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To study intracellular mechanisms of milk protein traffic to the cell surface and secretory vesicles biogenesis in mammary epithelial cells (MEC), we are establishing a in vitro system using the mouse MEC line HC11. HC11 cells grown on plastic, although heterogeneous, can easily be induced to synthesize ß casein (ßcn) in the presence of insulin, cortisol and PRL. When HC11 cells are grown on porous filters, they expressed ≥10 times more ßcn. Milk proteins secreted by these cells were essentially found in the apical medium and identified by western blotting as lactoferrin and γcn. Only a minor proportion of the cellular ßcn was found in the apical medium. Analysis by SDS-PAGE showed that ßcn from cells and medium migrated as a doublet of lower Mr. than the mature mouse milk ßcn. This increase in electrophoretic mobility suggested that this form had not underwent all post-translational modifications and was blocked in an early compartment of the secretory pathway. Consistent with this hypothesis, we observed by electron microscopy that the rough endoplasmic reticulum of these cells was highly dilated. In some experiments, HC11 cells also expressed αcns and, interestingly enough, the mature form of ßcn was observed in addition to the ßcn doublet. The amount of mature ßcn was found to be proportional to the level of expression of αcns.