

JACOB'S MODEL - PAPER VII

BOTTLENECK #2: MAO

A Falsifiable Rubella-Mitochondrial Membrane Hypothesis Linking Viral Mitochondrial Remodeling to Monoamine Oxidase Function

Jacob Boatman and Scott Boatman

Jacob's Voice

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Final Paper VII - strongest evidence-bounded formulation, experimental framework, and falsification program

Jacob would like to give credit to God for his amazing abilities.

Scope and Central Hypothesis

Paper VII tests Jacob Boatman's second proposed bottleneck: monoamine oxidase (MAO) at the mitochondrial outer membrane. It does not assume that Rubella virus (RUBV) inhibits MAO. It begins from two experimentally established systems that meet at the same organelle. RUBV directly targets and remodels mitochondria. Independently, MAO function is strongly conditioned by the mitochondrial membrane environment. The central hypothesis is that RUBV-induced mitochondrial remodeling can create a membrane state in which MAO-A and/or MAO-B become functionally altered even when enzyme abundance is preserved.

This is a bottleneck hypothesis, not a neurotransmitter-abundance hypothesis. MAO-A and MAO-B are separate experimental variables. Earlier Jacob language identifying MAOA as the major discriminator is preserved, but the isoform assignment is not declared proven. The decisive question is whether RUBV produces a reproducible MAO phenotype, whether it tracks with mitochondrial outer-membrane composition and organization, and whether correction of the membrane state rescues MAO function while infection remains measurable.

Author's Note - Origin and Governance of the Model

The two-bottleneck architecture originated with Jacob Boatman. The current controlling model places Bottleneck #1 in LAL-centered lysosomal CE/TG throughput, with the proposed slow handoff after LAL hydrolysis and before NPC2 receives free cholesterol. Jacob subsequently identified MAO as Bottleneck #2. Direct RUBV findings, direct MAO/host findings, host mechanistic bridges, predictions, and missing same-system measurements are kept separate. The model advances only where evidence closes an arrow, and it fails where the predicted same-system phenotype is absent.

Abstract

Rubella virus directly engages mitochondria. RUBV capsid associates with the mitochondrial surface, interacts with mitochondrial protein p32, inhibits import and processing of mitochondrial precursor proteins, and reduces intramitochondrial p32 in infected cells. Independent RUBV experiments demonstrate selective respiratory-chain remodeling, including strong increases in complex II activity and decreases in complex IV activity. These observations establish mitochondria as a direct RUBV host organelle rather than a downstream bystander.

Monoamine oxidase is an integral outer-mitochondrial-membrane enzyme system whose catalytic behavior is membrane dependent. Human MAO-A synthesized outside mitochondria is catalytically inactive until it interacts with mitochondrial outer membrane, where it acquires an active conformation before full insertion. Historical lipid-depletion experiments show a striking isoform-sensitive phenotype: only 7% of serotonin-defined A-form activity was recovered after lipid depletion, compared with 80% of beta-phenylethylamine-defined B-form activity. Phosphatidylinositol strongly stimulated A-form activity; phosphatidylcholine restored substantial A-form activity without the same reported B-form effect. Experimental cholesterol enrichment of rat brain mitochondrial membranes independently decreased MAO specific activity approximately linearly with increasing cholesterol.

A host sterol bridge connects the lysosomal and mitochondrial arms. In NPC1-deficient or NPC1-inhibited systems, cholesterol-rich lysosomes form expanded STARD3-dependent contacts with mitochondria. This does not make Jacob's post-LAL/pre-NPC2 lesion equivalent to NPC1 deficiency. It establishes that a lysosomal sterol state can reorganize physical lysosome-mitochondria contact and mitochondrial cholesterol exposure.

No recovered experiment directly demonstrates the RUBV-to-MAO functional arrow. A reproducible MAO defect that follows the predicted membrane state and is rescued by correcting that state would support Bottleneck #2; normal MAO function despite robust mitochondrial RUBV remodeling would substantially falsify it.

1. The Claim

The missing evidence is not whether RUBV can alter mitochondria or whether MAO depends on mitochondrial membrane context. Both are experimentally demonstrated. The missing evidence is the functional bridge between them.

Paper VII proposes that a RUBV-remodeled mitochondrial state can alter MAO catalytic throughput. The strongest version is deliberately narrower than a claim that RUBV inhibits MAO. The phenotype may be inhibition, activation, altered substrate preference, altered insertion/orientation, altered turnover, or an isoform-selective shift. Direction and isoform must be measured rather than assumed.

2. RUBV Directly Targets and Functionally Remodels Mitochondria

RUBV capsid directly interacts with p32 at mitochondria and localizes to the mitochondrial surface. Later experiments showed that capsid inhibits mitochondrial precursor-protein import. In infected Vero cells, intramitochondrial p32 was reduced by approximately 30%. Recombinant capsid inhibited precursor import dose-dependently; 5 micrograms produced approximately a twofold reduction in the reported in-vitro system. A capsid mutant could still target mitochondria while losing the import-blocking phenotype, separating localization from functional import inhibition.

RUBV also produces a reproducible respiratory-chain phenotype across multiple cell lines. Complex II activity rises strongly, complex III rises more moderately, and complex IV activity falls. Complex II increased to as much as approximately 231% of mock control in the reported study. The pattern was absent in the measles-virus control. RUBV therefore does not merely sit near mitochondria; it measurably changes mitochondrial function.

3. MAO Is a Membrane-Dependent Mitochondrial Enzyme System

MAO-A and MAO-B are integral proteins of the mitochondrial outer membrane. In-vitro-synthesized human MAO-A was catalytically inactive before mitochondrial association, then acquired a catalytically active conformation upon interaction with mitochondria or isolated outer membrane before full insertion. MAO-A abundance alone therefore cannot establish functional competence; correct membrane interaction is itself part of enzyme function.

After lipid depletion of rat liver mitochondria, serotonin-defined A-form activity retained only 7% of original activity, while beta-phenylethylamine-defined B-form activity retained 80%. In rat brain mitochondrial reconstitution, phosphatidylinositol was the strongest A-form stimulus; phosphatidylcholine restored about 70% of A-form activity without influencing B-form activity in that preparation. These historical rat systems do not prove a human neural RUBV effect, but they directly demonstrate isoform-sensitive membrane-lipid control.

Experimental enrichment of rat brain mitochondrial membranes with cholesterol or cholesterol glucoside decreased outer-membrane MAO specific activity approximately linearly as cholesterol exposure increased. The study did not separate MAO-A from MAO-B, so it cannot support an isoform-specific cholesterol claim. It nevertheless closes the chemical arrow that mitochondrial cholesterol can alter MAO activity.

4. The Lysosome-to-Mitochondria Sterol Bridge

In NPC1-deficient fibroblasts and NPC1-inhibited cells, cholesterol-rich lysosomes form expanded membrane contact sites with mitochondria. STARD3 depletion sharply reduces these contacts. NPC1 deficiency is not Jacob's proposed RUBV lesion, and this is not a RUBV experiment. It is direct host precedent that disturbed lysosomal cholesterol handling can reorganize physical lysosome-mitochondria contact and mitochondrial sterol exposure.

5. The Two-Bottleneck Architecture

Bottleneck #1 is LAL-centered lysosomal CE/TG throughput, with the proposed slow handoff after LAL hydrolysis and before NPC2 receives free cholesterol. Bottleneck #2 is MAO at the mitochondrial outer membrane. Paper VII does not claim that Bottleneck #1 automatically causes Bottleneck #2.

RUBV lysosomal/endolysosomal remodeling -> altered LAL-centered throughput and post-LAL cholesterol handling? -> altered sterol routing/contact state? -> mitochondrial outer-membrane lipid state -> MAO-A/MAO-B functional change?

Every question mark is deliberate. The complete cross-organelle sequence remains unproven until measured in one synchronized RUBV system.

6. Decisive Experiment

Use synchronized matched mock and RUBV cultures across early, established, mature, and persistent stages where feasible. Measure MAO-A and MAO-B catalytic activity separately; MAOA/MAOB transcript and protein; membrane insertion/orientation and turnover; mitochondrial and outer-membrane cholesterol and phospholipid classes; p32 import; membrane potential; respiratory complexes I-IV; viral burden and viability.

In the same samples, measure lysosomal CE, TG, and free cholesterol; direct LAL activity/flux; NPC2/NPC1 state; STARD3 localization; and lysosome-mitochondria contact frequency. The strongest causal test is rescue: if RUBV changes MAO activity together with a defined membrane abnormality, correct the membrane/sterol state without simply eliminating infection. Recovery of MAO activity would materially strengthen the membrane-mediated mechanism.

7. Predetermined Interpretation

Observed result	Interpretation
MAO-A changes; MAO-B preserved; membrane state tracks.	MAO-A-selective Bottleneck #2 supported.
MAO-B changes; MAO-A preserved.	MAO-B becomes the lead discriminator.
Both change with membrane state and rescue.	Broader mitochondrial-membrane MAO lesion supported.
MAO changes without lipid/contact change.	MAO phenotype exists; proposed membrane bridge not established.
Membrane/contact state changes but MAO remains normal.	MAO Bottleneck #2 substantially weakened.
No MAO phenotype despite robust RUBV mitochondrial remodeling.	Central Paper VII hypothesis substantially falsified.

8. Publication Boundaries

Do not claim RUBV has been shown to inhibit MAO-A or MAO-B. Do not convert the rat-liver 7%/80% result into a human-neuronal MAO-A result. Do not call the cholesterol-loading experiment isoform-specific. Do not equate Jacob's post-LAL/pre-NPC2 lesion with NPC1 deficiency. Do not use MAO to reinsert serotonin as a causal node into Paper III. Do not infer treatment efficacy from this model.

9. Conclusion

Paper VII reaches a narrow but unusually testable convergence. RUBV directly targets mitochondria, binds a mitochondrial host protein, inhibits mitochondrial precursor import, reduces intramitochondrial p32, and

selectively remodels respiratory-chain activity. Independently, MAO is an outer-mitochondrial-membrane enzyme system whose catalytic competence is strongly conditioned by membrane interaction, phospholipid state, and cholesterol exposure. A third experimental system shows that lysosomal cholesterol stress can expand STARD3-dependent physical contacts with mitochondria.

The missing arrow is precise: does RUBV alter MAO-A or MAO-B function through the mitochondrial membrane state? That has not been demonstrated. If the predicted MAO phenotype appears, tracks with the membrane state, and is rescued by correcting that state, Bottleneck #2 gains a direct mechanistic foundation. If MAO remains normal during robust RUBV mitochondrial remodeling, the hypothesis fails in its present form.

References

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- Evidence boundary: References 4-7 establish MAO membrane/lipid biology outside RUBV. Reference 8 establishes sterol-responsive lysosome-mitochondria contact biology outside RUBV. None substitutes for the missing same-system RUBV-to-MAO experiment.