



Mitotic Golgi is in a Dynamic Equilibrium Between Clustered and Free Vesicles Independent of the ER

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Golgi inheritance during cell division involves Golgi disassembly but it remains unclear whether the breakdown product is dispersed vesicles, clusters of vesicles or a fused ER/Golgi network. Evidence against the fused ER/Golgi hypothesis was previously obtained from subcellular fractionation studies, but left concerns about the means used to obtain and disrupt mitotic cells. Here, we performed velocity gradient analysis on otherwise untreated cells shaken from plates 9h after release from an S-phase block. In addition, we used digitonin and freeze/thaw permeabilization as alternatives to mechanical homogenization. Under each of these conditions, approximately 75% of the Golgi was recovered in a population of small vesicles that lacked detectable ER. We also used multicolor fluorescent microscopy with optical sectioning by deconvolution to compare the 3D metaphase staining pattern of endogenous Golgi and ER markers. Although both ER and Golgi staining were primarily diffuse, only the ER was excluded from the mitotic spindle region. Surprisingly, only 2% of the Golgi fluorescence was present as resolvable structures previously characterized as vesicle clusters. These were not present in the ER pattern. Significantly,

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