Advanced Sample Preparation and Quantitative Structural Analysis in Human, Mouse, and Cell Models: Linking Organelle Morphology to Mechanistic Function

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Abstract

Understanding how organelle structure encodes cellular function remains a central challenge in cell biology, particularly in the context of stress adaptation, metabolic regulation, and disease progression. While advances in imaging have enabled detailed visualization of cellular ultrastructure, a major gap persists in the ability to quantitatively link organelle morphology to mechanistic function across biological systems. Here, we present a unified and scalable framework that integrates advanced sample preparation, high-resolution electron microscopy, and quantitative structural analysis across human tissues, mouse models, and cultured cells.

Building on established methodologies for rigorous transmission electron microscopy acquisition and ImageJ-based quantification, we combine two-dimensional ultrastructural analysis with three-dimensional reconstruction approaches to systematically define organelle architecture. Using advanced segmentation and surface-rendering workflows with platforms such as Amira, our approach enables high-fidelity reconstruction and precise quantification of mitochondrial morphology, cristae organization, organelle contact sites, and degradation machinery. These multidimensional analyses capture volumetric features, surface area, and spatial relationships between organelles, moving beyond descriptive morphology to define geometric and functional complexity within intact cellular environments.

Importantly, this framework directly links structural remodeling to mechanistic pathways. We demonstrate that mitochondrial structural plasticity is coordinated by activating transcription factor 4 (ATF4), which regulates mitochondrial remodeling and tri-organelle coupling between mitochondria, the endoplasmic reticulum, and lipid droplets. Across Drosophila flight muscle, primary myotubes, and human skeletal muscle, ATF4 promotes increases in mitochondrial length, cristae density, organelle contact sites, lipid interactions, and nanotunnel formation, enhancing mitochondrial connectivity. In contrast, loss of ATF4 results in fragmentation, reduced inter-organelle communication, and impaired cellular function. Mechanistically, ATF4 regulates NRF1 and NFE2L2 to control MFN2 expression, linking mitochondrial fusion with organelle tethering and lipid metabolic pathways.

Collectively, this work establishes a robust platform for defining organelle structure–function relationships and identifies ATF4 as a central regulator of mitochondrial architecture and inter-organelle communication.