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Classification and Quantification of Autophagy in Quiescence Using Transmission Electron Microscopy

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Autophagy is a pathway of "self-eating" by which cells engulf non-specific areas of cytoplasm, traffic this material to lysosomes for degradation, and thereby reclaim amino acids to maintain metabolism during starvation [1]. It has been suggested that autophagy may not be exclusively a survival strategy to meet bioenergetic needs, but also a means to maintain genomic integrity and quality control of cellular proteins and organelles [2]. Quality control might be very important for cells in the body that spend most of their time in the reversibly arrested cell cycle phase of quiescence. Adult stem cells, lymphocytes, and fibroblasts spend much of their existence in the quiescent state waiting for cues to re-enter the cell cycle to perform tissue specific functions [3].

Autophagy has been recognized as a centrally important pathway with implications including aging and cancer, yet the tools for definitively assessing autophagy are less than perfect [4, 5]. Electron microscopy is an excellent technique for studying autophagy, as it allows researchers to morphologically classify and quantify autophagy-specific vesicles directly.

Using electron microscopy, we monitored autophagosomes (double-membrane vesicles containing mostly bulk cytoplasm prior to fusion with a lysosome) and autophagolysosomes (autophagosomes that have fused with the lysosomal compartment and may be multi-membranous) in fibroblasts under proliferating and quiescent conditions. We quantified the images with a grid method determining the percentage of cell area occupied by autophagy-specific vesicles. Quiescent fibroblasts induced autophagy as compared to proliferating cells (Figure 1). For fibroblasts maintained in the quiescent state for 7 or 14 days, autophagy-specific vesicles occupied 7.2% and 11.1% of the cell area, respectively, whereas in proliferating cells, 2.3% of cellular area was occupied with such vesicles.

Thus, electron microscopy has allowed for direct observation and quantification of autophagy in the distinct cellular states of proliferation and quiescence. Future work is aimed at elucidating why autophagy is induced, what cellular components are being ingested and degraded, and how autophagy deficiency affects quiescent cells.

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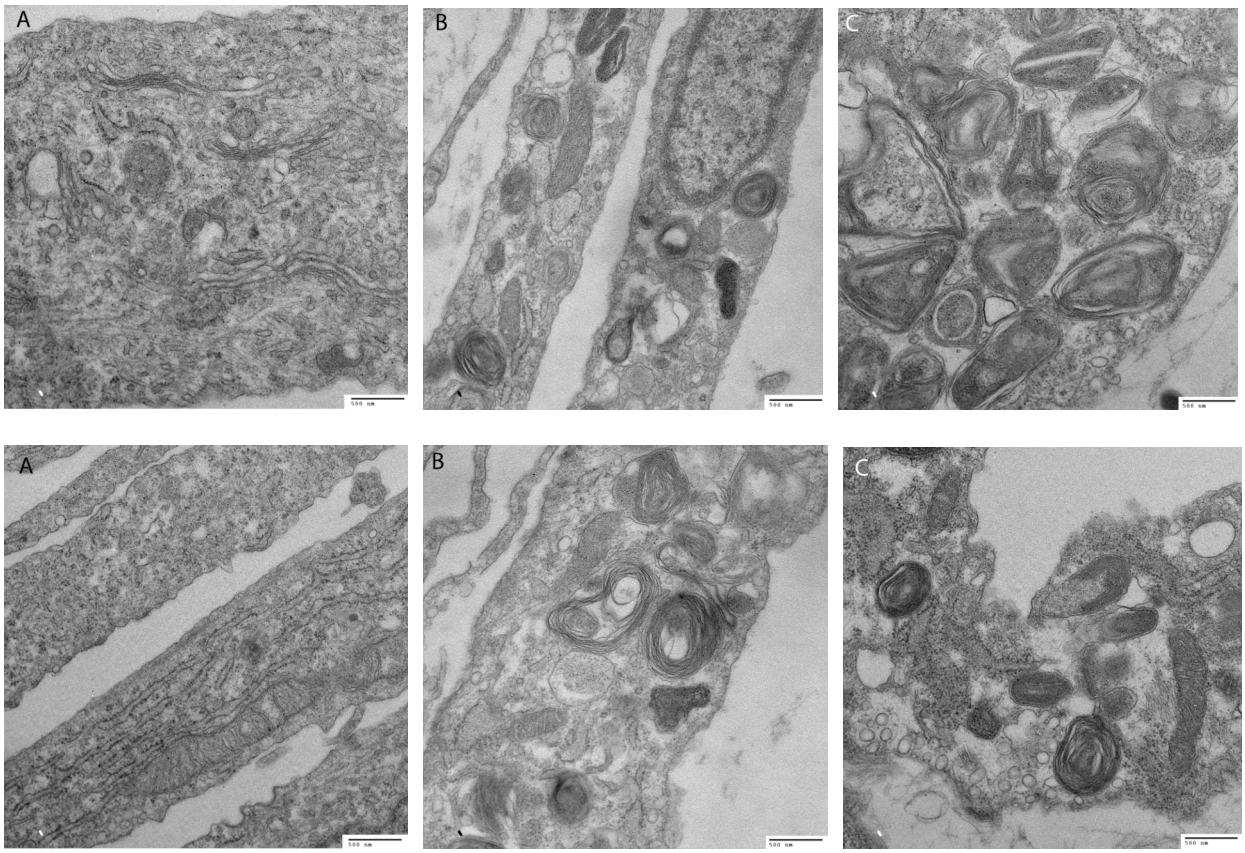

Figure 1: Autophagy induction in the quiescent state. Fibroblasts were pelleted and prepared for transmission electron microscopy using a slightly modified protocol [6]. Unstained 70 nm sections were imaged on a Zeiss912AB transmission electron microscope. (A) Proliferating fibroblasts were plated sparsely in complete growth media the day prior to sample preparation. (B) Quiescent fibroblasts were grown to confluence and maintained for 7 days with media changes once every two days. (C) Deeply quiescent fibroblasts were grown to confluence and maintained for 14 days with media changes every two days.

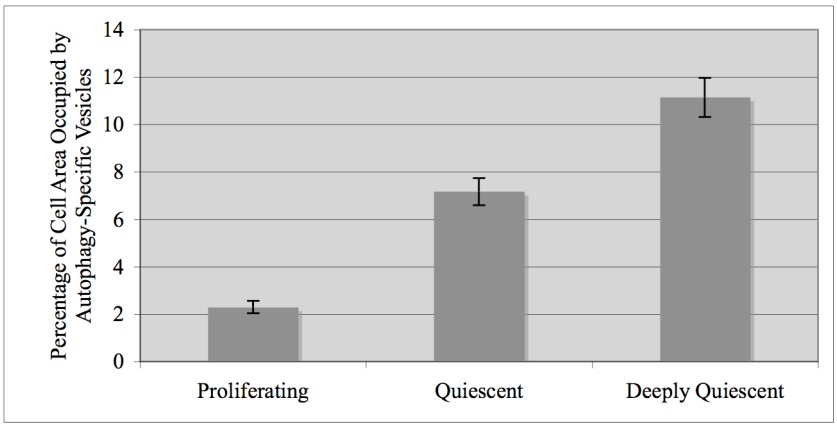

Figure 2: Quantification of autophagy induction in proliferating, quiescent, and deeply quiescent human fibroblasts. The ratio of the area occupied by autophagy-specific vesicles to total cellular area was quantified using a grid technique as described with 100 cells per condition as the sample size [7]. Error bars represent standard error.

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