

There's DNA in those organelles

Fractionation experiments in the early 1960s suggested that certain organelles may contain DNA (see Gibor and Granick, 1964, for review), but varying levels of nuclear contamination marred those results. Meanwhile, oddities in genetic transmission chipped away at the nucleus-only theory of genetic inheritance. But it took direct visualization to convince most skeptics that organelles could harbor genetic material.

Green DNA

First under the EM was the chloroplast of the alga *Chlamydomonas*. Ris and Plaut (1962) visualized chloroplast DNA both via direct staining by dyes and as DNase-sensitive fibers under the EM.

The fibers were evident only after the use of a staining procedure designed for bacterial cells, and this parallel gave the authors an idea. "With the demonstration of ultrastructural similarity of a cell organelle and free living organisms," stated Ris and Plaut, "endosymbiosis must again be considered as a possible evolutionary step in the origin of complex cell systems."

The idea of endosymbiosis, in which bacterial cells are engulfed and modified to become eukaryotic organelles, was first suggested for chloroplasts in 1905 and 1907. Now it was seized upon by a young graduate student in Plaut's laboratory, Lynn Sagan, who was then married to astronomer Carl Sagan. She had already seen cytoplasmic incorporation of labeled DNA precursors in amoeba (unicellular eukaryotes), but not connected this phenomenon to organelles (Plaut and Sagan, 1958). As similarities between organelles and bacteria mounted, however, Sagan was convinced that endosymbiosis was correct. She barraged over 20 journals before finding one that would publish her seminal paper (Sagan, 1967) and, after a name change to Lynn Margulis, became the consummate popularizer of this theory (Margulis, 1970).

More than respiration

The next organelles to be inspected for DNA were the mitochondria, which, says Margit Nass-Edelson, "were always looked at from the point of view of respiration [rather than replication]." Nass-Edelson was no exception to this rule. She started her postdoc intending to study whether different parts of the chick embryo showed different levels of mitochondrial respiration during development. An EM was a recently acquired tool at Stockholm University, so she and then-husband Sylvan Nass started looking. The DNA-containing regions of mitochondria "stood out because of their characteristic staining patterns," says Nass-Edelson. "As soon as this thing with the mitochondrial DNA came up, that pretty much changed the whole picture and I strictly focused on that."

Uranyl acetate treatment of the EM samples dispersed clumped fibers of mitochondrial DNA so that they resembled DNA seen elsewhere (Nass and Nass, 1963a), and after lighter fixation DNase was able to digest away the fibers (Nass and Nass, 1963b). "When we consider," said the authors, "that no other naturally occurring structure thus far studied has all the properties discussed, with respect to fixation, stabilization and staining, the unavoidable conclusion

appears to be that the mitochondrial fibers contain DNA."

Confirmation came with better isolation methods for mitochondrial (Luck and Reich, 1964) and chloroplast DNA (Sager and Ishida, 1963; Gibor and Izawa, 1963). Gibor and Izawa got around the contamination problem by extruding chloroplasts after enucleation of the giant unicellular alga, *Acetabularia*. Kiskey et al. (1965) also confirmed that DNA was present in the chloroplasts of higher plants. The eukaryotic cell had now officially become a more complicated genetic entity. **JCB**

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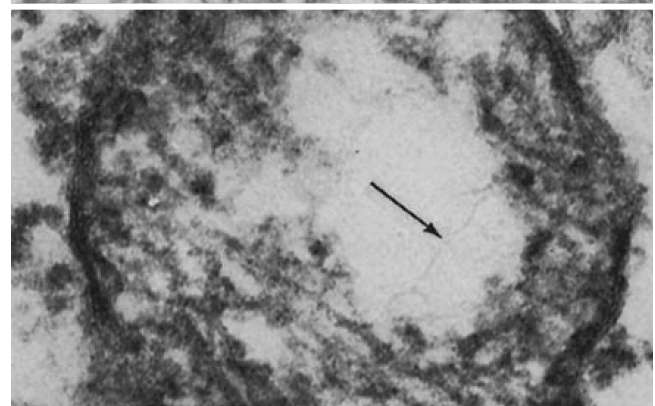
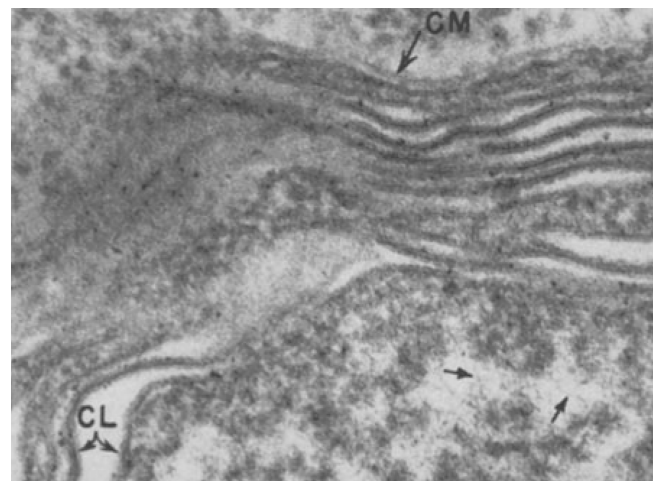
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Fibrils (arrows) represent the first sighting of DNA in chloroplasts (top) and mitochondria (bottom).