In vivo analysis of vessel fusion during DLAV formation in the zebrafish embryo

Lukas Herwig, Alice Krudewig, Yannick Blum, Elin Ellertsdottir, Anna Lenard, Heinz-Georg Belting and Markus Affolter
Biozentrum der Universität Basel, Klingelbergstrasse 70, CH-4056 Basel

To form an interconnected network of endothelial tubes, a number of vessels of the developing vasculature have to interact and fuse to each other. While much is known about vessel sprouting, very little is known about the vessel fusion, its molecular control and its regulation. We are studying the development of the intersegmental vessels (ISVs) of the zebrafish embryo. Angiogenic sprouts from neighbouring segments contact each other and fuse to give rise to a fully lumenized dorsal longitudinal anastomotic vessel (DLAV). In order to characterize how tip cells first contact each other and then eventually become part of a lumenized tube, we have generated a transgenic fish line expressing an eGFP-fused version of the AJ/TJ protein ZO1. eGFP-ZO1 co-localized with endogenous ZO1 and VE-Cad throughout ISV and DLAV formation and thus serves as a faithful marker for the presence and the de novo formation of AJ/TJ and the outline of endothelial cells. We find that during DLAV formation, tip cells contact each other via filopodial extensions and subsequently establish new contact points by localizing eGFP-ZO1 and VE-cad to such site of contact. These point-like contact sites subsequently extend into ring-like structures, suggesting that a pre-apical spot is elaborated into a larger apical membrane compartment between two tip/fusion cells. In 60% of our in vivo time-lapse movies, we find that the lumen grows or extends through the tip cells from the proximal-most position to the novel, more distal second apical side of the tip/fusion cell. This hollowing process appears to be connected to, or dependent on, the lumenization of the ISV stalk, suggesting that apical membrane growth and membrane invagination into the fusion cell are driven by luminal pressure. At the end of the lumen-forming process, most of the fusion cells are characterized by an intracellular lumen and three apical faces, one towards the stalk and one each on the anterior and posterior fusion point along the DLAV. This process of vessel fusion in the zebrafish embryo is very similar to the process described during fusion of adjacent tracheal metameres in *Drosophila melanogaster.* Interestingly, we also find that a second mechanism, similar to what has been described in the formation of the lumen in the notochord of *Ciona intestinalis*, is involved in vessel fusion. In this scenario, the two apical membrane domains of a fusion cell are brought together and eventually merge through cell rearrangements, resulting in an extracellular lumen embedded a regular, multicellular tube. Endothelial cells are thus rather plastic during the fusion process and vessel fusion can arise through distinct mechanisms.