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► To cite this version:

Raphaël Boisgard, Eric Chanat. Investigation of trans-golgi network (TGN) to cell surface transport of sulphated secretory proteins in mammary epithelial cells. ECBO 1997, 1997, Brighton, United Kingdom. 1997. hal-02834501

HAL Id: hal-02834501

<https://hal.inrae.fr/hal-02834501>

Submitted on 7 Jun 2020

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INVESTIGATION OF TRANS-GOLGI NETWORK (TGN) TO CELL SURFACE TRANSPORT OF SULPHATED SECRETORY PROTEINS IN MAMMARY EPITHELIAL CELLS.

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Mechanisms involved in the transport of milk protein to the apical cell surface of mammary epithelial cells are not well understood. A key step in the secretory pathway is the formation of secretory vesicles from the TGN. The approach we have adopted in order to investigate the molecular mechanisms underlying this budding event employs pulse-chase labelling with [³⁵S]sulphate, sulphation of proteins being a TGN specific post-translational modification, followed by subcellular fractionation. Labelling of rabbit mammary gland explants revealed three major, acidic and heatstable, sulphated proteins. These secretory proteins, which remained to be identified, are mostly sulphated on carbohydrates and are not associated with casein micelles. After short (4 min) pulse with [³⁵S]sulphate and fractionation using velocity sucrose gradient centrifugation, labelled proteins were detected in the middle of the gradient. When pulse-labelled cells were chased for 4 min, [³⁵S]sulphate-labelled proteins sedimented in the bottom half of the gradient due to their packaging into secretory vesicle. The respective density of the TGN and of secretory vesicles on equilibrium sucrose gradient was 1.13g/cm³ and 1.15g/cm³. The exit of [³⁵S]sulphate-labelled proteins from the TGN and their packaging into secretory vesicles during the chase has been confirmed by EM autoradiography.