In focus in HCB

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The following two review articles appear in this issue as part of the 60th anniversary of Histochemistry and Cell Biology.

Clathrin-independent endocytosis

Endocytosis encompasses a series of complex and diverse cellular mechanisms, divided into clathrin-dependent and -independent pathways. Although clathrin-dependent endocytosis is well-studied and universally recognized (Kaksonen and Roux 2018), multiple forms of clathrin-independent endocytotic mechanisms have been unveiled over the years. Sandvig et al. (2008) described some of the processes present 10 years ago in a review commemorating the 50th anniversary of *Histochemistry and Cell Biology*. Here, Sandvig et al. (2018) present an update on research in the field of clathrin-independent endocytosis over the past 10 years for this review highlighting the 60th anniversary of the journal. The review covers the myriad of newly discovered clathrin-independent endocytotic pathways, including those involving membrane lipid structure, dynamin-dependent mechanisms (RhoA, FEME, Shiga toxin-induced tubules, and ARF6, caveolae, and EGFR-reticulon 3 independent), and dynamin-independent mechanisms (Cdc42-dependent, CtBP/BARS and associated proteins, micropinocytosis, and flotillins). They mention methodological issues, such as ensuring that visualized vesicles are not actually still attached to the plasma membrane but are indeed independent in the cytoplasm, and the need for proper controls when interpreting inhibitor and siRNA knockout studies. They also discuss the effect of cell structure and morphology, for instance polarized versus non-polarized cell phenotype on

the mechanisms of endocytosis. The review is concise and thought-provoking, revealing that much is still unknown concerning the complexity and heterogeneity of the endocytotic process.

COPII: a coat for many occasions

The transitional elements of the endoplasmic reticulum are the exit gates for transport of cargo and membrane proteins and lipids to the Golgi apparatus (Bannykh and Balch 1997; Palade 1975; Saraste and Svensson 1991). The formation of membrane buds and the pinching-off of the canonical 60–90 nm vesicles as well as the formation of their multi-component COPII coat are intimately related. The review by McCaughey and Stephens (2018) in this issue represents an update of the review written by this laboratory at the occasion of the 50th anniversary of the journal (Hughes and Stephens 2008). It highlights new discoveries on the molecular interactions between the different COPII components during coat assembly and their ability for cargo recognition as well as the high flexibility of the coat. Disease-linked mutations of COPII components such as of Sec23A, Sec24B, or Sar1B causing cranio-lenticulo-sutural dysplasia, neural tube defects or chylomicron retention diseases, respectively, are discussed. Furthermore, the role of newly identified coat components such as TANGO1, cTAGE5 and TALI in the formation of COPII vesicles large enough to accommodate > 300 nm trimeric procollagens or large lipoprotein particles for export from the ER to the Golgi are detailed. Recent discoveries on the flexibility of the COPII coat formation and multiple modes of regulation as they relate to involvement of COPII in additional functions such as ER protein quality control and ER stress, autophagy, and virus replication are emphasized.

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High-resolution correlative focused ion beam/scanning electron microscopy

Several techniques are currently available for investigating the three dimensional (3D) reconstruction of cellular ultrastructure. Each of these techniques, including cryo-TEM tomography, serial block face sectioning, array tomography, and focused ion beam scanning electron microscopy (FIB/SEM) suffer from some limitations preventing them from becoming a routine high-resolution 3D method. Moreover, the ability to routinely correlate these ultrastructural 3D data sets with whole cell imaging by light microscopy would prove to be a most useful advantage. Luckner and Wanner (2018) now provide a most detailed methodological manuscript in which they present technical improvements to perform correlative light and electron microscopy (CLEM). Their improvements include: (1) use of laser-etched custom-labeled slides and coverslips with coordinates allowing precise relocation on the sample; (2) embedding of cells in thin or (ultra)thin resin layers; and (3) potential to achieve the highest resolution possible with the FIB/SEM technique in *x*, *y*, *z* planes. These methodological improvements allowing regions of interest defined by LM to be easily relocated by SEM resulted in an achieved voxel size of $2 \times 2 \times 2$ nm over hundreds of sections, demonstrated by visualizing several 3D ultrastructural features within mouse C2C12 myoblast and HeLa cells. In particular, features including ultrastructural changes occurring during mitosis such as shuttling between nuclear envelope and endoplasmic reticulum, formation of midzone and midbody, Golgi apparatus dissolution and reformation, and interconnected endoplasmic reticulum, endosomes, and lipid vesicles. The manuscript is beautifully illustrated, including several high-resolution movies provided in the Supplemental Data online. The authors conclude with suggestions of which 3D techniques to apply depending upon the specific sample and scientific question to be addressed.

Spermatid-Sertoli cell contacts during migration are mediated by VE-cadherin

A final step of spermiogenesis is the release of spermatozoa into the lumen of seminiferous tubules (O'Donnell et al. 2011; Wen et al. 2016; Xiao et al. 2014). During their migration along the seminiferous epithelium, spermatids are in continuous contact with Sertoli cells (Wen et al. 2016; Xiao et al. 2014), which involves cadherins (Byers et al. 1994; Preissner and Bronson 2007). In continuation

of previous studies (Aivatiadou et al. 2007), Berruti et al. (2018) now report the VE-cadherin distribution and that of tyrosine-phosphorylated (pY₆₅₈) VE-cadherin in the mouse seminiferous epithelium. They found cell surface VE-cadherin in early differentiating round spermatids which decreased progressively during spermatid elongation due to its internalization. On the other hand, pY₆₅₈ VE-cadherin was associated with elongated spermatids and decreasing contacts with Sertoli cells, which was interpreted to indicate its role in the acquisition of spermatid cell polarity. It was suggested that spermatid-Sertoli cell contacts through VE-cadherin take place along the entire surface of round spermatids, and that this cell–cell contact successively decreases with the acquisition of cell polarity. Support for this interpretation came from analyses of iRap1 transgenic mice.

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