

**P038** Trafficking of dense-core vesicles containing ZnT-8 during exocytosis in insulin-secreting beta cells  
**T.K.Taneja, T. Nicolson and G.A. Rutter**  
*Imperial College London, UK*

Aims: ZnT-8, encoded by *SLC30A8*, is a member of the zinc transporter family, restricted to the pancreas and previously shown to co-localise with insulin-containing secretory granules. Polymorphisms in the *SLC30A8* gene are associated with Type 2 diabetes whilst *SLC30A8* antibodies are found in ~50% of type 1 diabetic patients. Here, we investigated the intracellular trafficking of ZnT8 following cell stimulation. Methods: A *c-myc* tag was incorporated in the predicted extracellular and intracellular loops between 3<sup>rd</sup>-4<sup>th</sup> and 4<sup>th</sup>-5<sup>th</sup> transmembrane domains respectively of the human *SLC30A8* gene cloned into pcDNA3. A monoclonal anti-*c-myc* antibody was used to label vesicles containing ZnT-8 that were exposed at the surface of transfected INS-1(832/13) cells in response to 15min stimulation with D-glucose, forskolin, and IBMX. To assess co-localisation with insulin, cells were further incubated in RPMI without primary antibody and then fixed before staining with an anti-insulin antibody (Dako). Fixed cells were permeabilised and stained with Alexa-568 or -488 tagged secondary antibodies, respectively. Results: Following stimulation of exocytosis, *c-myc*-tagged ZnT-8 was internalised into vesicles that recycled to an insulin-containing compartment. Conclusions: These data suggests that ZnT8-containing dense core vesicles traffic to the cell surface upon stimulation, and are then either recaptured following partial release of insulin (“cavity recapture”) or traffic back into insulin-positive compartments.