

JACOB'S MODEL - PAPER V

THE TIMING OF RUBELLA

From Viral Entry to Mature Endolysosomal Replication Compartments

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Jacob's Voice

11 September 2026

Final definitive Paper V - stage-resolved evidence map, experimental framework, and falsification program

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

Scope and Central Hypothesis

Rubella virus (RUBV) is treated here as a temporally changing cellular system rather than a single static infection state. The central hypothesis is that early entry and low-pH-dependent events are biologically distinct from established and late states that emerge as P150/RNA-positive endolysosomal replication compartments mature. An assay performed before the relevant compartment has matured therefore cannot by itself exclude a phenotype predicted to emerge during established or late infection.

The mechanistic extension is narrower and falsifiable: as RUBV replication compartments mature, trafficking, acidification, membrane chemistry, soluble lysosomal-cargo availability, and cholesterol flux may diverge in an ordered temporal sequence that is absent or not yet detectable during early infection. That complete sequence has not yet been demonstrated in one RUBV experiment.

Author's Note - Origin and Governance of the Model

The timing model emerged from Jacob Boatman's repeated focus on biological state before versus after maturation. Paper V is not intended to shield any mechanism from negative evidence. It defines which stage an experiment actually interrogates and makes falsification stricter by requiring the predicted biology to be tested during the stage in which it is claimed to exist.

Scott Boatman serves as coauthor and literature investigator. Direct RUBV observations, direct host maturation biology, comparator/context evidence, hypotheses, and missing same-system measurements are kept separate. Clock time alone is not treated as biological stage when strain, cell type, viral burden, or compartment morphology indicates otherwise.

Abstract

RUBV infection is not a single cellular state. Early infection is dominated by entry, low-pH-dependent membrane events, and establishment of replication structures. P150/RNA-positive endolysosomal vacuoles then mature, and later 48-72-hour states can contain large tubular or lamellar endolysosomal networks accompanied by major reorganization of cytopathic-vacuole, mitochondrial, rough-ER, and Golgi architecture. Established multi-day infection additionally shows acidic-vesicle/autophagic and chemically specific lipid phenotypes that are not equivalent to entry-stage measurements.

The pH literature demonstrates why stage matters. RUBV shows direct low-pH membrane/fusion activity around pH 5.8; exposure below pH 6 can induce infected-cell fusion; virion exposure around pH 5 produces irreversible envelope changes; and capsid chemistry changes between pH 5.0 and 5.5. These are direct biochemical thresholds, not mature replication-compartment pH. In a five-day TO-336/SIRC system, established infection instead produces a major loss of strongly acidic acridine-orange vesicular signal. No calibrated absolute pH of an established RUBV-positive CPV has been recovered.

Timing also changes the interpretation of trafficking experiments. Direct host maturation biology places Rab7 and VPS41/HOPS before ARL8 acquisition and demonstrates partial ARL8A/ARL8B redundancy. The direct RUBV foothold is P150N-ARL8A binding. A P150-ARL8A perturbation could therefore be buffered early and become functionally consequential only as the compartment matures; this remains a prediction rather than a demonstrated RUBV mechanism.

Paper V proposes that RUBV experiments be indexed by elapsed time and biological compartment state. A stage-aware series spanning entry, 12 h, 24 h, 48 h, 72 h, five-day established infection, and persistent infection where feasible should measure compartment morphology together with pH, ARL8/HOPS state, Cl-M6PR routing, soluble lysosomal cargo, NPC2 availability/transfer, membrane chemistry, and cholesterol flux. A late-stage hypothesis is substantially falsified only when the predicted pathway remains normal through confirmed compartment maturation during robust infection.

1. The Core Experimental Problem: RUBV Is Not One Cellular State

A result obtained two hours after infection and a result obtained three or five days later can both be valid RUBV results while describing different biology. Entry, uncoating, replication-organelle establishment, organelle maturation, late membrane remodeling, and persistence are not interchangeable states.

Paper V separates two variables that are often treated as one: elapsed time after inoculation and biological state of the infected compartment. The second variable is essential because different strains and cell systems can progress at different rates.

The practical consequence is simple: a study can be internally correct yet biologically mismatched to the question it is later asked to answer.

2. Evidence Hierarchy for Timing

Evidence tier	What is established	What remains open
DIRECT RUBV - early	Low-pH fusion/conformational thresholds; early inhibitor-sensitive window; SM/cholesterol entry dependence	Exact mapping of all early events onto one continuous entry timeline
DIRECT RUBV - structural maturation	P150/RNA-positive compartments mature; later 48-72 h states can become large tubular/lamellar endolysosomal networks; organelle architecture changes over time	Same-cell linkage from structural maturation to cargo/pH/cholesterol phenotype
DIRECT RUBV - established/late	Five-day acidic-vesicle phenotype; late autophagic completion defect; late fucosylglyceride/lipid remodeling	Absolute mature CPV pH and species-resolved late membrane chemistry in the same cells
DIRECT RUBV - persistent	Persistent infection can coexist with altered cargo internalization/ingestion and continued virus production	Which acute phenotypes persist, adapt, or resolve
DIRECT HOST maturation	Rab7/VPS41 precede ARL8; ARL8 paralogs are partially redundant; HOPS/CI-M6PR/NPC2 biology is maturation-dependent	Whether RUBV P150-ARL8A produces this host phenotype during maturation

3. A Stage-Resolved RUBV Framework

Stage	Operational window	Direct/established biology	Primary question
Entry	Minutes to first hours	Low-pH membrane/fusion chemistry; SM/cholesterol dependence; early inhibitor-sensitive window	How does virus enter and initiate infection?
Establishment	~12-24 h, system-dependent	P150/RNA-positive replication structures form and interact with endolysosomal machinery	Which host machinery is recruited as the compartment forms?
Maturation	~48-72 h in key systems	Large tubular/lamellar endolysosomal networks; major CV-mitochondria-RER-Golgi reorganization	Which late trafficking, pH, cargo, and lipid phenotypes emerge?
Established multi-day	~5 d in TO-336/SIRC and comparable systems	Acidic-vesicle/autophagic phenotype; late lipid remodeling	Does mature infection alter lysosomal completion/throughput?
Persistent	Serial/chronic systems where supported	Continued virus production with altered cellular cargo movement in selected systems	Which acute phenotypes persist, adapt, or resolve?

4. The Early Functional Window Is Real - and Limited

Historical inhibitor-timing experiments define a major early sensitive period. Ammonium acetate and aminoadamantane had little or no antiviral effect when first added later than approximately five hours after infection in the recovered timing analysis. Pre-exposure followed by washout at inoculation did not reproduce the same inhibition.

The strong conclusion is stage-specific: an early infection step is vulnerable to those perturbations. The experiment does not establish the physiology of a mature P150/dsRNA-positive compartment and cannot directly test a phenotype assigned to late replication-organelle maturation.

This distinction is not semantic. Early-entry inhibition can prevent the late state from ever forming; it cannot tell us whether the mature state would have shown a different trafficking or metabolic phenotype.

5. The RUBV pH Staircase Must Be Kept on Its Correct Timeline

Experimental context	Direct pH result	Biological stage
Vaananan & Kaariainen	Low-pH membrane/fusion-related activity around pH 5.8	Entry/membrane triggering
Katow & Sugiura	Below pH 6 induces infected-cell fusion; virion exposure around pH 5 causes irreversible E1/E2 changes	Fusion/envelope conformation
Mauracher et al.	Capsid solubility/conformation transition between pH 5.0 and 5.5	Virion/capsid chemistry
TO-336/SIRC established infection	Five-day infection shows major loss of strongly acidic acridine-orange red-vesicle signal	Late cellular acidic-vesicle phenotype; not calibrated CPV pH

The numerical thresholds are direct and important. They do not establish that mature RUBV CPVs have luminal pH values of 5.0, 5.5, or 5.8. No calibrated absolute pH of an established RUBV-positive endosome, lysosome, autolysosome, or P150/dsRNA-positive CPV has been recovered.

The strongest current temporal hypothesis is therefore two-stage: RUBV exploits low-pH biology during entry and later established infection is associated with altered acidic-vesicle maturation. A continuous causal bridge between those observations remains unmeasured.

6. Replication Compartments Mature Structurally

Multiple independent RUBV programs show structural progression over time. Early replication complexes appear, P150/RNA-positive vacuoles mature, and later 48-72-hour structures become large tubular or lamellar endolysosomal networks.

Time-resolved ultrastructure in the Risco/Frey line of work independently shows major reorganization of the cytopathic-vacuole, mitochondrial, rough-ER, and Golgi architecture between earlier and later infection states.

This structural progression is the backbone of Paper V. The biological object being measured is changing with time. A 48-72-hour late network is not simply an older version of the entry vesicle.

7. Mature CPVs Remain Connected to Endocytic Cargo

Pulse-loaded extracellular BSA-gold has been recovered later inside mature, spherule-bearing CPV lumens. Mature RUBV replication structures therefore retain access from the endocytic system rather than becoming completely isolated viral sacs.

This creates a timing-dependent trafficking question. A compartment can be born from the endolysosomal system, become a replication organelle, and continue receiving cargo while its membrane composition, maturation machinery, and luminal function evolve.

8. Late Acidic-Vesicle and Autophagic Phenotypes Are Not Entry Phenotypes

In the five-day TO-336/SIRC system, established RUBV infection produced a major reduction in strongly acidic acridine-orange vesicular signal. Continuous bafilomycin exposure was not used across the full five-day course because of cytotoxicity; the displayed established-infection experiment interrogated the terminal 24 hours.

Recovered Pásztor/Megyeri work also supports a separation between early autophagy-associated signaling and later completion: upstream activation can coexist with impaired later autophagic flux and acidic-vesicle development.

The direct conclusion is stage dependence. The data do not show that early low-pH entry causes the later acidic-vesicle phenotype, and they do not establish an absolute mature-compartment pH.

9. Late Lipid Chemistry Provides an Independent Biological Clock

The RUBV-associated fucosylglyceride provides a biochemical timing marker independent of microscopy. It appears after approximately two days and was characterized around day four, is absent from uninfected controls and purified virions, and represents roughly 1% of infected-cell lipid.

The exact synthetic and degradative pathway remains unresolved. The timing is the key finding for Paper V: established infection develops a chemically distinct lipid phenotype that is not simply the entry state carried forward.

Historical BHK21 cells also show nearly unchanged bulk free cholesterol, approximately 5.6 in controls versus 5.8 in infected cells, despite broad phospholipid remodeling. Temporal and compartmental measurements are therefore more informative than a single whole-cell endpoint.

10. Host Endolysosomal Maturation Creates a Specific Late-Stage Mechanistic Prediction

Direct host biology places Rab7 and VPS41/HOPS before ARL8 acquisition during endosome-to-lysosome maturation. ARL8A and ARL8B are also partially redundant, with combined loss producing stronger cholesterol phenotypes than single-paralog loss in key systems.

The direct RUBV foothold is P150N-ARL8A binding. The timing prediction is therefore precise: an early P150-ARL8A perturbation could be functionally buffered while ARL8-dependent maturation is incomplete, then become consequential as the compartment acquires ARL8/HOPS-dependent functions.

This is not yet a demonstrated RUBV mechanism. It is a stage-specific prediction generated by combining a direct viral-host physical interaction with direct host maturation biology.

11. Lysosomal Identity Can Precede or Outlast Normal Lysosomal Throughput

Direct host Rab7/HOPS/CI-M6PR work shows that membrane-protein identity and soluble-cargo routing can diverge. A compartment can retain LAMP or acid-phosphatase identity while soluble lysosomal-cargo delivery is abnormal.

This matters temporally because a maturing RUBV CPV can look lysosomal before all of its cargo-delivery, acidification, and cholesterol-throughput functions are known. Morphologic identity should therefore be paired with functional cargo and flux measurements at each stage.

A LAMP-positive or acid-phosphatase-positive late CPV is evidence of organelle identity, not proof that NPC2, LAL, SMPD1, FUCA1, CI-M6PR retrieval, or cholesterol export remains normal.

12. Persistence Is a Distinct Biological State, Not Simply a Later Time Point

Persistent RUBV systems show that continued infection can coexist with altered cellular movement. Persistent M-33 infection increased EGF-binding capacity while profoundly reducing internalization of receptor-bound ligand. Persistent retinal-pigment-epithelial infection reduced particle ingestion despite preserved adhesion.

Primary human fetal endothelial systems can also sustain productive RUBV infection through serial passage with continued release of infectious virus.

These findings make persistence an experimental stage in its own right. Paper V does not assume that an acute 72-hour or five-day phenotype must persist indefinitely; persistence must be re-measured after the system adapts.

13. Clock Time, Viral Time, and Organelle Time Are Different Variables

Clock time is the elapsed interval after inoculation. Viral time reflects how far infection has progressed in the population. Organelle time reflects the maturity of the P150/RNA-positive compartment itself.

These variables can separate. A slowly progressing strain at 48 hours may not be biologically equivalent to a rapidly progressing strain at the same clock time. A culture with low infection penetrance may contain cells at multiple stages simultaneously.

The minimum stage annotation should therefore include viral burden, P150/dsRNA localization, CPV morphology, endolysosomal markers, and where possible quantitative ultrastructure or live-cell maturation markers.

14. Timing Changes the Meaning of a Negative Experiment

A negative experiment is informative only for the biological state it actually interrogates. If a hypothesis predicts a mature-compartment phenotype, a normal result obtained before maturation cannot by itself test that prediction.

The converse is equally important. A late abnormality cannot be projected backward onto entry. Paper V rejects both forms of temporal overreach.

The stricter falsification standard is longitudinal: demonstrate robust infection and confirmed compartment maturation, then show that the predicted pathway remains normal through the stage in which the phenotype is claimed to exist.

15. Common Stage-Mismatch Errors

Error	Why it fails	Correct interpretation
Using an entry inhibitor to infer mature-CPV physiology	The late compartment may never form	Valid for entry dependence only
Using pH 5-6 fusion thresholds as mature CPV pH	Biochemical trigger is not organelle measurement	Keep threshold and organelle pH separate
Using LAMP/acid-phosphatase positivity as proof of mature cargo function	Identity and soluble-cargo routing can diverge	Measure cargo delivery and flux directly
Using a 24 h negative to reject a 48-72 h phenotype	Biological state may not yet exist	Confirm compartment maturity before falsification
Using a 5 d phenotype to describe entry	Late adaptation may be absent early	Do not project backward
Using persistence as an extension of acute infection	Adaptation can change the phenotype	Measure persistent state independently

16. Decisive Longitudinal Experiment

Stage	Required biological anchor	Core measurements
0-5 h	Entry/endosomal markers	Binding, uptake, fusion, initial low-pH dependence
12 h	Early P150/dsRNA-positive structures	ARL8A/B recruitment, HOPS, CI-M6PR, early cargo localization
24 h	Established replication structures	Same panel plus calibrated compartment pH and membrane chemistry
48 h	Maturing CPVs	NPC2 lysosomal availability/transfer, CE->FC generation, lysosomal FC residence
72 h	Late tubular/lamellar state	Full cargo panel, SM/ceramide/PG/BMP, ER arrival, SOAT re-esterification
5 d	Established multi-day state	Acidic-vesicle/autophagy state plus persistence of flux phenotype
Persistent	System-specific chronic state	Determine whether acute phenotype persists, adapts, or resolves

The critical discriminator is temporal order. If pH, ARL8/HOPS, CI-M6PR, NPC2, membrane chemistry, and cholesterol flux diverge, the experiment should establish which variable changes first and whether targeted perturbation of that node changes later events.

The strongest design uses the same cell system, matched infection burden, stage markers at every collection point, and predefined criteria for what constitutes compartment maturation.

17. Predetermined Interpretation Matrix

Observed result	Interpretation
Only early entry variables change	Entry mechanism supported; no late maturation mechanism established
Structural maturation occurs but late functional variables remain normal	Late endolysosomal mechanism weakened
Late phenotype emerges only after confirmed maturation	Stage-dependent biology supported

Observed result	Interpretation
ARL8/HOPS change precedes CI-M6PR/NPC2/flux change	Trafficking-first sequence supported; causal perturbation still required
pH or SM/ceramide/BMP changes precede NPC2 transfer loss	Chemistry-first sequence supported
NPC2/flux changes precede pH/cargo changes	Downstream-first alternative favored
Persistent state no longer shows the acute phenotype	Acute maturation effect, not persistent mechanism
All variables remain normal through robust mature infection	Central late-stage endolysosomal model substantially falsified

18. Timing Does Not Make Every Late Hypothesis Safe

A timing framework can be abused if every negative result is reclassified as 'too early.' Paper V explicitly rejects that strategy.

A late-stage hypothesis must specify in advance the biological stage at which the phenotype should appear, the markers that define that stage, the measurements that constitute a positive result, and the negative pattern that will falsify it.

Once mature-compartment criteria are met, a reproducible normal result is a true negative for that stage and must be accepted.

19. Modern Dataset Doors: Useful, but Not Yet Timing Results

GSE192648 / PRJNA792562 is a 48-sample multi-strain RUBV HUVEC RNA-seq resource identified in the research archive. It can potentially test whether lysosomal/cargo signatures differ across strains or experimental states.

GSE225949 provides a complementary live Wb12 versus UV-inactivated RUBV and interferon-comparator design at a defined time point. It can help distinguish live-virus-specific effects from generic interferon responses.

No new gene-level timing claim is made from either resource here because the relevant matrices and exact metadata have not yet been directly analyzed for this purpose.

20. Why Paper V Matters Beyond the Jacob Model

The timing problem is broader than NPC2. RUBV studies spanning entry, replication, autophagy, membrane biology, lipids, neural systems, and persistence can appear contradictory when experiments performed at different biological stages are compared as though they interrogated the same state.

Paper V proposes a reusable rule: before two RUBV experiments are compared mechanistically, determine whether they tested the same stage of infection and the same maturity state of the virus-modified compartment.

This framework can therefore remain useful even if the specific cholesterol-throughput hypothesis in Paper I is ultimately falsified.

21. Relationship to Papers I-IV

Paper I supplies the downstream pathway most likely to benefit from stage-resolved testing: ARL8/HOPS/CI-M6PR, NPC2 availability, membrane chemistry, and LDL-derived cholesterol flux.

Paper II uses timing to prevent early assays from being treated as complete tests of a proposed late endolysosomal/neurodevelopmental mechanism. Paper III requires a stage-aware interferon and tryptophan-flux series. Paper IV asks whether Apo-defined input changes across entry, maturation, and persistence.

Paper V does not prove those mechanisms. It defines the temporal framework required to test them correctly.

22. Publication Boundaries

- Do not assign mature CPV pH from entry/fusion thresholds.
- Do not claim the early low-pH requirement causes the later acidic-vesicle phenotype.

- Do not claim P150-ARL8A produces a late trafficking defect until measured in RUBV.
- Do not define biological stage by clock time alone.
- Do not use an early negative assay as a complete test of a late-stage prediction.
- Do not project a late phenotype backward onto entry.
- Do not assume an acute phenotype persists during chronic/persistent infection.
- Do not treat unextracted RNA-seq target rows as timing results.
- Do not infer treatment efficacy or disease causation from this timing framework.

23. Evidence Ledger

Class	Highest-value timing evidence
DIRECT RUBV	Early low-pH thresholds; early inhibitor-sensitive window; time-dependent P150/RNA CPV maturation; later 48-72 h tubular/lamellar networks; time-resolved organelle reorganization; five-day acidic-vesicle phenotype; late fucosylglyceride; persistent cargo-handling phenotypes.
DIRECT HOST	Rab7/VPS41 precede ARL8 acquisition; ARL8 paralog redundancy; HOPS/CI-M6PR/NPC2 maturation and cargo biology; membrane identity can diverge from soluble-cargo routing.
HARD NEGATIVES	No calibrated absolute mature RUBV CPV pH; no demonstrated continuous early-pH-to-late-acidification sequence; no RUBV-specific temporal P150->ARL8/HOPS->CI-M6PR->NPC2->cholesterol-flux chain.
MISSING NAIL	One same-system longitudinal experiment linking compartment maturation to pH, ARL8/HOPS, CI-M6PR, NPC2, membrane chemistry, and cholesterol flux in temporal order.

24. Conclusion

The central conclusion of Paper V is methodological and mechanistic: RUBV infection changes state over time, and the experimental question changes with it.

Direct evidence separates early low-pH entry chemistry from later replication-compartment maturation, multi-day acidic-vesicle/autophagic phenotypes, late lipid remodeling, and persistent cargo-handling states. Later 48-72-hour structures can be morphologically and functionally different from early replication structures.

The strongest next experiment is longitudinal. It should follow the same infected system from entry through confirmed compartment maturation and persistence while measuring structure and function together.

A late-stage hypothesis should be rejected when the predicted pathway remains normal through the stage at which the phenotype is supposed to exist. It should not be rejected merely because an earlier biological state was normal.

Clock time is a coordinate. Compartment maturity is the biological state.

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SEPTEMBER 27, 2026 SYNCHRONIZATION ADDENDUM

Paper V remains the temporal control paper for the series. Its core rule is unchanged: clock time is not biological stage, and a negative result tests only the stage actually reached. Papers VI and VII add new endpoints and new temporal requirements; they do not weaken Paper V's falsification standard.

25. CURRENT BOTTLENECK #1 CHANGES THE LATE-STAGE FLUX TARGET

The current Jacob model places Bottleneck #1 at LAL-centered lysosomal cholesteryl-ester/triglyceride throughput and capacity, with the proposed slow handoff after LAL hydrolysis and before NPC2 receives free cholesterol. Older Paper V wording that treats NPC2 as the lead downstream bottleneck should therefore be read as superseded by this later formulation. NPC2 remains a downstream transfer discriminator.

The longitudinal experiment should now resolve CE/TG delivery and LAL hydrolysis before assigning a downstream defect. At established and maturing stages, measure substrate arrival, direct LAL activity/flux, free-cholesterol generation and residence, NPC2 receipt/transfer, NPC1-dependent export, ER arrival, and SOAT re-esterification. The timing question becomes: at what biological stage, if any, does the LAL/post-LAL/pre-NPC2 interval first diverge from mock?

A normal NPC2 measurement cannot by itself exclude a LAL-centered throughput defect, and a LIPA transcript change cannot establish one. Conversely, normal CE/TG hydrolysis and post-LAL transfer through confirmed mature infection would directly weaken Bottleneck #1.

26. PAPER VI SYNCHRONIZATION: CELLULAR TIME AND DEVELOPMENTAL TIME

Paper VI adds a second temporal axis beyond viral and organelle time: developmental chronology. For a disease-level causal model, the proposed exposure must precede the molecular lesion; the molecular lesion must precede or accompany the downstream lipid state; and that biological state must precede or accompany developmental loss. Temporal proximity alone is not causation.

This creates a nested timing requirement. Paper V determines whether a cellular phenotype appears before, during, or after RUBV replication-compartment maturation. Paper VI then asks whether the corresponding biological state is temporally positioned to precede or accompany the human neurodevelopmental phenotype. A cellular mechanism demonstrated at the wrong disease-level time cannot establish causation.

Any MMR extension adds an exposure-specific requirement: wild-type RUBV timing cannot be assigned automatically to attenuated rubella vaccine virus. The relevant vaccine strain must be tested directly, and any proposed temporal sequence must remain compatible with the population evidence summarized in Paper VI.

27. PAPER VII SYNCHRONIZATION: MITOCHONDRIAL / MAO TIME

Paper VII adds a mitochondrial time axis. RUBV directly engages mitochondria through capsid-p32 interaction and mitochondrial-surface capsid, inhibits mitochondrial precursor-protein import, reduces intramitochondrial p32 in infected cells, and selectively remodels respiratory-chain activity. These effects establish mitochondrial involvement but do not establish when a MAO-A or MAO-B phenotype would emerge relative to CPV maturation.

The stage-resolved design should therefore add p32 import/localization, mitochondrial membrane potential, respiratory-chain activities, mitochondrial and outer-membrane lipid composition, STARD3 localization and lysosome-mitochondria contact frequency, and MAO-A/MAO-B catalytic activity. The critical question is temporal order: does the mitochondrial/MAO phenotype precede the lysosomal flux phenotype, follow it, or arise independently?

A MAO abnormality that appears before any measurable LAL/post-LAL sterol phenotype would argue against a simple downstream Bottleneck #1 -> Bottleneck #2 sequence. A MAO phenotype that follows a defined lysosomal sterol/contact change and is rescued by correcting that state would strengthen the proposed bridge. Co-occurrence at one late time point is not enough to establish direction.

28. UPDATED LONGITUDINAL EXPERIMENT

Entry / first hours: binding, uptake, fusion, low-pH dependence, initial mitochondrial contact where measurable. Establishment / approximately 12-24 h: P150/dsRNA compartment state, ARL8/HOPS/CI-M6PR, early cargo localization, p32 import, early respiratory state. Maturation / approximately 48-72 h: direct LAL CE/TG flux, free-cholesterol residence, NPC2/NPC1 transfer, pH and membrane chemistry, STARD3 contacts, mitochondrial outer-membrane lipids, and MAO-A/MAO-B activity. Multi-day and persistent states: repeat the full functional panel to determine whether phenotypes persist, adapt, reverse, or separate.

These clock windows are operational starting points, not biological definitions. Each collection must still be anchored by viral burden, P150/dsRNA localization, CPV morphology, endolysosomal markers, and relevant organelle-state measurements.

29. UPDATED PREDETERMINED INTERPRETATION

If LAL/post-LAL flux diverges first and mitochondrial/MAO changes follow, a sequential two-bottleneck model gains temporal support but still requires causal perturbation. If mitochondrial/MAO changes precede lysosomal flux divergence, a direct or parallel RUBV mitochondrial route is favored. If both arise together, direction remains unresolved. If mature infection shows normal LAL/post-LAL flux and normal MAO-A/B function despite confirmed RUBV organelle remodeling, the integrated two-bottleneck model is substantially weakened.

30. UPDATED PUBLICATION BOUNDARY

Do not use timing to rescue a failed mature-stage experiment. Do not call NPC2 the lead bottleneck in the current model. Do not infer that a mitochondrial effect is downstream of lysosomal cholesterol merely because both occur late. Do not project wild-type RUBV timing onto vaccine strain. Do not convert cellular chronology into autism causation without the separate human temporal ordering required by Paper VI.

31. UPDATED CONCLUSION

Paper V now controls three nested clocks: viral/replication time, organelle maturation time, and, when the model is extended to Paper VI, developmental disease time. Paper VII adds a second organelle system whose temporal relationship to the lysosomal branch must be measured rather than assumed.

The strongest experiment is therefore not a single late endpoint. It is a synchronized longitudinal map that follows RUBV compartment maturation, LAL-centered CE/TG throughput, post-LAL cholesterol transfer, lysosome-mitochondria contact, mitochondrial membrane state, and MAO-A/MAO-B function in the same system. Timing does not protect the model from falsification. It specifies exactly when and where falsification must occur.

ADDITIONAL CROSS-PAPER REFERENCES

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