpoR. In addition, they found that challenge of CC cells with EPO and the hematopoietic growth factor SCF resulted in a synergistic effect on the cell cycle gene expression profiles of EPO, cyclinD1, and PCNA. These results highlight the role of EPO as a potential molecular mediator produced by hepatic cells during the process of liver injury and tumor progression.

Laminopathies represent a group of diseases resulting from mutations in the *LMNA* gene, which encodes proteins of the A-type lamins. Although evidence suggests that laminopathies are caused by a combination of mechanical and gene regulatory distortions, patients with identical *LMNA* mutations display widely varying disease symptoms, making predictive genetic screening problematic. Recently, it has been shown that repetitive nuclear ruptures

occur in fibroblasts cultured from patients with laminopathies, with a strong correlation with disease severity, making this a promising avenue for further investigations into a potential disease biomarker. Houben et al. (2013) report on their investigations of PML nuclear bodies, structures normally found in the nucleus which leak out into the cytoplasm (becoming cytoplasmic PML particles; PML CPs) upon nuclear rupture. Using immunostained fibroblast cells cultured from laminopathy patients, together with confocal microscopy imaging, they demonstrated an increased number of PML CPs in cells having nuclear abnormalities, and that the number of particles could be indicative of disease severity. Interestingly, they also found that in patient fibroblasts without demonstrable nuclear abnormalities the percentage of cells showing PML CPs was increased. The authors thus suggest that analysis of PML CPs in cultured fibroblasts from patients with laminopathies could serve as a diagnostic biomarker for disease diagnosis and severity.

Animal models for xenotransplantation of porcine pancreatic islet cells as a potential treatment for human diabetes type I hold much promise. However, expression of specific islet proteins, such as ZnT8, involved in endocrine hormone release and acts as a diabetes auto-antigen has not been established across species. Using double-labeling immunohistochemistry and nested RT-PCR, Schweiger et al. (2013) showed that (1) pig ZnT8 exclusively co-expressed in insulin-producing B cells, but was not found in glucagon- or somatostatin-producing cells; and (2) local expression of RT-PCR transcripts for ZnT8 was restricted to the pancreas. These localization results for ZnT8 should be considered when evaluating different approaches for xenotransplantation therapy to treat type 1 diabetes.

Organelles, subcellular structures, and compartments

A review of imaging active genes in live cells, based upon the lecture for the Robert Feulgen Prize 2012, awarded at the 55th Symposium of the Society for Histochemistry, was presented by Shav-Tal et al. (Yunger et al. 2013). They presented multiple examples of fluorescence images illustrating their kinetic, live-cell analysis of mRNA transcription, showing the high-resolution capabilities to image the transcription of single alleles. The power of using fluorescent proteins in live-cell imaging protocols to observe temporal aspects of gene transcription was beautifully illustrated in this review.

Immunohistochemistry and enzyme histochemistry are classical tools for investigations of peroxisomes. However, none of the currently used peroxisomal markers are optimal for this purpose since their presence, distribution, and abundance are dependent upon the cellular metabolism and varies also under various experimental conditions.

To overcome these limitations, Grant et al. (2013) have explored antibodies raised against the peroxisome biogenesis protein PEX14 and propose that they represent the ideal standard marker as compared to catalase and ABCD3. In their highly detailed, comparative analysis including Western blotting, immunohistochemistry, and immunoelectron microscopy, they demonstrated the superiority of PEX14 antibodies for studying peroxisomes in various cells and tissues from different animal species and humans. By using Quantum dots, they were able to increase the detection limit of immunofluorescence and to overcome commonly encountered problems due to photobleaching observed with fluorochromes. The authors also pointed out that the use of PEX14 antibodies provides optimal conditions for comparative morphometric analysis of peroxisomes. In another publication by Baumgart-Vogt et al. (Karnati et al. 2013), the usefulness of antibodies against superoxide dismutase (SOD2) as markers for peroxisomes and mitochondria was analyzed. Contrary to other reports, they found that in a large spectrum of cells and tissues SOD2 was unequivocally identified in mitochondria but not in peroxisomes. These data were also fully supported by published organelle proteome data indicating that SOD2 is solely a mitochondrial protein.

A further study from Baumgart-Vogt and coworkers (Stelzig et al. 2013) focused on the distribution of peroxisomes, their protein composition, and the expression of peroxisomal genes during dental development and in mature decalcified teeth in mice. Besides immunohistochemistry, laser-assisted microdissection of ameloblasts and odontoblasts for RNA isolation and subsequent RT-PCR was applied. Peroxisomes were already detected at the bud stage of dental development, an increase in abundance found during differentiation of ameloblasts and odontoblasts, a strong heterogeneity of peroxisomal enzyme content occurred within differentiated dental cell types, and a strong down-regulation of catalase was noted in maturing ameloblasts, whereas levels of lipid metabolizing enzymes were high.

Suzuki et al. (2013) aimed at establishing functions of protein kinase C for lipid droplets. They found seven PKC isoforms tagged with EGFP associated with lipid droplets. Specifically, they found that PKC η and to a lesser extent two other novel PKCs, PKC δ and PKC ϵ , tended to concentrate at the surface of lipid droplets. Moreover, overexpression of PKC γ reduced the average size of lipid droplets compared with the control even when synthesis of lipid esters was activated. It was proposed that PKC η is involved in regulating lipid metabolism in lipid droplets by phosphorylating substrates whose identity is currently unknown.

Exosomes and microvesicles are particular cellular structures released from the cell surface by an unconventional mode of exocytosis. As far as it is known, they are

involved in intercellular communication and transfer of protein, lipids, and mitochondrial DNA as well as RNAs. Falchi et al. (2013) reported that cultured human fetal astrocytes shed large membrane vesicles ranging from 1 to 8 μm in diameter that are considerably larger than the described microvesicles. The astrocyte-derived vesicle membrane was positive for $\beta 1$ -integrins, a marker for shedding, and the vesicle content consisted of mitochondria and lipid droplets and ATP. The shedding of the large vesicles occurred after repetitive ATP stimulation.

As will be mentioned in the section on the Golgi apparatus, bacterial toxins can hijack the cellular endocytosis machinery. Danielsen et al. (2013) analyzed the enterotoxins A and B produced by *Staphylococcus aureus* in regard to their binding and uptake by the apical (brush border) plasma membrane of organ cultures of porcine jejunal explants. In contrast to cholera toxin B binding, binding of both staphylococcal enterotoxins to the brush border membrane was scarce and patchy and sensitive to pretreatment with endoglycoceramidase II. Little evidence was found for partitioning with lipid rafts (detergent-resistant membranes). It was concluded that like cholera toxin B, both staphylococcal enterotoxins are transcytosed to eventually reach their target cell in the subepithelial lamina.

The Golgi apparatus

Although 115 years have passed since its first description, the Golgi apparatus still harbors many secrets to be revealed. A most intensely studied aspect of its highly complex function is transport. Warren (2013) reviewed and discussed transport through the Golgi apparatus in *Trypanosoma brucei*. The major advantage of analyzing this protozoan parasite is that it is “a stripped-down eukaryote” containing a single Golgi apparatus through which the GPI-anchored variant surface glycoprotein (VSG) is transported and delivered to the flagellar pocket membrane. Notably, the VSG represents >95 % of the surface proteins. By integration of the available morphological and biochemical data, Warren suggests that stable cisternae, as compared to cisternal maturation, represent the more likely transport mode in *Trypanosoma brucei*. In further reviews, transport through the Golgi apparatus of mammalian and plant cells and its enormous complexity were discussed. Day et al. (2013) proposed a three-stage model of Golgi apparatus structure and function. They categorized Golgi cisternae into three classes according to their function in import and export and proposed that the three classes correspond to functional stages of maturation. Their three-stage model is based on advanced electron microscopic analysis by electron tomography of high-pressure frozen/freeze-substituted cells. The first stage is related to the formation of new

cisternae by ER-derived COPII vesicles. The second stage corresponds to cisternae involved in glycosylation and polysaccharide synthesis. The third stage represents cisternae forming various types of transport carriers including clathrin-coated vesicles. It is emphasized that discrete transitions occur during the maturation of the cisternae from one stage to the next. Boncompain and Perez (2013) presented a very detailed review about aspects of Golgi apparatus transport as they relate to the diversity of the secretory cargos, the machineries involved in cargo transport and the specificity of Golgi-dependent transport routes and discussed them in view of the models of cisternal maturation or static cisternae. As also emphasized in the other reviews, depending on the functional situation, the models of cisternal maturation or static cisternae are not mutually exclusive, and Boncompain and Perez (2013) propose an integrative model of intra-Golgi transport. Likewise, Mironov et al. (2013) compared different models of intra-Golgi transport from the point of view of their ability to explain existing experimental facts. In their view, the kiss-and-run (KAR) model they had proposed in 2008 (Mironov and Beznoussenko 2008) has the highest potential for the explanation of the majority of experimental observations. The review by Machamer (2013) provided a very timely discussion of the transport of large cargo—procollagen and lipoprotein particles or coronaviruses—within the Golgi cisternal stack. Transport of large rod-shaped procollagen or of large spherical chylomicrons or coronaviruses requires the reversible, local dilation of part of Golgi cisternae. Aspects of the possible importance of the activation of the Src family kinase (SFK) signaling, the flux of osmotically active ions and water, and the importance of GRASPs and Golgins for the trafficking were highlighted.

Farhan and coworkers (Tillmann et al. 2013) reviewed evidence indicating that the functional organization of the ER–Golgi system is also regulated by signal transduction. The various signaling events occurring at the ER–Golgi apparatus interface involve several kinases such as PCTAIRE, the serine/threonine kinase AKT, or CK2 that appear to regulate the efficiency of the ER export by regulating the production and progression of COPII vesicles. In contrast, only little is known about signaling at the level of the ERGIC, alias intermediate compartment, alias pre-Golgi intermediates, whereas signaling by Ras GTPases and Src family kinases at the level of the Golgi apparatus is well established. Signaling in the Golgi apparatus appears also to involve the Golgi matrix proteins GRASP65 and GRASP55 as well as ion channels for Mn^{2+} and Ca^{2+} transport. As emphasized by the authors, the field of signal transduction for the organization of the ER–Golgi system is still in its infancy. The focus of the review by Cancino et al. (2013) was also on Golgi signaling and the control of cargo movement from the ER to the Golgi apparatus. The authors

emphasize the importance of the KDEL receptor as a regulator of Golgi traffic and point to structural and functional similarities with the function of G protein-coupled receptors (GPCRs) that operate at the plasma membrane. Additionally, the role of heterotrimeric G proteins, Src family kinases, the serine/threonine protein kinase A as well as protein kinase C and D isoforms in the control of secretion and Golgi apparatus organization were reviewed.

The review by Martinez-Alonso et al. (2013) focused on tubules of the Golgi apparatus with respect to their structure and formation and their role in intra-Golgi transport. This comprehensive review also covers aspects of experimental induction or inhibition of tubule formation, the relation of Golgi tubules to particular lipids and lipid-modifying enzymes as well as coat complexes and the role of tubules in connecting Golgi cisternal stacks. Valente et al. (2013) reviewed recent advances of the fission-inducing protein CtBP1/BARS (C-terminal-binding protein/brefeldin A ADP-ribosylated substrate) as they relate to the formation of different carriers at the *cis*- and *trans*-side of the Golgi apparatus. The authors emphasized aspects of the molecular structure of CtBP1/BARS and the mechanisms behind the functional switch of the protein from a transcriptional co-repressor in the nucleus to an inducer of membrane fission in the cytoplasm, specifically of the Golgi apparatus. Recently, discovered similarities between the machinery proteins and the fission process in animal and plant cells were also discussed.

Willett et al. (2013) concentrated in their review on the important role the conserved oligomeric Golgi (COG) complex plays in targeting of vesicles at the Golgi apparatus. The interaction of the various COG complex subunits with different classes of molecules involved in intra-Golgi trafficking was nicely illustrated by the analysis of the COG complex interactome. Thus, COG appears to act as a docking station, seems to bridge membranes, or may mediate SNARE assembly. The authors proposed a unified model of COG's molecular function.

The review by Zhu and Kaverina (2013) summarized a more recently discovered Golgi apparatus function: microtubule nucleation. This function involves the formation of a subset of non-centrosomal microtubules at the Golgi apparatus that are indispensable not only for the organization of the Golgi apparatus as an organelle but also for post-Golgi trafficking and cell polarity. The authors detailed the molecular machinery involved in the nucleation of microtubules at Golgi membranes and the functions of Golgi-derived microtubules in Golgi assembly and directional post-Golgi trafficking, as well as their role for establishing cell polarity and polarized cell migration. Another component of the cytoskeleton, actin, together with its binding and regulatory proteins, is important for the structural integrity of the Golgi apparatus and for Golgi-associated

trafficking. In their review, Egea et al. (2013) summarized the current knowledge of actin function as it relates to the Golgi apparatus in terms of its structural organization and its function in trafficking, including sorting as well as the formation and movement of Golgi-derived transport carriers. They also reviewed the relation between the Golgi apparatus and actin in plant cells, yeast, *Drosophila*, *Dictyostelium discoideum*, and *Caenorhabditis elegans*.

In addition to its many other important functions, the Golgi apparatus is central for normal copper homeostasis. It contains copper-transporting ATPases that transfer copper in the Golgi cisternal lumen, where it becomes incorporated in copper-dependent enzymes. Polishchuk and Lutsenko (2013) reviewed structural and functional aspects, as well as the Golgi localization of the copper-translocating ATPases. Furthermore, details of the cell and tissue expression of the two different ATPases, their trafficking from the Golgi, and their retrieval from the plasma membrane to the Golgi apparatus were reviewed. The review also included data about mutations in the copper-transporting ATPases that result in the disruption of the copper homeostasis and cause either copper deficiency—Menkes disease—or copper overload—Wilson disease.

It has been amply demonstrated that endocytic traffic merges with the Golgi apparatus. Chia et al. (2013) reviewed the various pathways of retrograde transport from endosomes to the *trans*-Golgi network (TGN) whose main function is to retrieve the endocytosed cargo for recycling. The authors distinguished four groups of cargo proteins and provided details about the early endosome-to-TGN pathway, the late endosome-to-TGN pathway, and the recycling endosome-to-TGN pathway, as well as the special case of endocytosis in gastric parietal cells which have a fragmented Golgi apparatus. In addition, they report on the combination of flow cytometry and imaging as a novel tool for monitoring intracellular cargo transport.

The endocytic pathway can be hijacked by various plant and bacterial toxins that travel to and through the Golgi apparatus to eventually exit to the cytosol for their usually poisonous action. Sandvig et al. (2013) discussed in their review aspects of the mode of cell surface binding of different toxins and their endocytic uptake by clathrin-dependent and clathrin-independent mechanisms. With the example of the plant toxin ricin, the bacterial Shiga toxin and the cholera toxin, the authors illustrated how these toxins are sorted to the Golgi apparatus which is followed by their retrograde transport to the ER from where they become dislocated to the cytosol through components of the ERAD machinery.

Like animal cells, plant cells possess a TGN. Uemura and Nakano (2013) reviewed recent progress providing new insight into the dynamics and functions of the plant TGN. Like the TGN of animal cells, the TGN of plant cells is important for exocytic and endocytic trafficking.

However, in contrast to animal cells and yeast, the TGN of plant cells appears to have functions independent from the Golgi apparatus. The authors emphasized the importance of advanced microscopic techniques such as *in vivo* imaging with fluorescent-tagged proteins to unravel the TGN dynamics and of electron tomography for the analysis of the relationship between the Golgi cisternal stack and the TGN in plant cells.

Traditionally, electron microscopy and immunoelectron microscopy were indispensable tools for the analysis of the structure and functional organization of the Golgi apparatus. The advanced techniques of cryo-electron microscopy and tomography involving the analysis of resin thin sections of high-pressure frozen and freeze-substituted cells, or of frozen-hydrated sections have confirmed the known structural organization of the Golgi apparatus and provided novel insight in regard to the presence and 3D structure of supramolecular complexes. Han et al. (2013) gave an excellent overview about the possibilities provided by novel techniques of tissue preparation and imaging for the high-resolution analysis of the Golgi apparatus and associated structures.

The Golgi apparatus may exhibit characteristic structural alterations induced by experimental manipulation or in disease conditions. Rendon et al. (2013) reported on the cause of Golgi fragmentation as observed in Parkinson's disease and other chronic neurodegenerative diseases. They used a cellular model of Parkinson's disease to unravel the mechanism involved in Golgi fragmentation. They found that Golgi fragmentation preceded and perhaps triggered aggregation of α -synuclein and subsequent aggresome formation. In addition, in their model, a relationship between altered Golgi morphology and the levels of certain Rab proteins, such as Rab 1, 2, and 8, and SNARE proteins was established.

Intermediate filaments and cytoskeleton

A special issue of *Histochem Cell Biol* was devoted to intermediate filaments, the main topic of the 2013 annual symposium of the Society for Histochemistry held in Prague, Czech Republic.

In her "*Histochem Cell Biol Lecture 2013*," Nam-on Ku reviewed keratin function in simple type epithelia with emphasis on the lung (Yi and Ku 2013). The review provided an excellent overview on intermediate filament proteins in lung epithelial cells with regard to their cellular distribution, their role in cell integrity, their marker function in non-small cell lung carcinomas, epithelial–mesenchymal transition, and cell migration. As pointed out by the authors, this knowledge is an important prerequisite for further investigations on the role of lung intermediate

filaments and their disease-causing mutations in animal models and human lung diseases. Pant and coworkers (Binukumar et al. 2013) reviewed the topographic regulation of neuronal intermediate filaments by phosphorylation with emphasis on the role of peptidyl-prolyl isomerase 1 (Pin1) and the significance of defects of these phosphorylation processes for neurodegeneration. Lepinoux-Chambaud and Eyer (2013) reviewed various aspects of nervous system intermediate filaments such as their abnormal accumulation and perturbed metabolism in neurodegeneration, and their potential use as biomarkers to assess progression of neurodegeneration or targets for brain tumors. From the group of Pekny (de Pablo et al. 2013), original work on the importance of intermediate filaments for astrocyte response to oxidative stress induced by oxygen-glucose deprivation and reperfusion was reported. They demonstrated the importance of intermediate filaments in the elimination of reactive oxygen species. Olbrich et al. (2013) gave an account on their work on neuronal growth cones and reported that following VEGF stimulation a fast rearrangement of the neuronal growth cone's actin cytoskeleton occurs which was mediated through VEGF receptor 2. Wiche and coworkers (Castanon et al. 2013) presented an impressive overview on the molecular structure and function of plectin, an intermediate filament-associated large protein. In addition, they reviewed the role of plectin isoforms in stress-prone tissues such as skin, skeletal muscle, and Schwann cells of peripheral nerves and implicated diseases. Tsoupry and Capetanaki (2013) reviewed the muscle-specific intermediate filament desmin and myospryn, a novel desmin-associated TRIM-like protein. They focused on the importance of the association for mechanochemical signaling in the regulation of muscle development and homeostasis, as well as aspects of the dysregulation of the desmin–myospryn interactome resulting in diseases of the cardiac and skeletal muscles. The nuclear lamina consists of a proteinaceous network along the inner nuclear membrane with lamin proteins being its major constituents. Zwerger and Medalia (2013) reviewed high-resolution and molecular structural aspects of lamins and of the nuclear lamina organization as they classically relate to the shape of the nuclear envelope and the nucleus, as well as the more recently recognized involvement of lamins in various nuclear functions such as DNA replication and repair, regulation of gene expression, and signaling. This knowledge is critical for the understanding of disease-causing lamin mutations.

Connective tissue and extracellular matrix

The intervertebral disc (IVD) is composed of an outer region, the annulus fibrosus, which is a collagen-rich tissue that provides tensional properties, and a central

proteoglycan-rich nucleus pulposus, which is a viscoelastic weight-bearing cushion for the absorption of axial spinal loads. Hayes et al. (2013) examined the localization of fibrillin-1 and perlecan in the fetal human, wild-type C57BL/6, and HS-deficient $hspg2\Delta^{3-}/\Delta^{3-}$ exon 3 null mouse IVD by confocal immunofluorescence. Fibrillin-1 fibrils were prominent components of the outer posterior and anterior annulus fibrosus of the fetal human IVD. Finer fibrillin-1 fibrils observed in the inner annulus fibrosus displayed an arcade-type arrangement in the developing lamellae. Short fibrillin-1 fibrils were present in the central region of the IVD and presumptive cartilaginous endplate and defined the margins of the nuclear sheath in the developing nucleus pulposus. Likewise, fibrillin-1 was also demonstrated in the annulus fibrosus of C57BL/6 wild-type mice, but to a far lesser extent in the HS-deficient $hspg2\Delta^{3-}/\Delta^{3-}$ exon 3 null mouse. It was concluded that the HS chains of perlecan may contribute to fibrillin-1 assembly or its deposition in the IVD. The cell–matrix interconnections formed by the fibrillin fibrils were suggested to facilitate communication between disc cells and their local biomechanical microenvironment in mechanosensory processes which regulate tissue homeostasis. The ability of fibrillin-1 to sequester TGF- β , a well-known anabolic growth factor in the IVD also suggests potential roles in disc development and/or remodeling.

Leitch et al. (2013) reported findings on the retinol-binding protein 4 during osteogenesis, since it is not only known that retinoid signaling has an essential role in skeletal development, but as also shown in their previous gene microarray study, retinol-binding protein 4 was the most highly down-regulated gene in suture tissue during the pathological process of premature bony fusion. To analyze the function of retinol-binding protein 4 in cranial suture, they studied primary cell cultures derived from human cranial suture mesenchyme and found that retinol-binding protein 4 was down-regulated during mineralization. They also observed that retinol-binding protein 4 was not secreted, which they interpreted as evidence for a cell-autonomous action. Based on the intracellular distribution of retinol-binding protein 4, the authors speculated that it has functions in addition to being a serum transporter of retinol, and hence a broader role in osteogenesis than currently assumed.

The family of Fras1/Frem extracellular matrix proteins comprises four structurally related members, Fras1, Frem1, Frem2, and Frem3, which are colocalized in embryonic epithelial basement membranes and contribute to epithelial–mesenchymal adhesion. Makrygiannis et al. (2013) have studied the Fras1/Frem family in the developing mouse brain and revealed an exclusively meningeal basement membrane protein deposition. Furthermore, Fras1 displayed a segmental localization pattern since it was

restricted to certain regions of the meningeal basement membrane. The Frem2 protein exhibited a similar localization pattern, while Frem3 was rather uniformly distributed throughout the meningeal basement membrane. On the other hand, Fras1 and Frem2 proteins were detected in regions of the basement membrane that underlie organizing centers, such as the roof plate of diencephalon, midbrain, and hindbrain, and the roof plate-derived structures of telencephalon. The authors propose that the spatial diversity in the composition of the meningeal basement membrane may reflect functional differences that could affect the function of the respective brain areas.

Autophagy

Autophagy is a mode of type II cell death, in which cellular components are degraded in newly formed double-membraned structures called autophagosomes, which subsequently fuse with primary lysosomes. The activity of autophagosomes can be increased by nutrient starvation and may be a critical mechanism by which cells survive under severe conditions. The protein Beclin-1 has been shown to be involved in the regulation of autophagy in mammalian cells. Kim et al. (2013) examined the effect of Beclin-1 knockdown in cultured HT22 neurons challenged with amino acid starvation (AAS) on the autophagic response. In wild-type cells, 3 h post-AAS exposure led to an induction of light chain-3 (LC-3)-immunopositive and monodansylcadaverine (MDC) fluorescent dye-labeled autophagosome formation, as well as an increase in autophagosome-targeting LC3-II. Moreover, AAS treatment also led to slight-to-moderate activation of the levels of apoptotic factors caspase-3 and AIF. In contrast, knockdown of Beclin-1 resulted in reduced AAS-induced LC3-II increase, autophagosome formation, and enhanced neuronal cell death. These results indicate that a decrease in autophagy resulting from Beclin-1 knockdown leads to an increased susceptibility to AAS-induced pro-apoptotic signals, emphasizing the idea that autophagy may represent a cell protective, rather than destructive mechanism under extreme nutrient conditions.

Similar to apoptosis, autophagy is also believed to play a role in programmed cell death during development. Yang et al. (2013) sought to investigate the role of autophagy in murine tooth development and whether it interacts with the apoptotic developmental mechanism. They used a variety of experimental protocols, including RT-PCR for autophagy-related genes, Western blot analysis, immunohistochemistry, dual immunofluorescence analysis, and transmission electron microscopy. Results from their analyses showed that autophagy is involved, and in some cases overlapped with apoptotic mechanisms during tooth development in

murine embryos and neonates. Their studies reveal that the cell death mechanisms of apoptosis and autophagy need not be mutually exclusive during tissue development and that the developing tooth germ in the mouse may represent an attractive model for investigating these mechanisms.

Live-cell analysis of cellular functions requires the use of specific probes, and autophagy as a cell process is no different. The development of fluorescent probes to mark specific cellular compartments and molecules has been an intense area of research and investigation over the past couple of decades. Oeste et al. (2013) have investigated the specificity of two recently developed fluorescent probes for the autophagocytic/lysosomal pathways: (1) Lyso-ID Red (Lyso-ID), an amphiphilic compound sequestered in acidic compartments, and (2) Cyto-ID green reagent (Cyto-ID), a cationic amphiphilic compound reported to specifically localize to autophagosomes. They performed colocalization fluorescence analyses of Lyso-ID and Cyto-ID with well-known markers for the autophagic and endo-lysosomal pathways in live cells. They found that Lyso-ID specifically labeled acidic compartments also stained with the late endosome/lysosome markers Lamp1 and CINC-KVL. Likewise, they observed that Cyto-ID labeled intracellular compartments also stained with LC3, a marker for autophagosomes (see above). Dual labeling of cells with Lyso-ID and Cyto-ID resulted in compartments labeled with either one, or both of the probes, showing that these fluorescent probes can be used to specifically label the lysosomal or autophagosomal pathways and compartments in live cells, making them useful and specific probes for evaluating the autophagosomal response in cultured cells.

Stem cells

Mesenchymal stem cells (MSCs) are multipotent stromal cells with the capacity to differentiate into chondrocytes, osteoblasts, and adipocytes. Differentiation into chondrocytes requires the activation of the Wnt signaling pathway; regulation of this Wnt pathway is dependent upon glycogen synthase kinase-3. Eslaminejad et al. (2013) performed experiments to investigate the effect of two glycogen synthase kinase-3 (GSK-3) inhibitors, lithium chloride (LiCl) and the small-molecule SB216763 on a human-derived MSC chondrogenic culture. Cell cultures were treated with the two GSK-3 inhibitors in the presence of TGF- β 1 or TGF- β 3 for 21 days and then analyzed for the expression of cartilage-specific genes. The results showed an up-regulation of these cartilage-specific genes by both GSK-3 inhibitors, with SB216763 appearing to be more potent than LiCl. Moreover, treatment with SB216763 resulted in a greater deposition of glycosaminoglycans in the cell culture. These results demonstrate that addition of both LiCl

and SB216763 to a chondrogenic cell culture leads to an up-regulation of cartilage-specific genes through a Wnt/ β -catenin specific pathway.

Li et al. (2013b) also studied the differentiation of bone-derived MSCs into chondrocytes, focusing on the importance of chondroitin/dermatan sulfate (CS/DS) chains in this process. Exposing cultured MSCs to p-nitrophenol xyloside (PNPX), a competitive acceptor of CS/DS chains on proteoglycans, resulted in a delay in the onset of chondrogenesis as evidenced by a rounding-up and cell aggregation into spheroids. Moreover, PNPX exposure also caused a reduced expression of the cartilage-specific genes SOX-9, aggrecan, and collagen type II, as well as a reduction in collagen type II protein. Additionally, a delayed expression of a native CD/DS sulfation motif epitope specifically recognized by the antibody 6C3 was evident in cultured cells treated with PNPX. In aggregate, these results suggest that disruption of CS/DS chain attachment to proteoglycan core proteins alters the process of chondrocytic cell aggregation, affecting subsequent cartilage formation. CS/DS chains thus appear to be intimately involved in the initial stages of chondrogenesis, likely through specific sulfation motif sequences within their glycosaminoglycol chain structure.

In cases of oral and maxillofacial surgery requiring stem cells, it would be advantageous to have a local source of bone stem cells, rather than requiring a second source, such as tibia or sternum. Pekovits et al. (2013) isolated stem cells from intraoral alveolar bone and compared their properties with those from mesenchymal stromal cells to determine whether they would be a viable alternative for applications in oral and maxillofacial tissue engineering. Assays included the ability of the cells to proliferate in culture with a well attached, spread morphology, as well as the presence of a consistent set of cell surface markers. Their results showed that human alveolar bone contains mesenchymal progenitor cells (hABDCs) that can be isolated and expanded in vitro. Further, upon appropriate stimulation, these hABDCs were shown to differentiate into an osteogenic, adipogenic, and chondrogenic cell lineage as do mesenchymal cells from other tissue targets, thereby providing a source of multipotent stem cells from an accessible tissue. Morphologically, the hABDCs resembled bone marrow mesenchymal cells in that they were mononuclear, fibroblast-like, spindle-shaped, and adhered well to plastic substrates. Thus, these hABDCs should be of special interest in cases of dental surgery, where the alveolar bone is readily accessible, requiring no additional surgical intervention, nor extra-oral donor site.

In the mammalian brain, neural stem cells produce neuroblasts, which migrate along the rostral migratory stream (RMS) while assuming a typical bipolar cellular morphology. This morphological appearance, which is maintained by the cellular cytoskeleton, seems to be a critical

characteristic for the ability of the cells to migrate. Moon et al. (2013) examined this process by investigating the immunohistochemical expression of ezrin, a member of the ezrin–radixin–moesin (ERM) family of proteins known to be involved in linking the cytoskeleton with the cell plasma membrane, in murine developing brain, adult brain, and adult neural stem cells *in vitro*. ERM proteins were found to be highly expressed in: (1) early postnatal RMS; (2) ventricular zone of embryonal cerebral cortex; and (3) *in vivo* and *in vitro* neuroblasts. Moreover, ezrin expression was found to diminish as neuroblasts migrated. *In vitro* differentiation of adult neural stem cells showed that ezrin was expressed by neural stem cells, neuroblasts, and astrocytes, but not by cells with oligodendrocytic progeny. These results suggest that ERM proteins may be involved in the process of neuroblast migration along the RMS via their actin-binding properties.

Given their potential applications in translational medicine and research, a long-term culture matrix for human pluripotent stem cells (hPSCs) has been sought. Pakzad et al. (2013) report the development of a safe and cost-effective extracellular matrix (ECM) for hPSCs which they call “RoGel,” and is derived from conditioned medium of human fibroblasts under serum- and xeno-free culture conditions. In a variety of cell assays, they showed that hPSCs survival properties, including self-renewal, pluripotency, plating efficiency, and cloning efficiency of cells grown on RoGel compared very favorably with those grown on a traditional Matrigel substrate. Since the cells could be passaged mechanically on a cold surface, long-term maintenance with a normal karyotype could be sustained. Moreover, since coated plates stored for 1 year at room temperature proved to be viable substrates for hPSC expansion, this ECM product should find wide-spread use in stem cell research and clinical applications.

Musculoskeletal system

The actin-based molecular family of myosins represents a very diverse group with structurally and functionally variable isoforms. Karolczak et al. (2013) investigated the function of the unconventional isoform myosin VI (MVI) in rat striated muscle. In rat hindlimb skeletal muscle, they detected MVI in the muscle periphery as well as in the myocyte nuclei. In longitudinally cut cryosections from both hindlimb skeletal muscle and cardiac ventricular muscle, the immunostaining for MVI revealed a 3- μ m pattern of striations, suggestive of the sarcoplasmic reticulum; MVI was also detected in sarcoplasmic reticulum fractions from both muscle types as well. Moreover, MVI was detected at the postsynaptic region of the neuromuscular junction; in denervated muscle tissue (sciatic nerve

cut model), MVI was localized to the muscle fibers themselves. Using MS on muscle homogenates, they identified at least six novel potential binding partners for MVI. Their results suggest that MVI may be involved in intra-fiber trafficking, in maintenance of the sarcoplasmic reticulum and sarcomere, and potentially in neuromuscular signal transmission and transcription.

Similar to the myosin family just mentioned, a family of myosin heavy chain (MyHC) proteins also exists, manifesting diverse cellular functions. For instance, MyHC isoforms compose part of muscle spindles, skeletal muscle mechanoreceptors that provide information on stimuli to the central nervous system, and are found in variable numbers depending upon the muscle source. Osterlund et al. (2013) performed an exhaustive combined immunohistochemical and morphometric analysis of the expression of MyHC in muscle spindle intrafusal fibers in young masseter and biceps muscles using a panel of monoclonal antibodies recognizing different MyHC isoforms. Eight MyHC isoforms were detected in both the masseter and biceps muscle sections, while individual intrafusal fibers were found to co-express 2–6 MyHC isoforms. The results demonstrated similar expression of many MyHC isoforms in intrafusal fibers from both muscle types, yet many differences were also revealed, suggesting muscle-specific proprioceptive control. Comparing their results to those previously published on adult masseter and biceps muscles again revealed many similarities of MyHC expression, suggesting early muscle spindle maturation.

Duchenne muscular dystrophy (DMD) is a muscular degenerative disease resulting from a mutation in the dystrophin gene. Cycles of degeneration and regeneration followed by proliferation characterize DMD, and these processes may be affected by prostaglandins. Since prostaglandin synthesis is catalyzed by isoforms of cyclooxygenase (COX), Flavia et al. (2013) used a knockout mouse model of DMD to investigate the expression of isoform COX-2 in the skeletal tissue. They used histochemical staining with Sirius red to assess collagen in fibrotic tissue, and immunohistochemistry to stain for COX-2 antigen. The results showed an increase in both the amount of type I collagen and immunostaining for COX-2 in intercellular spaces in the knockout animals as compared to their control littermates, suggesting that fibrotic lesions may lead to an increase in COX-2 expression in DMD.

Maximal eccentric exercise on an isokinetic dynamometer has been often used to create skeletal muscle damage for research purposes. Paulsen et al. (2013) have applied this protocol to examine the inflammatory response by determining the expression of leukocyte markers, CD11b, CD16, CD66b, CD68, myeloperoxidase, and neutrophil elastase on sections from skeletal muscle biopsies from volunteers subjected to unaccustomed eccentric exercise,

followed by an additional round of exercise 3 weeks later. Subjects received either a COX-2 inhibitor or a placebo and were then divided into three groups representing mild, moderate, and severe effects of eccentric exercise based upon reduction and recovery of force-generating capacity. The following experimental assays were then performed: (1) staining for leukocyte markers on smears prepared from whole blood; (2) immunohistochemical evaluation of leukocyte markers on sections from skeletal muscle biopsies; (3) quantitative evaluation of CD16-, CD66b-, and CD68-positive cells on immunohistochemically stained muscle cross-sections; and (4) transmission electron microscopic analysis of leukocyte cell types present in the muscle biopsies. Their results may be summarized as follows: (1) CD66b antibody can be used to detect neutrophils in human skeletal muscle, though the number of positive cells was low and there was no discernible pattern of tissue distribution in exercised versus non-exercised samples; (2) neutrophils may not necessarily be involved in exercise-induced skeletal muscle fiber injury; (3) though anti-CD68 antibody is commonly used as a macrophage marker, it recognized cells other than monocytes/macrophages in human skeletal muscle and thus cannot be used to quantify inflammatory cells in this model; (4) CD16 positive cells may represent a macrophage population involved in skeletal muscle exercise-induced degeneration; (5) muscle-force-generating capacity after a single round of exercise seems to be a strong indicator of muscle damage; and (6) individual responses to eccentric exercise must be considered when evaluating averaged data sets.

Hunt et al. (2013) also investigated the role of inflammatory cells in skeletal muscle damage and regeneration, using a contusion injury mouse model; specifically, they investigated the role of the cytokine leukemia inhibitor factor (LIF). After a contusion injury, LIF is up-regulated; moreover, muscle regeneration was adversely affected in an LIF-knockout mouse model based upon morphometric analysis. They also examined an inflammatory component of LIF-mediated muscle regeneration by injection of the myotoxic compound notexin and employing the LIF agonist MH35-BD. After injection with notexin, an up-regulation of both LIF protein and mRNA was detected to occur in a two-phase response: the first phase included an increased expression of pro-inflammatory cytokines, while the second phase included myogenic differentiation and the formation of new myotubes. Treatment with MH35-BD during the second phase of LIF up-regulation did not result in an effect on genes required for myocyte differentiation or associated with inflammation; on the other hand, treatment with the LIF agonist during the initial response phase resulted in an increased expression of gene transcripts for several pro-inflammatory cytokines, concomitant with an increased number of neutrophils infiltrating

the damaged muscle tissue. Subsequently, a decrease in myogenin mRNA was noted, with a diminished number of myotubes formed in the MH35-BD-treated versus control groups. Their results suggest that LIF functions in skeletal muscle regeneration not by directly effecting muscle cells, but rather by regulating the local inflammatory response to tissue damage.

Repair and regeneration of muscle fibers damaged by activities such as eccentric exercise as discussed above requires membrane trafficking and fusion events at the myocyte level. Since *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins are involved in membrane trafficking and fusion, Takahashi et al. (2013) used immunoblotting and immunofluorescence techniques to investigate the expression of the SNARE family member vesicle-associated membrane protein 5 (VAMP5) in skeletal and cardiac muscle tissue. Immunofluorescence analysis of skeletal muscle tissue determined variable levels of VAMP5 expression depending upon fiber type; samples expressing the highest levels were determined to be type IIa fibers based upon their myosin heavy chain subtypes (see above). Moreover, the expression patterns for VAMP5 and glucose transporter 4 were similar in skeletal muscle. In cardiac muscle sections, VAMP5 was detected in the vicinity of the intercalated discs. Thus, VAMP5 may be involved in membrane trafficking functions during repair processes in both skeletal and cardiac muscle.

Damage to skeletal muscle at the cellular level, as may occur during prolonged exercise, can be the result of the action of oxidants. Vitamin C is a potent antioxidant, and the expression of the vitamin C/sodium co-transporter SVCT2 has been shown to be preferentially expressed in oxidative slow muscle fibers and in early fusion of myoblasts. To investigate the expression of SVCT2 during development, Sandoval et al. (2013) examined chicken slow anterior and fast posterior latissimus dorsi muscles from 7-day postnatal through adulthood. They found a greater expression of SVCT2 in slow versus fast muscle fibers at all developmental stages examined; interestingly, its expression was down-regulated during postnatal development. Moreover, utilizing a myogenic cell culture system of C2C12 cells, they found that SVCT2 was expressed in a biphasic manner, with highest expression during early myoblast fusion, and tapering off during myotube growth phase. Nevertheless, the levels of SVCT2 expression may be sufficient to offset oxidative damage processes in skeletal muscles.

Respiratory and cardiovascular systems

Hypersecretion of mucin by goblet cells in the respiratory pathway may be a consequence of asthma-related

inflammation and airway remodeling. Huang et al. (2013b) performed a combined histochemical/morphometric investigation to test the effect of histamine-induced inflammation on rat trachea goblet cell population and physiology. Utilizing tracheal whole mount preparations and tissue sections from histamine-treated animals, a temporal investigation yielded the following results: (1) 5 min after treatment, leaky mucosal venules were observed and subepithelial edema ratio (the per cent of length of edema along the mucosal epithelial circumference of tracheal cross-section) was increased compared with saline-treated animals, whereas the number of goblet cells was not increased; (2) 1-day following treatment, the number of goblet cells was decreased to approximately one-half that observed after the 5-min histamine treatment, suggesting that mucus hypersecretion had occurred at this time; (3) 3 or 5 days following histamine treatment, the subepithelial edema ratio decreased, while the number of goblet cells was still diminished on day 3; in contrast, by day 5 following treatment, the number of goblet cells had returned to the number observed 5 min after treatment; and (4) 7 days following treatment, the subepithelial edema ratio had returned to the level of the saline-treated group. Their results suggest that degranulation and diminishment of tracheal goblet cells as well as mucous hypersecretion were retarded with respect to histamine-induced plasma leakage and edema.

Lung development is retarded in preterm infants, with the pulmonary cell structure lagging behind the physiological requirements for breathing. Indeed, ambient air breathing requires a specified number of mature alveolar epithelial type II cells; these cells synthesize the lipid-containing surfactant molecule, responsible for maintaining surface tension at the pulmonary air–liquid interface. Since normal alveolar oxygen pressures lead to pro-inflammatory effects, the presence of local antioxidants is necessary to negate such effects. Since the surfactant-stimulating recombinant human keratinocyte growth factor (rhKGF) has been proposed to assist in diminishing the injurious effects of hyperoxia on pulmonary epithelial cells, Koslowski et al. (2013) performed experiments to investigate the use of rhKGF in improving expression parameters of lung maturity in newborn rats subjected to hyperoxia. In response to rhKGF treatment, they found the following results: (1) increased PCNA mRNA expression, indicating enhanced cell proliferation; (2) increased expression of three genes involved in oxygen radical protection (ATP-binding cassette protein A3, thioredoxin, and thioredoxin reductase); and (3) increased mRNA levels of acyl-coA:lysophosphatidylcholine acyltransferase I (catalyzes dipalmitoyl-PC synthesis) and adipose triglyceride lipase (supplies fatty acids for surfactant PC synthesis). Given these results showing that rhKGF stimulated genes involved in alveolar type II cell proliferation and differentiation, PC

synthesis, and anti-oxidative capacity, this growth factor may be a useful alternative to glucocorticoid treatment in preventing potential impaired lung development complications in preterm infants.

Likewise, treatment of premature newborns with hyperoxic ventilation may also result in myocardial dysfunction, in addition to pulmonary distress as described above. Zara et al. (2013) examined the role of the signaling molecule NF- κ B in response to moderate and severe hyperoxia exposure in a rat neonatal model. In the first two prenatal weeks, newborn rats were exposed to either room air (normal control), 60 % oxygen (moderate hyperoxia), or 95 % oxygen (severe hyperoxia). Under these conditions, morphological analysis revealed less compact heart tissue in rats exposed to moderate hyperoxia, and a decreased number of nuclei, and thus cells, in tissue sections from animals exposed to severe hyperoxia. Moreover, the following results were observed: (1) in animals exposed to severe hyperoxia, an increased number of NF κ B-positive nuclei and p-I κ B α expression were observed compared to control air- and moderate hyperoxia-exposed animals; (2) increased pAkt/Akt ratio in animals exposed to moderate and severe hyperoxia compared with room air; (3) enhanced cytochrome *c*/Apaf-1 immunocomplex and decreased Bcl2 expression in severe hyperoxia versus moderate hyperoxia animals. Their findings suggest that the NF- κ B signaling pathway (involved in oxidative stress and inflammation) and Akt signaling may be involved in regulating mechanisms of myocardial damage in newborns induced by exposure to hyperoxia; thus, potential therapeutic interventions should take these results into consideration.

The protein kinase C (PKC) family, consisting of 12 different isoforms, is involved in a variety of cell functions, including differentiation, proliferation, and survival. One family member, PKC ϵ has been shown to assume a cardioprotective function in cardiac ischemia–reperfusion injury models. Galli et al. (2013) investigated the functioning of PKC ϵ in early cardiac gene expression using mouse cardiomyocytes and rat bone marrow mesenchymal cells. PKC ϵ overexpression resulted in a down-regulation of two early cardiac genes, *NK2 transcription factor related, locus 5 (nkx2.5)*, and *GATA binding protein 4 (gata4)*, as well as in phospho-extracellular regulated mitogen-activated protein kinase1/2 (p-ERK1/2); in contrast, p-ERK1/2 expression was decreased in *pkc ϵ* siRNA-treated cells. Pharmacological inhibition of ERK1/2 in cells resulted in a restoration of *nkx2.5* and *gata4* expression levels, indicating that MAPK signal transduction is involved in the diminished expression of cardiac genes in PKC ϵ overexpressing cells.

Saphenous vein segments are commonly used in coronary artery bypass grafting (CABG) for the surgical treatment of coronary artery disease in patients older than 70 years of age, due to the presence of atherosclerosis in their arteries. The

saphenous vein grafts used in CABG are subsequently prone to atherosclerosis, with an early step in this process the infiltration of macrophages. Malinska et al. (2013) investigated the presence of CD68-positive macrophages in saphenous veins harvested from patients arranged into the following two groups: (1) those between 50 and 70 years of age and (2) those 70 years of age or older. Using immunohistochemistry and transmission electron microscopy, they found no differences in the content of macrophages between the two age groups. They also found that macrophages were only observed in the *tunica intima* layer if also present in the *tunica media* or *tunica adventitia* layer. When macrophages were detected in the *tunica intima*, the authors found that these patients had a higher number of bypass stenoses compared with patients without CD68-positive cells in this layer. The authors speculate that the presence of CD68-positive macrophages in the *tunica intima* may serve as an early indicator of potential saphenous vein graft occlusion.

Gastrointestinal tract

Mas-related gene (Mrg) receptors comprise a family of G protein-coupled receptors proposed to be involved in pain reception. Avula et al. (2013) performed an exhaustive investigation of 19 cloned Mrg subtypes in normal and two inflamed models of mouse intestine. They employed the methods of RT-PCR, QT-PCR, and multiimmunofluorescence microscopy, to analyze the expression of these Mrg subtypes in mouse ileum and related dorsal root ganglia (DRG) in control, versus two models of inflammation utilizing either exposure to intestinal schistosomiasis or treatment with 2,4,6-trinitrobenzene sulfonic acid. They observed that most of the Mrg subtypes were only sparsely expressed (displaying a neuron-specific) pattern in the non-inflamed control ileum. In contrast, in experimentally inflamed ileum, they found the following results: (1) MrgA4, MrgB2, and MrgB8 were detected in an increased number of enteric sensory neurons and nerve fibers located in the intestinal lamina propria; (2) MrgB10 was found in both enteric sensory neurons and mast cells newly recruited to the intestinal mucosa; and (3) the Mrg10B expressing mast cells were noted to be in close proximity to nerve fibers in the lamina propria. While some differences were noted in Mrg subtype expression between the two inflammatory models investigated, in general their results have shown that multiple Mrg subtypes are up-regulated in response to intestinal inflammation; interestingly, the up-regulation of these Mrg receptors was mainly restricted to the sensory afferent neurons in the ileum and in newly recruited mast cells present therein. These results should have potential therapeutic implications for the treatment of intestinal inflammatory conditions.

Beyer et al. (2013) also investigated the characterization of enteric neuronal subpopulations. Using immunofluorescence techniques, they performed quadruple immunostaining on human intestinal segment wholemounts and double immunostaining on intestinal cryosections. The wholemounts were immunostained with antibodies against calretinin (CALR), somatostatin (SOM), substance P (SP), and choline acetyltransferase (ChAT). By immunofluorescence observation they found: (1) all SOM-positive neurons co-stained for ChAT, while 50–75 % co-stained with SP (50 % in small intestinal external submucosal plexus (ESP) and 75 % in colonic ESP); (2) by contrast, between 77 and 92 % of CALR-positive neurons co-stained with ChAT, while <4 % co-stained with SP; and (3) wholemounts stained with an antibody against peripherin (marker allowing morphological analysis) revealed that both SOM-positive and SOM/SP double-positive neurons showed uniaxial morphology. Cryostat sections doubly immunostained for SOM and SP revealed: (1) co-positive nerve fibers only detected in the mucosa; (2) SOM singly stained neuronal fibers seen around submucosal arteries; and (3) SP-singly stained fibers located in the muscularis propria. Their results indicate that the chemical code of SOM-immunoreactive human submucosal neurons is ChAT+/SOM+/SP+– and that the overall target of SOM(/SP)-neurons is the mucosa.

In mice and rats, the proximal and distal stomach are composed of mucosa of the non-glandular and glandular types, respectively. The stratified squamous epithelium of the esophagus intersects with the simple columnar epithelium of the stomach at a “squamocolumnar” epithelial junction. Since Eph receptor family proteins have been proposed to be involved in tissue interactions, including tissue-border formation, Ogawa et al. (2013) investigated the expression of EphB2 and ephrin-B1 in epithelial cells at the rodent squamocolumnar border, as well as EphB2/ephrin-B1 signaling in cultured rat gastric keratinocytes. On immunostained tissue sections they found unique staining patterns for the two proteins along the proximal-to-distal axis of the gastric epithelium across the squamocolumnar junction: staining for EphB2 was most intense near the junction, and greatly diminished in the stratified squamous epithelium a short distance away, while in contrast staining for ephrin-B1 was maximal in the stratified squamous epithelium, and sharply decreased upon approach to the squamocolumnar junction. These immunostaining results suggest that EphB2/ephrin-B1 signaling occurs mainly in the epithelium across the squamocolumnar junction, where the expression of the receptor and ligand is maximal. Moreover, their results indicate that EphB2 preferentially bound ephrin-B1. Finally, using a cell culture system, they found that cell repulsion/lateral migration was induced in gastric keratinocytes on ephrin-B1- and EphB2-coated substrates. Taken together, their results suggest that EphB2 and

ephrin-B1 molecules may be involved in boundary formation at the rodent squamocolumnar junction.

As mentioned above, protein kinase C and ERK1/2 are involved in mechanisms of cardiac muscle ischemia/reperfusion tissue damage (Galli et al. 2013). Ischemia/reperfusion damage also occurs in other tissues, such as intestine. Chen et al. (2013) have used their well-documented *in vivo* cryotechnique (IVCT), known to preserve soluble cellular components (such as signal transduction molecules) to investigate the involvement of the ERK1/2 signal transduction pathway after mouse intestinal ischemia/reperfusion by immunostaining for pERK1/2 and pCREB on frozen, freeze-substituted tissue sections. In control tissue sections, ERK immunoreactivity was widely observed in the cytoplasm of intestinal epithelial cells, whereas pERK1/2 was much more restricted in distribution to some epithelial cells present in the crypt region and at the tops of villi. Following a 5-min exposure to ischemic conditions, more pERK1/2 immunoreactivity was detected in epithelial cells of the crypts; after 60 min of reperfusion, pERK1/2 expression was detected in wide areas of intestinal epithelial cells. After 20 and 60 min of ischemia, pCREB immunoreactivity was found in the nuclei of epithelial cells from the same areas found to be stained for pERK1/2. 20 min of ischemia followed by 60 min reperfusion resulted in a reduction in pERK1/2 immunoreactivity in the crypt epithelial cells, whereas, in contrast, 60 min of ischemia followed by 60 min of reperfusion resulted in strong pERK1/2 immunoreactivity in crypt epithelial cells. These results indicate a rapidly changing expression of the signaling molecule pERK1/2 in intestinal epithelial cells in response to an ischemic insult, with or without reperfusion, suggesting a role in regulation of cell survival.

Central nervous system

Asan et al. (2013) published a most informative and timely review on the serotonergic innervation of the amygdala. The importance of amygdala lies in the fact that it is a core component of neural circuits that mediate processing of emotional, particularly anxiety and fear-related stimuli, across species. Furthermore, it plays a key role in the central nervous system stress response, and alterations in amygdala responsivity are found in neuropsychiatric disorders, especially those precipitated or sustained by stressors. Specifically, the authors in their review focused on organizational principles of the amygdala in rodents, non-human primates, and humans. They summarized and critically analyzed findings on the origin, morphology, and targets of serotonergic innervation, the distribution patterns and cellular expression of serotonin receptors, and the consequences of stress and pharmacological manipulations of serotonergic

transmission in the amygdala with focus on the extensively studied basolateral complex and central nucleus.

By using their advanced *in vivo* cryotechnique, Ohno and coworkers analyzed angiotensin II receptors in the cerebellum and adrenal gland of mice (Huang et al. 2013c). By immunohistochemistry, the cerebellar molecular layer showed strongest staining that overlapped with immunostaining for glial fibrillary acidic protein. Reduction in immunostaining intensity for angiotensin II receptors was observed following 5 and 10 min of hypoxia or the administration of the angiotensin II receptor antagonist losartan. The immunoreactivity in the adrenal gland which was defined to the zona glomerulosa did not change following hypoxia. These data provided another example of the superior performance of the *in vivo* cryotechnique for the immunolocalization of receptors.

Elongation factor 1A (eEF1A) is well known for its function in protein *de novo* synthesis. In addition, it has non-canonical functions related to actin bundling and it interacts with microtubules. Becker et al. (2013) investigated the effects of two mammalian eEF1A isoforms on the scaffold protein gephyrin, which plays an important role for concentrating receptors in postsynaptic membranes of the central nervous system. They observed partial colocalization of gephyrin and F-actin along filamentous structures in rat hippocampal neurons. Following a 3-week period of eEF1A overexpression, cultured hippocampal neurons showed an increase in number, size and density of postsynaptic gephyrin clusters. The authors proposed that the observed effects of eEF1A overexpression indicate the stabilization of gephyrin at inhibitory synapses and that this may serve to maintain the synaptic homeostasis in neurons.

Detailed information concerning the influence of brain 5-hydroxytryptamine (HT) deficiency on the GABAergic networks in specific limbic areas is lacking. Waider et al. (2013) have analyzed heterozygous and homozygous tryptophan hydroxylase-2 knockout mice to assess the influence of 5-HT deficiency on the GABA system. In heterozygous, but not in homozygous knockout or wild-type mice, the amygdala had significant increased GABA concentrations, whereas GABA concentration of the prefrontal cortex was significantly decreased. Remarkably, the hippocampus of homozygous knockout mice had increased GABA concentrations. Likewise, although the total cell density was unchanged, the density and number of GABAergic interneurons in the anterior basolateral amygdala of homozygous knockout mice was significantly decreased. The authors conclude that the effects of reduction or complete lack of brain 5-HT transmission in limbic regions they observed provide a basis for additional detailed analysis and will help to explore more deeply the regulation of emotional behavior.

Walton et al. (2013a) have performed an immunohistochemical analysis on the usefulness of neural stem and progenitor cell (NPC) markers in the dog. The choice of a canine model resulted from the notion that the canine brain as compared to rodent brains is more similar to the human brain in its physical organization and that many well-defined neurological diseases in the dog serve as analogs of human brain diseases. The distribution of NPCs in the three major postnatal neurogenic regions, the rostral subventricular zone (SVZ), hippocampus, and cerebellum was analyzed using antibodies against CD15, CD133, nestin, GFAP, and phosphacan. Taken together, their results demonstrated that in the dog, immunoreactivity for these markers was detected in regions known to be neurogenic in rodents and primates.

Zeng et al. (2013b) reported on the expression of P2X₅ receptor subunit in neurons of dorsal root ganglia of various mammalian species. They observed quantitative differences with low levels of immunostaining in rat dorsal root ganglia, but high levels in mouse and guinea pig. In contrast, only a few neurons were immunoreactive for P2X₅ receptors in cat. At the cellular level, the P2X₅ receptor in mouse dorsal root ganglia was expressed largely by medium-diameter neurons and less in small and large neurons. However, in the guinea pig, P2X₅ receptor expression was greatest in small-diameter and less in medium- and large-diameter neurons. In addition, results of codistribution analysis with the neurofilament marker NF-200, the calcitonin gene-related peptide, calbindin, and nitric oxide synthase were reported.

Uno et al. (2013) have performed a comparative immunohistochemical investigation on the spatial relationship between Rab family GTPases and neuropeptides in the brain of the silkworm, *Bombyx mori*. Although the importance of Rab proteins for vesicular transport is well established in many animal species, this was the first report to indicate that Rab 1, 7, and 14 may be involved in the transport of various neuropeptides in the brain of insects.

Peripheral nervous system

Taste buds of the oral cavity are composed of gustatory cells which sense nutrients. Protein-rich foods elicit a taste perception called umami, which is mainly caused by monosodium glutamate and mediated by the heterodimer taste receptor T1R1 and T1R3. Haid et al. (2013) reported that the gustatory sensory cells additionally express a peptone receptor known to be expressed in gastric enteroendocrine cells. This receptor, GPR92, is activated by peptones which represent a mixture of small molecular mass peptidic fragments and free amino acids. They found that T1R1-positive gustatory cells also express the peptone receptor GPR92.

It was concluded that the co-expression of the amino acid receptor T1R1 + T1R3 together with the peptone receptor GPR92 in umami cells may make these cells more effective for monitoring the protein content of ingested food.

GABA as the main inhibitory neurotransmitter in the central nervous system appears to exist also in elements of the peripheral nervous system such as neuroepithelial bodies which are clusters of pulmonary endocrine cells found in the airways. Schnorbusch et al. (2013) have conducted a study of the mouse airways to gain insight into the GABAergic system of its neuroepithelial bodies. They detected not only immunoreactivity for glutamic acid decarboxylase 65/67 and for the vesicular GABA transporter in the neuroepithelial bodies, but also the two GABA_A and GABA_B (R1 and R2) receptors by combined laser microdissection—RT-PCR and by immunohistochemistry. Additional support was obtained in elegant GAD67-green fluorescent protein knockin mice showing expression of the hybrid protein in all pulmonary neuroepithelial bodies. The authors rightfully propose that the GAD67-GFP mouse model will be of great importance for future functional imaging and gene expression analysis of the mouse neuroepithelial body microenvironment.

The carotid body is composed of two main cell types, the chemoreceptor cells (glomus cells or type I cells) and sustentacular cells (type II cells), and represents a peripheral chemoreceptor station for sensing changes in the pO₂ and the pCO₂/H⁺. Since it was proposed that serotonin (5-HT) has an impact on the chemosensory activity of carotid bodies, Yokoyama et al. (2013) asked whether 5-HT synthetic activity and 5-HT transporter were detectable. Few glomus cells were immunoreactive for 5-HT, whereas all of them were positive for tryptophan hydroxylase 1 and the 5-HT transporter SERT. In addition, nerve fibers were positive for SERT as well as varicose nerve fibers that were immunoreactive for dopamine beta-hydroxylase, but not in nerve fibers immunoreactive for vesicular acetylcholine transporters or nerve terminals immunoreactive for P2X₃ purinoreceptors. It was concluded that the chemosensory activity of carotid bodies is regulated by 5-HT synthesized and released by glomus cells and sympathetic nerve fibers.

Neuroepithelial and carotid bodies constitute highly confined and specialized elements of the peripheral nervous system. However, the largest part of the peripheral nervous system is represented by the enteric nervous system. It not only consists of an intricate network but is also composed of different neuronal subclasses with different phenotypes and functions. Both the fibroblast growth factor-2 (FGF2) and the glial cell line-derived neurotrophic factor are important for the formation and function of the enteric nervous system. Hagl et al. (2013) investigated primary myenteric plexus cultures and wholemount preparations from FGF2-knockout mice. To analyze complex

geometric shapes, they used fractal dimension (D) to classify the subclasses of enteric neurons. By their fractal analysis, seven different neuronal subtypes could be differentiated according to a rising D . Within the same D , the neurite length revealed significant differences between wild-type and FGF2-knockout cultures. The subclass distribution was also altered in the knockout mice and showed a reduction in a secretomotor neuronal type. In wholmount preparations, a reduction in up to 40 % of these neurons was observed. It is concluded that FGF2 as compared to the glial cell line-derived neurotrophic factor plays a more important role in the fine tuning of the enteric nervous system during development.

Schmidt–Lanterman incisures are characteristic features of peripheral myelinated nerves and formed by Schwann cells. The presence of various adhesion molecules in these structures indicates their importance for maintaining the internodes. Previously, Ohno and coworkers and others reported on the membrane skeletal molecular complex composed of 4.1G, a membrane-associated guanylate kinase, membrane protein palmitoylated 6 (MPP6), and cell adhesion molecule 4 in Schmidt–Lanterman incisures. In continuation of these studies, Terada et al. (2013) identified and localized a signal transduction protein, Src, in the Schmidt–Lanterman incisures and analyzed its phosphorylation state in Y527 and Y418 under normal conditions and after deletion of 4.1G. In normal nerves, strong staining for P527-Src was detected in the Schmidt–Lanterman incisures. In 4.1G-deficient nerve fibers, which were devoid of both 4.1G and MPP6, P418-Src immunostaining in Schmidt–Lanterman incisures was strong in contrast to the weak staining in normal nerve fibers. Apparently, Src and MPP6 formed complexes as indicated by immunoprecipitation analysis. Together, it was concluded that the Src-MPP6-4.1G complex in Schmidt–Lanterman incisures functions in signal transduction.

Excretory glands

As already mentioned, gustatory cells of taste buds of the oral cavity contain various taste receptor types. In the vallate taste buds of guinea pigs, gustducin, which belongs to the taste reception–transduction system, was previously detected. In extension of their earlier studies, Ibirá et al. (2013) demonstrated gustducin-immunoreactive cells in von Ebner's glands, a minor salivary gland, and compared their characteristics with the vallate taste bud cells. Apparently, gustducin-immunoreactive cells in both locations had many features in common, such as being immunoreactive for type III inositol 1,4,5-triphosphate receptor, phospholipase C β 2, and villin and partly immunoreactive for neuron-specific enolase and calbindin D-28K. In von Ebner's

gland, gustducin-immunoreactive cells corresponded to clear cells devoid of secretory granules which were situated in the gland end portion. Based on these findings, it appears that the gustducin-positive cells in vallate papillae and von Ebner's glands function in chemoreception.

Protease-activated receptors (PARs), as their designation implies, become activated by proteolytic cleavage, and constitute a family of proteins playing a role in inflammation and pain. Activation of PAR2 in excretory glands triggers secretion. Oikawa et al. (2013) investigated the possible influence of PARs on intracellular Ca²⁺ concentration in acinar cells of rat lacrimal gland. They identified solely PAR2 in lacrimal glands; its activation by trypsin or an activating peptide, resulted in an increase in intracellular Ca²⁺ concentration. Several other experimental conditions confirmed this finding and altogether indicated that PAR2 activation primarily caused mobilization of Ca²⁺ from intracellular Ca²⁺ stores. It was proposed that the PAR2-activating peptide predominantly regulates non-capacitative calcium entry.

Sjögren's syndrome is an autoimmune disease of lacrimal and salivary glands characterized by inflammation and neovascularization associated with destruction of the secretory gland cells. Lisi et al. (2013) have analyzed the role of pro-inflammatory cytokines in Sjögren's syndrome. Specifically, they analyzed the growth related oncogene-alpha (GRO- α) and its receptor CXCR2 and detected high levels in tissue of patients suffering from Sjögren's syndrome. In addition, in cultured human salivary gland epithelial cells exposed to the pro-inflammatory cytokines IL-6 and TNF- α , CXCR2 was found to be increased in a cytokine- and time-dependent manner. They propose that the GRO- α /CXCR2 complex could represent a novel therapeutic target for chronic inflammatory diseases such as Sjögren's syndrome.

Kidney and urinary bladder

Intracellular vitamin C counteracts damaging effects caused by reactive oxygen and nitrogen species and additionally represents a cofactor in enzymatic reactions involved in the synthesis of collagen, steroids, and neuropeptides. Two different isoforms of specific ascorbate sodium-dependent co-transporters, namely SVCT1 and SVCT2, have been identified. In the kidney of adult human and mouse, SVCT1 is present in the apical plasma membrane of proximal tubule epithelia. Nualart et al. (2013) analyzed the expression of both co-transporter isoforms during perinatal development of mouse kidney. By RT-PCR and immunohistochemical analyses, SVCT1 expression increased progressively during postnatal kidney development, although in embryonic kidney SVCT1 transcripts were barely detected, if not

absent. However, SVCT2, the high-affinity transporter, was strongly expressed in the developing kidney from embryonic day 15, but decreased during postnatal stages. Immunohistochemical analyses showed a changing pattern of subcellular distribution of SVCT2 during kidney development. In cortical epithelial cells, intracellular distribution of SVCT2 was observed at embryonic day 19 and along the basolateral plasma membrane domain at postnatal day 1. On the other hand, SVCT2 was detectable in the apical and basolateral membrane domains between embryonic day 17 and 19 in medullary tubular epithelia, but was intracellular at postnatal day 1. In support of these findings, functional expression of SVCT2, but not SVCT1 was observed in human embryonic kidney-derived HEK293 cells. These changing expression patterns suggested differential roles of the SVCTs during the ontogeny of kidney tubular epithelial cells.

The urothelium covers the surface of the urinary tract, and its primary function is to act as a barrier against bacterial infection and for the control of solute passage. The upper cell layer of the urothelium is made up by the specialized superficial or umbrella cells that function in maintaining the permeability barrier. A characteristic morphological feature of umbrella cells is discoidal/fusiform endocytic vesicles. They have a well-known function as a membrane recycling reservoir which is related to the filling and voiding phases of the urinary bladder. In their review, Grasso and Calderon (2013a) highlight and discuss newly discovered functions of the fusiform cell's vesicles in addition to their role in membrane recycling and in the control of membrane permeability. The same authors (Grasso and Calderon 2013b) reported original work on the functionality of endocytic vesicles depending on the membrane lipid composition of umbrella cells. They observed differences in the traffic of fluid phase and membrane-bound probes. In animals fed an oleic acid-enriched diet, as compared to linoleic acid-enriched or control diet, a lower endocytic capacity and a reduced delivery to lysosomes was observed.

It was proposed that the membrane lipid composition/organization may be involved in the regulation of the balance between membrane endocytosis followed by lysosomal delivery on one side and plasma membrane recycling on the other.

As already mentioned, a main function of the umbrella cells of the urothelium is related to the permeability barrier. An injury of the epithelium requires a fast regenerative response. Erman et al. (2013) investigated aspects of regeneration of the urothelium after chitosan-induced injury in an ex vivo system of side-by-side diffusion chambers. As a measure for a functional permeability barrier, they analyzed the high transepithelial electrical resistance (TEER). Following a 20-min treatment with chitosan, a 60 % decrease in the TEER was measured, leaky tight junctions were

found and undifferentiated urothelial cells normally forming the basal layer of the urothelium were surface exposed. After about 200-min recovery, TEER returned to normal and structural regeneration continued over the entire observation period of 350 min. Importantly, a rapid formation of functional tight junctions occurred in the ex vivo system preceding the terminal differentiation of umbrella cells. The ex vivo system of urinary bladder is proposed to represent a useful system to study aspects of regeneration.

Mitsui and Hashitani (2013) performed an immunohistochemical study on the suburothelial microvasculature of mouse bladder. In their previous work, they observed that suburothelial venules, in contrast to arterioles, exhibit spontaneous phasic constrictions indicating their possible role in active drainage of the microcirculation. Here, they used wholemount bladder mucosal preparations immunostained for α -smooth muscle actin. Suburothelial arterioles showed circular smooth muscle cells, whereas the venules, especially larger ones, displayed smooth muscle cells with a more complex morphology which formed a complex meshwork with processes extending from their cell bodies, e.g., stellate-shaped cells. In contrast to mouse bladder, the venules and arterioles of the rat bladder had circular smooth muscle cells. Additional studies on the innervation of mouse bladder submucosal venules and arterioles demonstrated that both were targets of sympathetic, cholinergic, and primary afferent nerve fibers.

Male and female reproductive systems

Germ line cells contain a unique compartment which is known as nuage, and based on morphological grounds, six structurally different nuages can be distinguished. Many of the nuage proteins are RNA-binding proteins, and mice with null mutations of nuage proteins have defects in spermatogenesis, and hence are infertile. In continuation of their previous work, Yokota and coworkers (Takebe et al. 2013) investigated the MAELSTROM (MAEL) protein. They focused on the localization and chronology of expression of MAEL in developing spermatogenic cells, the localization of MAEL in nuage and non-nuage structures, and the spatial relation of MAEL with DDX4, DDX25, and MIWI in nuage and non-nuage structures. In mice, MAEL is a component of meiotic nuage, in which MAEL interacts with DDX4, MILI, and MIWI. MIWI proteins are critical for integrated localization of MAEL in male germ cells. Various lines of evidence indicate that MAEL is essential for spermatogenesis and transposon repression in meiosis. In the present work, MAEL was localized by immunofluorescence microscopy in inter-mitochondrial cement, irregularly shaped perinuclear granules and satellite bodies of pachytene spermatocytes, and in chromatoid bodies

of spermatids. The satellite bodies appeared exclusively in pachytene spermatocytes at stages IX–X and were strongly positive for MAEL. In step 12–19 spermatids, many granules stained for MAEL but not DDX4. By immunoelectron microscopy, these granules were confirmed to be non-nuage structures, such as mitochondria-associated granules, reticulated body and granulated body. In the neck region of late spermatids and sperm, MAEL-positive small granules were found. MAEL was colocalized with MIWI in nuage and non-nuage structures. The results suggest that MAEL may function in non-nuage and nuage structures and interacts with MIWI.

The ubiquitin–proteasome system is best known for its role in ER-associated degradation of misfolded proteins. However, it has other, quite different functions, and there is evidence that it may play a role in sperm head shaping. Zhao et al. (2013) set out to disclose aspects of the underlying mechanism. For this, they detected by Northern blotting, in situ hybridization, and immunohistochemistry the E3 ubiquitin ligase MARCH7 in rat testis. By in situ hybridization, spermatogonia showed a weak signal which gradually increased in intensity during their development. By immunohistochemistry, MARCH7 was detected in spermiogenic cells from late round spermatids to elongated spermatids and in epididymal spermatozoa. In addition, MARCH7 immunostaining was detected in the caudal end of the developing acrosome of late round and elongating spermatids in codistribution with β -actin, a component of the acroplaxome. MARCH7 was also detected in the developing flagella, and its expression levels were prominent in elongated spermatids. Altogether, these results were interpreted as evidence that MARCH7 is involved in spermiogenesis by regulating the structural and functional integrity of the head and tail of developing spermatids.

Liprin α 3 is a synaptosomal protein with a known function in membrane fusion during neurotransmitter release. Somewhat surprising, it was identified in sperm by proteomic analysis. Based on similarities between its function at synapses and the acrosome reaction, Joshi et al. (2013) reported a detailed analysis of liprin α 3 in sperm. Immunoblotting of protein extracts from testis and epididymis showed it to be a 113-kDa protein. In testis, immunoreactivity for liprin α 3 was observed in pachytenes, diplotenes, and round spermatids. In spermatogenic cells, it was localized to developing acrosomes and to acrosomes of sperm. Furthermore, liprin α 3 was found to contain estrogen responsive elements by in silico analysis, and their functionality was proven by tamoxifen treatment of rats. Most important, liprin α 3 was detected by immunoblotting in epididymosomes as strong indication for a role in vesicle trafficking.

Glycosylation is a major post-translational protein modification. Protein *N*-glycosylation and protein *O*-glycosylation represent two major types of glycosylation

reaction. Among the various types of protein *O*-glycosylation, a main one is the so-called mucin-type *O*-glycosylation initiated by the transfer of *N*-acetylgalactosamine to serine or threonine residues by the family of polypeptide *N*-acetylgalactosaminyltransferases. Miyazaki et al. (2013) studied the importance of the polypeptide:*N*-acetylgalactosaminyltransferase 3 for spermatogenesis of mice. Previously, mice deficient in this glycosyltransferase have been shown to be infertile. In homozygous knockout mice, spermatozoa were not only rarely observed but also immotile and exhibited structural abnormalities of their heads. In addition, they had abnormal acrosomes and the arrangement of the mitochondria in the flagella was disturbed; this disturbance of acrosomal structures was due to inhibition of the fusion of proacrosomal vesicles into mature acrosomes. Additional analysis showed the expected drastically reduced *O*-glycosylation of acrosomal glycoproteins, in particular of equatorin. Therefore, the above-mentioned structural abnormalities due to *O*-glycosylation deficiency in polypeptide:*N*-acetylgalactosaminyltransferase 3 knockout mice resulted in oligoasthenoteratozoospermia.

The human placenta is an organ of crucial importance for the exchange of gases and solutes, as well as biomolecules between mother and fetus. Larger molecules such as immunoglobulins of the mother must be carried by endocytic vesicles to the fetus. For this vital process, syncytiotrophoblasts are important. Walton et al. (2013b) reported novel aspects of placental transcytosis based on results of their proteomic findings that syncytiotrophoblast apical plasma membranes contain flotillin-1 and flotillin-2. By immunomicroscopical analysis of first trimester and term placentas, they showed the presence of flotillin-1 and flotillin-2 most prominently in villous endothelial cells and cytotrophoblasts, and less prominently in syncytiotrophoblasts. Further experiments with BeWo cells indicated that flotillin protein expression was reduced following trophoblast syncytialization. Single or combined knockdown of the flotillin protein(s) resulted in reduced endocytosis of cholera toxin B subunit. The authors concluded that flotillin-mediated endocytosis is important in cytotrophoblasts and endothelia, but does not appear to be a major pathway in syncytiotrophoblasts.

In humans, intrauterine fetal demise represents a multifaceted problem with far reaching consequences. Londero et al. (2013) analyzed cases of intrauterine fetal demise that occurred after 34 weeks of gestation in regard to placental human chorionic gonadotropin expression at the third trimester and free β -hCG levels measured at 11–13 weeks of gestation. Furthermore, immunohistochemical data from TMA were presented. Their detailed analysis indicated that low human chorionic gonadotropin expression was an independent risk factor for intrauterine fetal demise.

Members of TGF- β superfamily such as nodal play a major role in the endometrial changes involved in the establishment and maintenance of pregnancy. Arganaraz et al. (2013) have studied the possible role of nodal in the cattle reproductive process by analyzing its expression pattern and localization in the oviduct and uterine horn during the estrus cycle and early pregnancy. They were able to detect nodal both in oviduct and uterus during either the estrus cycle or pregnancy. However, nodal showed a differential expression profile in the uterine horn at diestrus and pregnancy, decreasing 1.5- and 1.4-fold, respectively, in comparison with estrus. Nodal immunostaining intensity was observed in stromal and in epithelial cells of the surface and the glandular epithelium. This first analysis of nodal in the bovine reproductive tract indicated that it may be involved in the remodeling occurring in the endometrium of cattle during the estrus cycle and in the embryo implantation, and as such may represent a predictor for successful pregnancy in cattle.

Poon et al. (2013) investigated aspects of blastocyst implantation. Although the non-receptive uterine luminal epithelium is refractory to blastocyst invasion, this barrier is dismantled during implantation in an organized fashion to permit blastocyst attachment. The authors focused on claudin 7, a tight junction protein that in contrast to other members of the claudin family exhibits a distinct basolateral membrane localization, and investigated its localization, abundance, and hormonal regulation. Their results showed a distinct basal and lateral localization of claudin 7 in the uterine luminal and glandular epithelium throughout early pregnancy. On day 1, claudin 7 was abundantly present in response to ovarian estrogen, but at the time of implantation its abundance decreased independent of the presence of the blastocyst. The authors concluded that claudin 7 acts as an adhesion molecule that facilitates adhesion between adjacent uterine epithelial cells as well as between uterine epithelial cells and the underlying basal lamina. Furthermore, they suggest that the drastic reduction in claudin 7 as pregnancy progresses toward uterine receptivity for blastocyst implantation is a further contributor to the plasma membrane transformation, which sees uterine luminal epithelial cells detach from the underlying connective tissue.

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