THE WAX AND THE WELD

Ceramide, the Mitochondria-Associated ER Membrane, and the Lipid Contact Where a Disorder of Fat and a Disorder of Energy Become One Disorder of Alzheimer's Disease

The Weld That Never Fuses · The Wax Made at the Contact · The Presenilin at the Seam
C99, the Lesion That Ties Wax to Weld · The Two Currents and the Shared Executioner

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The organelle-contact volume of the corpus. It welds two lines that had run in parallel — the ceramide thesis of The Janus Lipid and the mitochondrial thesis of The Bioenergetic Collapse — by locating them at a single physical site: the

mitochondria-associated ER membrane, the contact where the cell manufactures ceramide, ferries calcium to the mitochondrion, and — by an accident of localisation that reorganised the field — cuts the amyloid precursor protein. It argues that the accumulating C99 fragment, acting there as a cholesterol-sensing peptide, expands the contact and deranges its sphingolipid machinery, so that the disease's lipid disorder and its energy failure are one buckling of one weld — graded, connection by connection, and honest about a retracted citation the argument was built to avoid.

"A lipid disorder and an energy disorder were told for thirty years as two diseases in one brain. They are one disorder of one contact, read from two ends. The wax is made at the weld; and the weld, we will argue, is where the disease begins — before the plaque, before the tangle, at a junction too small to stain."

— ONS Methodology Working Notes, 2026

For the two literatures that scarcely cite one another — the students of ceramide and the students of the failing mitochondrion — who have been describing, in two vocabularies, the manufacturing output and the mechanical failure of one organelle contact.

THE COLLAPSE FRAMEWORK LIPID & BIOENERGETIC COMPANION

The Janus Lipid — *ceramide as a conserved membrane death signal*

The Bioenergetic Collapse — *mitochondrial dysfunction & the energy failure of the neuron*

The Narrow Gate — *TOM40 & the mitochondrial import channel*

The Wax and the Weld (ceramide, the MAM & the ER–mitochondria contact) — this volume

This volume welds two lines of the corpus that had run in parallel: the ceramide line, which established the sphingolipid as a conserved membrane death signal; and the bioenergetic line, which located the disease's energy failure in the mitochondrion. It argues that both are seated at one organelle contact — the mitochondria-associated ER membrane — where a substantial share of the cell's ceramide is manufactured, where calcium is delivered to the mitochondrion, and where presenilin, γ-secretase, and the accumulating C99 fragment of the amyloid precursor protein reside. The claim is that ceramide is not a pathway parallel to the mitochondrial story but one of the contact's own products, whose executioner — the channel it forms in the outer mitochondrial membrane — is built on the very membrane the contact feeds. The wax is made at the weld, and the weld may be where the disease begins.

Contents

Abstract

Two organelles that never fuse are nonetheless joined. Along a scaffold of protein tethers, patches of the endoplasmic reticulum are drawn to within a few tens of nanometres of the mitochondrion and held there, forming a specialised subdomain of the reticulum known as the mitochondria-associated endoplasmic reticulum membrane, or MAM. This dissertation argues that the MAM is the physical place where two of the best-documented derangements of Alzheimer's disease — a disorder of ceramide and a disorder of mitochondrial bioenergetics — turn out to be the same derangement, read from two ends. The MAM is a lipid-raft-like domain that carries out cholesterol esterification, phospholipid shuttling, calcium transfer to the mitochondrion, and — the fact on which this thesis turns — a substantial share of the cell's sphingolipid and ceramide metabolism. It is also, by a discovery that took the field by surprise, the subcellular home of presenilin-1, presenilin-2, and γ-secretase activity, and of the β-secretase that precedes them; the entire amyloidogenic apparatus is enriched at this contact. The 99-residue carboxy-terminal fragment of the amyloid precursor protein, C99, accumulates at the MAM in models of the disease, behaves there as a cholesterol-sensing peptide that expands the contact, and drives an increase in sphingolipid turnover that alters the lipid composition of both the MAM and the adjacent mitochondrial membrane — corrupting the assembly of the respiratory supercomplexes and starving the cell of energy. Ceramide is therefore not a parallel pathway running alongside the mitochondrial story; it is one of the MAM's own manufactured products, made by ceramide synthases resident at the very contact, and its terminal weapon — the ceramide channel that renders the outer mitochondrial membrane permeable to cytochrome *c* — is assembled on the same membrane the MAM feeds. The wax is made at the weld, and the weld is where the disease appears to begin. Throughout, the argument is graded connection by connection in an explicit validity ledger: the enrichment of the amyloid machinery at the MAM and the in-vitro reality of ceramide channels are firm; the claim that upregulated MAM function is *upstream* of plaques and tangles is bold and incompletely proven; the direction of ceramide accumulation is complicated, as it is in the wider literature, by reverse causation from membrane breakdown. The synthesis is offered as a heuristic that unifies otherwise scattered features of the disease under one organelle contact — and as a warning that the therapeutic instinct to sever, drain, or silence that contact is aimed at a structure the neuron cannot live without.

Keywords: mitochondria-associated ER membranes; MAM; ceramide; sphingolipids; presenilin; γ-secretase; C99; APP; cholesterol; respiratory supercomplexes; calcium; lipid rafts; Alzheimer's disease.

1. Introduction: Where Two Corpora Meet

There are, in the lipid and organelle biology of Alzheimer's disease, two literatures that have grown up almost independently of one another and that this dissertation is written to weld together.

The first is the ceramide literature. It records, with unusual consistency, that the aging and Alzheimer's brain accumulates ceramide and cholesterol; that this accumulation is accompanied by membrane-associated oxidative stress; that amyloid-β can provoke it; and that ceramide, once raised, is a competent executioner — it drives oxidative injury, impairs mitochondria, and engages the intrinsic apoptotic programme (Cutler et al., 2004). A companion volume to this one, *The Janus Lipid*, treated that literature at length and argued that ceramide is a conserved membrane death signal whose pathological sign in the brain is set by the fact that the neuron is post-mitotic and cannot afford the death the lipid commands.

The second is the bioenergetic literature. It records that mitochondrial function and dynamics are disturbed early in the disease, that respiration falls, and that these deficits appear before, not after, the accumulation of plaques and tangles. That literature is the subject of another companion, *The Bioenergetic Collapse*, which located the disease's energy failure in the mitochondrion itself.

These two accounts are usually told separately, as if a lipid disorder and an energy disorder were two independent insults that happen to co-occur. This dissertation makes a stronger claim: that there is a single subcellular structure at which the two stories are not merely adjacent but identical, and that this structure is a contact site between the endoplasmic reticulum and the mitochondrion — the mitochondria-associated ER membrane, or MAM. The MAM is where the cell makes much of its ceramide; it is where the cell delivers calcium to the mitochondrion; it is where cholesterol is esterified and phospholipids are exchanged between the two organelles; and — the coincidence that gives the whole argument its force — it is where presenilin, γ-secretase, and the rest of the machinery that generates amyloid-β turn out to live. A lipid factory, a calcium conduit, and the amyloid apparatus occupy one address. The proposal, first advanced by Area-Gómez and Schon and here extended toward the ceramide side, is that the disease begins as a disorder of that address (Area-Gómez & Schon, 2017).

The metaphor of the title should be introduced honestly, because it is imperfect in an instructive way. A weld, in the ordinary sense, fuses two pieces of metal into continuity. The MAM does no such thing: the ER and the mitochondrion remain two organelles with two membranes, separated by a cleft of roughly ten to thirty nanometres and held in register by protein tethers that span the gap. It is a *weld that never fuses* — a controlled apposition, close enough for lipids and calcium to pass between the two surfaces, distant enough that each organelle keeps its identity. That gap is not a flaw in the joint; it is the joint's function. Everything this dissertation describes — the transfer of a lipid, the flux of an ion, the processing of a peptide — happens *because* the two membranes are held near but apart. And the "wax" of the title is ceramide, named for *cera*, the Latin for wax, and genuinely waxy: intensely hydrophobic, small-headed, self-associating. The wax is manufactured at

the weld. When the manufacture goes wrong, the weld distorts, and — this is the claim to be graded — the neuron begins to die.

The argument proceeds in ten movements. Sections 2 and 3 establish the two halves of the title as matters of settled cell biology: what the weld is, how it is held, and what it does (Section 2); and that ceramide is made and sorted there, with its executioner apparatus co-located on the adjacent membrane (Section 3). Section 4 introduces the discovery that reorganises everything — that the Alzheimer machinery is enriched at this contact. Section 5 is the mechanistic heart: how the C99 fragment, accumulating at the MAM, ties the wax to the weld and corrupts both. Section 6 adds the second current that crosses the contact — calcium — and shows it converging on the same mitochondrial membrane as the ceramide. Section 7 asks which cells and regions actually exhibit the lesion, and concedes the selective-vulnerability problem the mechanism does not solve. Section 8 reads the two companion theses together as one lesion. Section 9 is the validity ledger, where each connection is graded and the weakest links, including one retracted paper, are named rather than hidden. Section 10 turns to therapy and its dangers. A short coda closes.

A note on method and voice, carried over from the companion volumes. This is a synthesis, not a primary report. Where a claim rests on a single study, that is said. Where a mechanism is established in cultured cells and unproven in the human brain, the gap is named. The reader is invited to disagree with the grades of Section 9 on the evidence, not on the prose.

2. The Weld: A Contact That Does Not Fuse

2.1 Vance's Fraction X

The MAM was not discovered as an object; it was discovered as a contaminant. In 1990, Jean Vance, studying where the cell builds its phospholipids, fractionated a crude rat-liver mitochondrial preparation and found that certain biosynthetic activities — phosphatidylserine synthase, the methyltransferase that converts phosphatidylethanolamine to phosphatidylcholine, and a cholinephosphotransferase — were present in the crude preparation but absent from highly purified mitochondria. They belonged, she showed, to a distinct membrane that sedimented *with* mitochondria at ten thousand times gravity but was not mitochondrial: a fraction she labelled, with deliberate reticence, "fraction X" (Vance, 1990). Fraction X resembled microsomes — that is, endoplasmic reticulum — but it was not ordinary ER; its specific activities differed, and it clung to the mitochondrion through purification. Vance proposed that the combined mitochondria-plus-fraction-X could account for a long-standing puzzle, the compartmentalised pool of serine-labelled phospholipids, and that it "might be involved in the transfer of lipids between the endoplasmic reticulum and mitochondria."

That guarded sentence founded a field. Fraction X is what we now call the MAM: a specialised, biochemically distinct subdomain of the endoplasmic reticulum that is physically apposed to the mitochondrion and that serves as the conduit for lipid and calcium exchange between the two organelles. The discovery's shape matters for what follows. The MAM announced itself first as a *lipid-synthesising* membrane — a factory — and only later as a signalling platform. When Alzheimer's disease arrived at this address, it arrived at a factory floor.

2.2 What Holds It: The Tethers

For two membranes to remain apposed at a fixed distance while their organelles move and divide, something must hold them. Over two decades, several of these tethers have been identified, and their names recur throughout the disease story.

The best-characterised is the calcium tether. On the ER side sits the inositol-1,4,5-trisphosphate receptor, IP$_3$R, the channel that releases calcium from the reticulum; on the mitochondrial side sits the voltage-dependent anion channel, VDAC, in the outer membrane. Szabadkai and colleagues showed that these two channels are not merely near one another but physically linked, bridged by the cytosolic chaperone glucose-regulated protein 75 (grp75); knocking grp75 down abolishes the functional coupling by which IP$_3$R-released calcium is delivered efficiently into the mitochondrion (Szabadkai et al., 2006). The IP$_3$R–grp75–VDAC bridge is thus both a structural tether and a calcium relay — a point to which Section 6 returns.

A second tether is mitofusin-2 (MFN2). De Brito and Scorrano reported that MFN2, better known for fusing mitochondria to one another, is enriched at the ER–mitochondria interface and that ablating it loosens the contact and reduces the efficiency of mitochondrial calcium uptake; they

proposed that MFN2 on the ER engages mitofusins on the mitochondrion to bridge the two organelles (de Brito & Scorrano, 2008). This assignment has since been genuinely contested: other groups, deleting the same protein, found that contacts *increased*, and argued that MFN2 is not a tether but a spacer that holds the organelles apart. The controversy is unresolved, and it is flagged here rather than smoothed over — it is treated again in the validity ledger — because it is a caution against reading any single tethering protein as the master regulator of a contact that is plainly built from several.

A third tether, the VAPB–PTPIP51 pair, links an ER-resident protein (VAPB) to an outer-mitochondrial one (PTPIP51). Stoica and colleagues showed that this interaction regulates ER–mitochondria apposition and calcium homeostasis, and that it is disrupted by TDP-43, a protein central to amyotrophic lateral sclerosis and frontotemporal dementia; strikingly, they found the interaction to be regulated by glycogen synthase kinase-3β (GSK-3β) (Stoica et al., 2014). That last detail is worth holding, because GSK-3β is also the kinase most implicated in the hyperphosphorylation of tau; a tether whose set-point is tuned by a tau kinase is a small but suggestive thread connecting the contact to the second pathology of the disease. The VAPB–PTPIP51 evidence comes from an ALS/FTD context and should not be over-read into Alzheimer's, but it belongs in the inventory of what holds the weld.

The lesson of the inventory is that the MAM is a *composite* junction — several tethers, partly redundant, some pulling the membranes together and at least one perhaps pushing them apart — whose net apposition is a tunable variable rather than a fixed distance. A disease that shifts that variable does not need to sever the weld; it needs only to lean on the dial.

2.3 A Raft on the Inside

The plasma membrane, as *The Janus Lipid* discussed at length, is not uniform: cholesterol and sphingolipids can pack into ordered, raft-like microdomains that concentrate particular proteins and thereby organise signalling. The surprising finding about the MAM is that this raft-like organisation is not confined to the cell surface. Area-Gómez and colleagues showed that the MAM is itself an intracellular *detergent-resistant, lipid-raft-like domain* of the endoplasmic reticulum — enriched in cholesterol and sphingolipids, resistant to cold non-ionic detergent, and possessing the ordered character of a raft (Area-Gómez et al., 2012). This is a structurally consequential fact. It means the MAM is precisely the kind of membrane in which raft-dependent enzymes prefer to work, and it forecasts the two occupants that matter most for this thesis: the sphingolipid machinery, which is at home in ordered membranes, and γ-secretase, which is a raft enzyme. The MAM is, in effect, an internal raft moored to the mitochondrion — and it will turn out to be the raft on which the amyloid apparatus and the ceramide apparatus are both staged.

The raft caveat carried through the companion volume applies here too, and is inherited into Section 9: "raft" denotes a real but dynamic nanoscale ordering, not a fixed platform one can purify

intact, and the detergent-resistant fraction that defines the MAM biochemically is not, by itself, proof of a discrete structure in the living cell. That the MAM can also be visualised morphologically as apposed membranes by electron microscopy is what keeps the construct anchored to a real object.

2.4 What the Weld Is For

The MAM discharges a small set of functions, each of which turns out to be disturbed in Alzheimer's disease, and it is the *coincidence* of that list with the disease's non-amyloid features that first drew the field's attention.

First, **phospholipid shuttling.** The synthesis of phosphatidylserine, its transfer to the mitochondrion, its decarboxylation there to phosphatidylethanolamine, and the return of that lipid for conversion to phosphatidylcholine is a relay that physically requires the two organelles to be apposed — Vance's founding observation. The rate of this relay is a standard readout of MAM function.

Second, **cholesterol esterification.** The MAM houses acyl-CoA:cholesterol acyltransferase (ACAT1/SOAT1), which converts free cholesterol to cholesteryl esters; the rate of cholesteryl-ester synthesis is the other canonical readout of MAM activity (Area-Gómez et al., 2012). Cholesterol homeostasis and MAM function are, through this enzyme, coupled.

Third, **calcium transfer.** Through the IP₃R–grp75–VDAC bridge, the MAM delivers calcium from the ER directly into the mitochondrion, where it tunes the dehydrogenases of the citric-acid cycle — and, in excess, triggers the permeability transition and death (Szabadkai et al., 2006). The contact is a calcium synapse between organelles.

Fourth, **mitochondrial dynamics and bioenergetics.** The sites of ER–mitochondria contact mark where mitochondria divide, and the composition of the membranes at the contact feeds directly into the assembly and function of the respiratory machinery — the link that Section 5 shows to fail in the disease.

And fifth, running through all of these and central to this thesis, **sphingolipid and ceramide metabolism**, which Section 3 treats on its own terms.

Set beside the clinical phenomenology of Alzheimer's disease — disturbed phospholipid and cholesterol metabolism, aberrant calcium handling, altered mitochondrial dynamics, reduced bioenergetic function, all appearing early — this list is nearly a match, item for item (Area-Gómez & Schon, 2017; Schon & Area-Gómez, 2012). That match is the empirical coincidence from which the MAM hypothesis was built. It is a coincidence of *function*, and a coincidence of *location*, as the next two sections establish.

3. The Wax: Ceramide Is Manufactured at the Weld

3.1 A Lipid at the Crossroads

Ceramide, as its companion volume set out, is the metabolic hub of the sphingolipids — the molecule through which the cell passes to build sphingomyelin, glucosylceramide, and the gangliosides, and through which it passes again to dismantle them. It is made by three roads: *de novo* synthesis at the endoplasmic reticulum, beginning with serine and palmitate and running through six ceramide synthases that set the length of the fatty-acyl chain; liberation from sphingomyelin by the sphingomyelinases, the fast signalling route; and salvage from complex sphingolipids in the lysosome. And it is not one molecule but a family: ceramide synthase 1 makes the C18 species enriched in neurons, ceramide synthases 5 and 6 make C16, ceramide synthase 2 the very-long-chain species — and these differ in what they do. This primer is not repeated here; the point that matters for the present argument is *where*, in the cell, ceramide is made.

3.2 The Ceramide Synthase Lives at the Contact

The naïve textbook answer is that ceramide is made "at the ER." The more exact answer, and the one that welds this thesis together, is that a substantial part of ceramide synthesis occurs at the MAM and at the mitochondrion itself. Bionda and colleagues, working with highly purified rat-liver fractions and taking deliberate care to separate the MAM from bulk mitochondria, showed that both the MAM and purified mitochondria generate ceramide *in vitro*, through ceramide synthase and through reverse ceramidase, and that ceramide synthase activity is recoverable in both the outer and the inner mitochondrial membranes (Bionda et al., 2004). Their conclusion is the sentence on which Section 3 turns: because the topology of ceramide formation may determine ceramide's function, it matters that a pool of the lipid is generated at the very membrane where it can act on the mitochondrion.

The MAM, then, is not merely near the mitochondrion; it is a place where the cell chooses to *make* ceramide, adjacent to the organelle that ceramide is most competent to kill. Independent protocols developed to purify the MAM note the same coupling from the other direction: the fraction is defined, in part, by its documented role in the transfer of lipids and ceramide from the ER to the mitochondrion (Williamson et al., 2015). Manufacture and delivery are co-located.

3.3 The Topology Principle

Bionda's phrase — that the topology of ceramide formation could determine its function — deserves to be stated as a principle, because it resolves much of the apparent contradiction in the ceramide literature and it motivates the whole architecture of this dissertation. A molecule of ceramide generated slowly in the bulk ER, destined for glycosylation and export to the plasma membrane, means something entirely different from a molecule generated at the MAM, in a raft-ordered membrane, a few nanometres from the outer mitochondrial membrane. The first is a housekeeping intermediate; the second is positioned to become a weapon. "Total ceramide," measured by lipidomics on a whole-brain homogenate, averages over this distinction and can therefore look flat or ambiguous even while a small, lethal, correctly-placed pool has changed. The disease, this thesis argues, is a disease of the second pool — of ceramide made in the wrong amount at the right place.

3.4 From Synthesis to Channel: The Executioner Is Co-Located

The final fact of Section 3 is the most dramatic, and it closes the geometry. Ceramide is not only made near the mitochondrion; it can, in sufficient local concentration, *self-assemble into a channel across the outer mitochondrial membrane*. Siskind, Kolesnick, and Colombini showed that C_2- and C_{16}-ceramide — but not dihydroceramide, which lacks the crucial double bond — form large, stable channels in the mitochondrial outer membrane and in defined planar bilayers, raising the membrane's permeability to proteins with a molecular-weight cutoff near sixty thousand, large enough to release cytochrome *c* itself; the channels are reversible and are not a detergent effect (Siskind et al., 2002). Colombini's later work established their architecture — barrel-stave pores roughly ten nanometres across, visible by electron microscopy — and their regulation: the anti-apoptotic Bcl-2 proteins disassemble ceramide channels, while pro-apoptotic Bax acts synergistically with ceramide to permeabilise the membrane (Colombini, 2013; Ganesan et al., 2010). Ceramide and activated Bax, at concentrations at which neither does much alone, together commit the outer membrane to the leak that begins apoptosis (Ganesan et al., 2010).

Assemble the geometry. The synthase that makes ceramide sits at the MAM and on the mitochondrial membranes (Bionda et al., 2004). The channel that ceramide forms punches through the outer mitochondrial membrane a few nanometres away (Siskind et al., 2002). The regulators of that channel are the Bcl-2 family proteins stationed on the same membrane. The entire apparatus of ceramide-driven mitochondrial death — synthesis, delivery, pore, and regulation — is built into the ER–mitochondria contact and the membrane it feeds. The weld is where the wax is made, and the wax, made there in excess, is a key to the mitochondrion's release of its death signals. What remains is to show that Alzheimer's disease acts on exactly this structure — which is the burden of Sections 4 and 5.

4. The Presenilin at the Seam

4.1 The Amyloid Machinery Has an Address, and It Is the MAM

For most of the field's history, the subcellular localisation of the presenilins was described as "controversial" — they had been reported at the ER, the Golgi, the nuclear envelope, endosomes, lysosomes, the plasma membrane, and the mitochondria, a promiscuity that read more like an artefact than a fact. In 2009, Area-Gómez and colleagues resolved the confusion by asking a sharper question. Using three complementary approaches — subcellular fractionation, direct γ-secretase activity assays, and immunocytochemistry — they found that presenilin-1, presenilin-2, and γ-secretase activity are *highly enriched* in the MAM: the subcompartment of the ER that bridges to the mitochondrion (Area-Gómez et al., 2009). The proteins had appeared to be everywhere because the MAM touches everything; concentrated at a contact site, a protein reports to every fraction the contact contacts. Pin the localisation to the MAM and the promiscuity dissolves.

This is the discovery that reorganises the disease. The catalytic core of γ-secretase — the enzyme that makes the carboxy-terminus of amyloid-β, and the enzyme whose mutations cause early-onset familial Alzheimer's disease — resides at the ER–mitochondria contact. The mutations that cause the disease are mutations in a MAM protein. And because γ-secretase is a raft enzyme and the MAM is a raft-like domain (Section 2.3), the localisation is not incidental; it is where the chemistry prefers to happen.

4.2 The Whole Processing Line

Presenilin and γ-secretase perform the second of the two cuts that liberate amyloid-β. The first cut is made by β-secretase (BACE1). If the MAM claim is to explain amyloidogenesis rather than only its last step, the earlier machinery ought to be there too — and an independent group, working in a different city with different models, found that it is. Del Prete and colleagues (from the Nice laboratories of Checler and Chami) showed that the amyloid precursor protein and its proteolytic fragments are present in the MAM in cells expressing wild-type or familial-mutant APP and in the brains of transgenic mice; that *both* β- and γ-secretase are present and enzymatically active in the MAM; and that cells expressing the Swedish familial mutation show increased ER–mitochondria contact and increased accumulation of neutral lipids that tracks with amyloid-β production and is reversed by inhibiting either secretase (Del Prete et al., 2017). Two independent lines of evidence, then, place not just the last enzyme but the whole processing line — substrate, β-secretase, and γ-secretase — at the contact, and tie the *act* of processing to a change in the contact's lipids. The amyloidogenic route is a MAM event.

4.3 The Upregulated Weld

Localisation is one claim; dysregulation is another. The MAM hypothesis requires not only that the machinery lives at the contact but that the contact's function is *altered* in the disease — and here the evidence is direct. Area-Gómez and colleagues measured the two canonical readouts of MAM activity, cholesteryl-ester synthesis and phospholipid synthesis, and found both increased significantly in presenilin-mutant cells and, crucially, in fibroblasts from patients with *both* familial and sporadic Alzheimer's disease (Area-Gómez et al., 2012). The upregulation is not confined to the rare genetic form; it appears in the common sporadic disease, in a patient's own cells, measured as a rate of lipid synthesis. Alongside the functional increase they documented an anatomical one: ER–mitochondrial connectivity itself is increased. The weld is not loosened in Alzheimer's disease; it is *tightened and overworked*.

The finding that most resists a dismissive reading came from the human brain. Hedskog and colleagues examined ER–mitochondria contacts in human Alzheimer brain tissue and in mouse and neuronal models, and reported that MAM-associated proteins are upregulated in the AD brain and in the APP-Swedish/London mouse — and that in the mouse the upregulation appears *before* the deposition of plaques (Hedskog et al., 2013). They further showed that nanomolar amyloid-β raises the expression of IP_3R and VDAC, increases the number of ER–mitochondria contact points, and elevates mitochondrial calcium — a result that reappears in Section 6. The temporal ordering is the load-bearing observation: if MAM upregulation precedes plaques, then it cannot be merely a scar left by plaques, and the possibility opens that it lies upstream. That possibility is a hypothesis, not a proof — the human-brain data are cross-sectional and correlational, and Section 9 grades them accordingly — but it is the observation that gives the MAM hypothesis its ambition.

4.4 The Hypothesis Stated

From these strands Area-Gómez and Schon assembled a claim of unusual scope: that Alzheimer's disease is, fundamentally, a disorder of ER–mitochondrial communication — the "MAM hypothesis" (Schon & Area-Gómez, 2012; Area-Gómez & Schon, 2017). Its logic is subtractive and elegant. The disease has always had features the amyloid cascade struggles to explain — the disturbances of calcium, cholesterol, phospholipid, and mitochondrial function that arrive early and do not obviously follow from plaques. Those features are precisely the functions of the MAM. The proteins whose mutations cause the disease are enriched at the MAM. MAM function is upregulated in the disease, in patients' cells, and before plaques in models. Rather than treat the lipid, calcium, and bioenergetic abnormalities as a scattered periphery around a proteinaceous core, the MAM hypothesis gathers them into the centre and proposes that a single perturbed organelle contact generates them all — and generates the amyloid too, as one of its outputs.

This dissertation accepts the MAM hypothesis as its frame and adds to it the argument the original formulation left implicit: that ceramide is not one more item on the list of disturbed MAM

functions but the through-line that connects the contact to the death of the cell. To show that, we need the lesion that ties the wax to the weld — the C99 fragment.

5. C99: The Lesion That Ties Wax to Weld

5.1 The Overlooked Fragment

The amyloid cascade has trained two generations to watch amyloid-β — the peptide that aggregates, deposits, and gives the disease its plaques. But amyloid-β is the *product* of the second cut; the *substrate* of that cut is C99, the 99-residue carboxy-terminal fragment left in the membrane after β-secretase has removed the APP ectodomain. C99 is normally short-lived: γ-secretase cleaves it promptly to release amyloid-β and the APP intracellular domain. In the disease, and in cells that model it, C99 accumulates. And C99, it turns out, does its most consequential damage not as a precursor to a plaque but as a lipid-active peptide at the MAM — which is where this thesis's two halves finally meet.

5.2 C99 as a Cholesterol-Sensing Peptide That Expands the Contact

Montesinos and colleagues asked what C99 does at the MAM and found that it behaves as a *lipid-sensing peptide*. Because of its affinity for cholesterol, C99 delivered to the ER for cleavage nucleates transient, cholesterol-rich, detergent-resistant membrane domains — that is, it nucleates MAM. When C99 accumulates, as it does early in the disease, it drives the *internalisation of extracellular cholesterol and its trafficking from the plasma membrane to the ER*, expanding these regulatory domains and, as a homeostatic consequence, inducing cholesterol esterification while attenuating new cholesterol synthesis (Montesinos et al., 2020). C99 is thus not a passive by-product; it is an active agent that *builds and expands the very contact at which it sits*, and that reroutes the cell's cholesterol in doing so. Here is the mechanistic root of the "upregulated MAM" of Section 4.3: the fragment that accumulates in the disease is itself a MAM-nucleating, cholesterol-mobilising signal. The same phenomenon has since been recapitulated *in vivo* in a model of traumatic brain injury — a known risk factor for later dementia — where injured cortex and hippocampus show increased C99 together with increased MAM activity measured as phospholipid synthesis, sphingomyelinase activity, and cholesterol turnover (Agrawal et al., 2022). C99 raises MAM function; MAM function, we will see, deranges the mitochondrion through lipids.

5.3 The Sphingolipid Consequence

If C99 expanded the contact and rerouted cholesterol but left sphingolipids alone, this would be a cholesterol thesis, not a ceramide one. It does not leave them alone. In the paper that is the keystone of this dissertation, Pera and colleagues showed that C99, in addition to its endosomal pool, is present in the MAM, where it is normally processed rapidly by γ-secretase — but that in cell models of Alzheimer's disease the concentration of *unprocessed* C99 rises in the MAM, and that this rise drives **elevated sphingolipid turnover and an altered lipid composition of both the MAM and the mitochondrial membranes** (Pera et al., 2017). The accumulating fragment does not merely sit in the contact; it accelerates the sphingolipid machinery of the contact — the ceramide-synthesising, sphingomyelin-hydrolysing apparatus that Section 3 placed there — and thereby rewrites the lipid composition of the adjacent mitochondrial membrane. This is the wax tied to the weld: an Alzheimer lesion (accumulated C99), acting at the Alzheimer organelle contact (the MAM), driving a ceramide-and-sphingomyelin disturbance (elevated sphingolipid turnover) at the mitochondrion.

5.4 The Bioenergetic Catastrophe

Why should a change in mitochondrial-membrane lipid composition matter to the energy budget of the neuron? Because the respiratory chain is not a set of freely diffusing complexes; its complexes assemble into higher-order *supercomplexes* whose formation and stability depend on the lipid environment of the membrane. Pera and colleagues showed that the C99-driven change in mitochondrial membrane composition **interferes with the proper assembly and activity of the respiratory supercomplexes**, and that this, rather than any direct action of amyloid-β on the matrix, plausibly accounts for the bioenergetic defects characteristic of Alzheimer's disease (Pera et al., 2017). The chain of causation is now unbroken from the earliest amyloidogenic event to the energy failure of the cell: β-secretase makes C99 C99 accumulates at and expands the MAM (Montesinos et al., 2020) it drives sphingolipid turnover and remakes the mitochondrial membrane's lipids (Pera et al., 2017) the altered lipid membrane cannot assemble its supercomplexes respiration falls. The bioenergetic thesis and the lipid thesis are, at this contact, one thesis.

5.5 The Loop, Drawn

It remains to close the loop into a feed-forward cycle, because the pieces assembled across Sections 3 through 5 do not merely form a line; they form a spiral. Consider the elements now in hand:

- Accumulating **C99** nucleates and expands the **MAM** and mobilises **cholesterol** into it (Montesinos et al., 2020; Agrawal et al., 2022).
- The expanded, over-active MAM drives **sphingolipid turnover**, raising **ceramide** and reshaping the mitochondrial membrane (Pera et al., 2017).

- Ceramide made at this contact is positioned to form **channels in the outer mitochondrial membrane**, permeabilising it to cytochrome *c* (Siskind et al., 2002; Bionda et al., 2004).
- The altered lipid membrane disassembles **respiratory supercomplexes**, lowering respiration and raising the **reactive oxygen species** that leak from a poorly-assembled chain (Pera et al., 2017).
- Oxidative stress, in the wider ceramide literature, *activates the sphingomyelinases* and drives further ceramide accumulation, while also promoting the membrane-associated lipid derangement that Cutler and colleagues documented in aging and Alzheimer brain (Cutler et al., 2004).
- More ceramide, more membrane derangement, more supercomplex failure, more oxidative stress — and the loop turns again.

This is a self-amplifying cycle seated entirely at one organelle contact, in which a peptide fragment, a lipid, an ion (Section 6), and the respiratory chain drive one another toward the permeabilised, energy-starved, calcium-overloaded state that commits a post-mitotic neuron to a death it cannot undo. The single most important claim of this dissertation is that the cycle does not require amyloid-β *plaques* to run — it runs on C99 and ceramide at the MAM, upstream of deposition — and that this is why the disease's metabolic signature precedes its histology.

6. Calcium: The Second Current Across the Weld

6.1 The Transfer Machinery and Its Presenilin Tuning

The MAM carries two currents between the organelles: a lipid current, which has occupied us so far, and a calcium current. The calcium current runs through the IP$_3$R–grp75–VDAC bridge of Section 2.2, and it is exquisitely sensitive to how tightly the two membranes are apposed — the closer the contact, the more efficiently a pulse of ER calcium is captured by the mitochondrion (Szabadkai et al., 2006). This is where the presenilins re-enter the story not as proteases but as calcium proteins. Zampese and colleagues showed that presenilin-2 — and specifically not presenilin-1 — modulates the calcium shuttling between ER and mitochondrion, and that familial-AD mutants of presenilin-2 *strongly favour* calcium transfer between the two organelles, not by acting on the uptake machinery directly but by *increasing the physical interaction between ER and mitochondria*, multiplying the calcium hot-spots generated at the outer mitochondrial surface (Zampese et al., 2011). A disease mutation in a MAM protein tightens the contact and increases the calcium delivered to the mitochondrion — the same directional change, toward a tighter and busier weld, that Section 4.3 found for lipid synthesis.

6.2 Amyloid-β Tightens the Contact

The convergence deepens when amyloid-β is added. Hedskog and colleagues found that nanomolar amyloid-β increases the expression of the IP$_3$R and VDAC channels, raises the number of ER–mitochondria contact points, and elevates mitochondrial calcium concentration (Hedskog et al., 2013). Amyloid-β, in other words, does to the calcium tether what C99 does to the lipid domain: it tightens and multiplies the contact. Whether one enters the disease through the peptide or through the fragment, the vector points the same way — toward more contact, more calcium transfer, and more lipid traffic across a weld that is being progressively overworked.

6.3 The Shared Executioner

Excess mitochondrial calcium is not benign. Sustained calcium overload is a canonical trigger of the mitochondrial permeability transition and of the release of pro-apoptotic factors — the same outer-membrane permeabilisation that ceramide channels achieve by a different route. Here the two currents of the weld converge on one event. Ceramide, made at the MAM, forms channels or acts with Bax to permeabilise the outer membrane (Siskind et al., 2002; Ganesan et al., 2010); calcium, delivered across the tightened MAM, drives the permeability transition from the matrix side. Both roads end at the mitochondrion's commitment to death, and both are downstream of the same structural change — a MAM that the disease has made too tight and too active. The lipid weapon and the calcium weapon are wielded from one platform. This is the deepest sense in which the ceramide thesis and the bioenergetic thesis are a single thesis: they share not only a location but an executioner.

7. The Cells and the Regions: Where the Lesion Is Seen

7.1 A Universal Contact, Read First in the Fibroblast

A caution must precede any answer to the natural question — *which neurons?* — because the honest reply begins by declining the premise. The MAM is not a neuronal specialisation. Every nucleated cell holds patches of endoplasmic reticulum against its mitochondria; the contact was discovered, after all, in rat *liver* (Vance, 1990), and its tethering and calcium machinery was characterised largely in HeLa cells and fibroblasts (Szabadkai et al., 2006; de Brito & Scorrano, 2008). The process this dissertation describes is general cell biology, and that generality is not a defect of the argument but one of its most useful features.

It is useful because it means the Alzheimer lesion can be read in a cell that is not dying. The single most consequential demonstration that MAM function is upregulated in the disease was made not in a neuron but in **skin fibroblasts taken from living patients** — and in fibroblasts from patients with the *sporadic* disease as well as the familial form (Area-Gómez et al., 2012). Much of the mechanistic dissection that followed used cells chosen for tractability rather than authenticity: mouse embryonic fibroblasts, the SH-SY5Y neuroblastoma line, and — closer to the neuron — induced-pluripotent-stem-cell-derived cells (Montesinos et al., 2020; Del Prete et al., 2017). That a peripheral, replaceable, dividing cell from a patient reproduces the contact-level abnormality is what makes the MAM attractive as a systemic, biopsy-accessible biomarker (§10.3), and it is a standing reminder that the disease's earliest biochemistry is not confined to the neurons that eventually die.

7.2 The Neurons in Which It Has Been Seen

Within the brain, the contact is neither patchy nor restricted to a rare cell. Hedskog and colleagues, examining neurons directly, reported a **uniform distribution of MAM** in them, and found MAM-associated proteins upregulated in **human Alzheimer cortical tissue** and in the hippocampus and cortex of the APP-Swedish/London mouse, before plaques (Hedskog et al., 2013). The calcium arm of the mechanism was demonstrated in **SH-SY5Y cells and in primary neuronal cultures** (Zampese et al., 2011), and the APP-processing machinery was localised to the MAM in neuroblastoma and in transgenic-mouse brain (Del Prete et al., 2017). The demonstrated neuronal footprint is therefore broad — cortical and hippocampal, in human tissue and in models — rather than pinned to a single named population.

Why the neuron should suffer a universal contact's dysregulation more than the fibroblast that shares it is a question of what the cell can absorb, not of where the contact is. The neuron is

post-mitotic and cannot dilute a mislocated lipid or a damaged mitochondrion by dividing; it runs at a steep and continuous energy demand; and it depends on precise, moment-to-moment mitochondrial calcium buffering to sustain synaptic transmission (Proulx et al., 2021). A perturbation that a dividing fibroblast tolerates as an altered synthesis rate is, in a neuron, delivered to a cell that cannot replace what the perturbation destroys — the "irreplaceability" argument *The Janus Lipid* made for ceramide, applied now to the contact that manufactures it. There is, besides, a neuron-specific lipid detail that sharpens the point: the brain relies heavily on the C18 ceramide made by ceramide synthase 1, an enzyme enriched in neurons (§3.1), so that a disturbance of ceramide *topology* at the neuronal MAM falls on the very species the neuron most depends upon.

7.3 The Vulnerability the Contact Does Not Explain

Here the section must concede what the evidence does not supply. Alzheimer's disease is not a uniform dissolution of the cortex; it has a stereotyped topography, ascending from the transentorhinal and entorhinal cortex to the hippocampus and only later to the neocortex, with the basal-forebrain cholinergic neurons and — earliest of all, by the tau-staging evidence treated in this corpus's coerulean volumes — the noradrenergic neurons of the locus coeruleus among the first to tangle. The obvious hope is that the MAM lesion would prove *selectively worse* in exactly these populations and would thereby explain why they fall first. **That selectivity has not been shown.** The MAM data come from bulk cortex and hippocampus, from cell lines, and from fibroblasts; no study has yet demonstrated that entorhinal or coerulean neurons carry a heavier or earlier MAM burden than the resistant neurons beside them.

This is a real limitation, and it is graded as such (Tier III, §9.3). A universal organelle contact cannot, by itself, account for a selective pattern of death; some further variable — regional lipid composition, APOE genotype acting on the entorhinal lipidome, differential calcium-buffering reserve, the net-poor exposure of the locus coeruleus described elsewhere in this corpus — must be layered on top to convert a general mechanism into a regional one. The MAM hypothesis, in short, offers a strong account of *what goes wrong* and a much weaker account of *where it goes wrong first*. A synthesis that claimed otherwise would overreach; the correct posture is to hold the mechanism as established in principle and to name selective vulnerability as the open problem it remains.

8. Reading the Two Theses Together

8.1 One Lesion, Two Vocabularies

Return now to the two literatures with which this dissertation opened. The ceramide literature and the bioenergetic literature described, it can now be said, the two ends and the middle of one lesion. Ceramide is a *product* of the MAM's sphingolipid machinery, made worse by the C99 that accumulates there; the bioenergetic failure is a *consequence* of the same C99-driven change in the mitochondrial membrane that the ceramide disturbance is part of. The two are not parallel insults that happen to co-occur in the Alzheimer neuron. They are the manufacturing output and the mechanical failure of a single overworked contact — the wax that is made at the weld, and the weld's own buckling under the load. A researcher measuring ceramide and a researcher measuring respiration, working in different buildings and citing different journals, have been describing the same organelle from two windows.

8.2 C99, Not Aβ, as the Membrane's Pathogen

The MAM account also quietly reframes the identity of the disease's pathogenic species, and it is worth making the reframing explicit because it bears on the long argument between the amyloid cascade and its critics. In the plaque-centred view, the toxic agent is aggregated amyloid-β. In the MAM view assembled here, the agent that does the early lipid and bioenergetic damage is *C99* — the uncleaved fragment — acting as a cholesterol-sensing, MAM-expanding, sphingolipid-deranging peptide *before* amyloid-β is even released, let alone deposited (Montesinos et al., 2020; Pera et al., 2017). This does not overturn the amyloid cascade so much as relocate its origin: the same amyloidogenic pathway is implicated, but the damage is done at the membrane, by the substrate, upstream of the aggregate. A companion volume of this corpus, *The Case for the Cascade*, deliberately steelmanned amyloid primacy and withheld rebuttal; the MAM reading is not that rebuttal either, but it is the reason the rebuttal has room to stand — because it offers a mechanism by which the disease's earliest, pre-plaque, metabolic phase is generated by the amyloidogenic machinery without requiring the plaque.

8.3 The Boldest Claim and Its Cost

Intellectual honesty requires naming the strongest and least-proven claim in the edifice. It is the claim of *primacy*: that upregulated MAM function is not a downstream consequence of the disease but its upstream cause — that the contact fails first. The evidence gestures toward it: MAM upregulation precedes plaques in mouse models (Hedskog et al., 2013); the causal proteins are MAM proteins; C99 is a MAM-active peptide that accumulates early. But "precedes" is not "causes," the human data are correlational, and no experiment has yet shown that correcting MAM function in an intact brain prevents the disease. The primacy claim is the engine of the whole hypothesis and simultaneously its most exposed flank. Section 9 grades it as such. What can be said with confidence is weaker but still substantial: that the MAM is a genuine site of convergence at which lipid, calcium, bioenergetic, and amyloidogenic abnormalities are demonstrably co-located and mechanistically linked, whether or not it is the first domino.

9. The Validity Ledger

The argument of this dissertation is a chain, and a chain is only as strong as its weakest verified link. What follows grades each major connection on a three-tier scale — Tier I (established, multiply replicated, mechanistically direct), Tier II (strong but incomplete, often proven in cells and unproven in the human brain), Tier III (plausible, contested, or extrapolated) — and names, without softening, the places where the evidence thins or has been retracted.

9.1 Tier I — Established

- **Presenilins and γ-secretase activity are enriched at the MAM.** Demonstrated by three complementary methods and consistent with γ-secretase's known raft preference (Area-Gómez et al., 2009). The whole APP-processing line, including active β- and γ-secretase, was independently localised there by a separate group (Del Prete et al., 2017).
- **The MAM is a detergent-resistant, lipid-raft-like domain** enriched in cholesterol and sphingolipids (Area-Gómez et al., 2012). (Carries the general raft caveat of §2.3.)
- **Ceramide is synthesised at the MAM and the mitochondrial membranes**, via ceramide synthase and reverse ceramidase, in carefully purified fractions (Bionda et al., 2004).
- **Ceramide forms large channels in the outer mitochondrial membrane** *in vitro*, permeabilising it to cytochrome-*c*-sized proteins, regulated by Bcl-2-family proteins (Siskind et al., 2002; Colombini, 2013; Ganesan et al., 2010).

- **MAM function is upregulated in AD cells**, including fibroblasts from sporadic as well as familial patients, measured as cholesteryl-ester and phospholipid synthesis (Area-Gómez et al., 2012).
- **The calcium tether is real and chaperone-coupled** (IP_3R–grp75–VDAC) (Szabadkai et al., 2006).

9.2 Tier II — Strong but Incomplete

- **C99 accumulates at the MAM and drives sphingolipid turnover and altered mitochondrial-membrane composition, disrupting respiratory supercomplexes** (Pera et al., 2017). This is the keystone mechanism; it is demonstrated in cell models of AD, and its extension to the human brain is inferred, not shown. It is graded Tier II for that reason, not for any weakness in the cellular data, which are strong.
- **C99 acts as a cholesterol-sensing peptide that nucleates and expands MAM** (Montesinos et al., 2020), recapitulated *in vivo* in a TBI model (Agrawal et al., 2022). Cellular and single-model *in vivo* evidence; not yet shown in human AD brain.
- **MAM-associated proteins are upregulated in the human AD brain, and before plaques in mouse models** (Hedskog et al., 2013). The mouse temporal ordering is the strongest support for primacy; the human data are cross-sectional and correlational.
- **Presenilin-2 FAD mutants tighten the contact and increase ERmitochondrial calcium transfer** (Zampese et al., 2011). Robust in cell models; a familial-mutation finding whose generalisation to sporadic disease is an inference.
- **Ceramide and cholesterol accumulate in aging and AD brain with membrane oxidative stress, and Aβ induces this in neurons** (Cutler et al., 2004). Solid for the association and for an *in-vitro* causal arrow; the direction in the human brain remains partly open (see §9.3).

9.3 Tier III — Plausible, Contested, or Extrapolated

- **Primacy of MAM dysfunction — that the contact fails *upstream* of plaques and tangles.** The hypothesis's boldest claim (§8.3). Supported by pre-plaque timing but not by any intervention proving causation in an intact brain. Plausible; unproven.
- **Ceramide channels operate in the living AD neuron.** The channels are established *in vitro* in isolated mitochondria and planar bilayers (Siskind et al., 2002); that they form and release cytochrome *c* in the intact human neuron *in situ* is a reasonable but unproven extrapolation.
- **MFN2 as the tether.** Genuinely contested: the original tethering assignment (de Brito & Scorrano, 2008) has been challenged by work finding that MFN2 loss *increases* contacts,

implying a spacer rather than a tether. The net role is unresolved; no conclusion here rests on MFN2 specifically.

- **Direction of ceramide accumulation.** Carried from *The Janus Lipid*: elevated brain ceramide may be partly a *consequence* of membrane breakdown rather than only a cause, and Mendelian-randomisation evidence on sphingolipids and AD is mixed. Reverse causation cannot be excluded for the accumulation, even where the MAM mechanism for a lethal sub-pool is sound.
- **The VAPB–PTPIP51 / GSK-3β thread to tau.** Suggestive but derived from an ALS/FTD context (Stoica et al., 2014); its relevance to Alzheimer tau pathology is an analogy, not a result.

9.4 A Retraction Named

One connection frequently drawn in the ceramide–AD literature must be flagged for a specific reason. The widely cited claim that **ceramide post-translationally stabilises BACE1 and thereby increases amyloid-β production** traces to a 2003 paper (Puglielli et al., *J. Biol. Chem.* 278:19777) that has since been **retracted**. This dissertation therefore does *not* rest any part of its argument on a direct ceramideBACE1 stabilisation step, and the reader should treat that particular sub-branch of the ceramide story as unsupported by its foundational citation. The MAM mechanism assembled here does not need it: the C99sphingolipidmembranesupercomplex axis (Pera et al., 2017) and the C99cholesterolMAM axis (Montesinos et al., 2020) stand on non-retracted, independently corroborated work. Naming the retraction is part of the method, not an aside — a synthesis that quietly inherited a retracted result would be building on sand.

10. Therapeutic Implications: Tuning a Contact

10.1 The First Rule: Do Not Sever the Weld

The therapeutic reflex that this analysis most sternly warns against is the reflex to *abolish* the offending structure. The MAM is not a lesion to be excised; it is an essential organelle contact through which the cell synthesises phospholipids, esterifies cholesterol, transfers calcium to fuel respiration, and divides its mitochondria. Two of its resident proteins — the calcium-handling PACS2 and the sigma-1 receptor — are required for neuronal survival, and silencing them degenerates neurons (Hedskog et al., 2013). A drug that broke the ER–mitochondria contact to stop the disease would kill the cell faster than the disease does. The therapeutic target is not the existence of the weld but its *set-point*: the goal is to return an over-tight, over-active contact toward normal apposition and normal flux, not to dissolve it.

10.2 Candidate Nodes

Within that constraint, the mechanism identifies several rational points of intervention, each with an honest caveat.

- **Cholesterol esterification (ACAT1/SOAT1).** Because cholesteryl-ester synthesis is both a readout of MAM overactivity and a homeostatic response to the C99-driven cholesterol influx (Montesinos et al., 2020; Area-Gómez et al., 2012), ACAT inhibition is a mechanistically motivated node — and one for which brain-penetrant inhibitors have shown benefit in AD models in the broader literature. The caveat is that cholesterol esterification is protective detoxification as well as a marker; blunting it could, in principle, expose the cell to free-cholesterol toxicity.
- **Sphingolipid synthesis and the sphingomyelinases.** The keystone mechanism runs through elevated sphingolipid turnover (Pera et al., 2017); and inhibiting sphingomyelin synthesis prevented ceramide/cholesterol accumulation and protected neurons against amyloid-β in Cutler's experiments (Cutler et al., 2004). Modulating the ceramide-generating enzymes at the MAM is therefore a direct strike at the lethal sub-pool. The caveat is severe and is inherited from *The Janus Lipid*: ceramide is a systemic death signal that oncology labours to *raise* in tumours, and a drug that lowers it brain-wide is, at the level of whole-body lipid biology, pointed at the opposite therapeutic goal elsewhere in the patient.
- **Clearing C99 rather than only blocking Aβ.** If the early membrane damage is done by the *fragment*, then the design logic of anti-amyloid therapy shifts. A γ-secretase *inhibitor* would *raise* C99, potentially worsening the MAM lesion even as it lowers amyloid-β — a possible clue to why some γ-secretase inhibitors failed clinically. A γ-secretase *modulator*, or a strategy that lowers C99 production or accelerates its non-amyloidogenic clearance, is more consonant with this mechanism than one that merely mops up the downstream peptide. This is a hypothesis to be tested, not a settled recommendation.

- **The tethers themselves.** In principle the apposition could be loosened pharmacologically toward its healthy set-point, but the contested biology of the individual tethers (§9.3) makes any single-protein strategy premature. This node is flagged as conceptually apt and practically immature.

10.3 A Measurement Agenda

The mechanism also implies what to measure. If the disease's earliest phase is an over-active contact making a mislocated lipid, then the biomarkers worth developing are not only the plaque and tangle markers of late disease but *contact-level* readouts: the degree of ER–mitochondria apposition, the rate of MAM lipid synthesis, and — hardest and most valuable — the size and location of the lethal ceramide sub-pool rather than total brain ceramide, whose averaging (§3.3) hides the signal. A cell-biological biomarker of MAM overactivity, validated against the pre-plaque window, would test the primacy claim of §8.3 more decisively than any correlation in end-stage tissue.

11. Coda: The Wax, the Weld, and the First Lesion

The pathology of Alzheimer's disease has for decades been narrated as a story of two proteins that misfold and accumulate — a plaque outside the neuron and a tangle within it. This dissertation has tried to tell the part of the story that happens before either, at a place too small to see in a stained section: a contact where the endoplasmic reticulum leans against the mitochondrion and is held there, a few tens of nanometres away, by a scaffold of tethers. At that contact the cell makes ceramide, ferries calcium, esterifies cholesterol — and, by an accident of localisation that reorganised the field, cuts the amyloid precursor protein. When a fragment of that protein lingers where it should be cleaved, it expands the contact, mobilises cholesterol into it, and drives the sphingolipid machinery too hard; the ceramide made there in excess and the calcium delivered across a contact drawn too tight converge on the mitochondrion, disassemble its respiratory chain, permeabilise its outer membrane, and commit a cell that can never be replaced to a death summed silently over years.

If that account is right — and Section 9 has been careful about how much of it is proven — then the wax and the weld are not two problems but one, and the first lesion of the disease is neither the plaque nor the tangle but the buckling of a junction that most of neurology has never had cause to name. The plaques and tangles would then be the smoke; the MAM would be the fire, or at least the hearth in which it is laid. The metaphor of the title was chosen to hold both the material and the join — the manufactured lipid and the contact that manufactures it — because the argument's

whole burden is that they cannot be understood apart. The weld that never fuses is where two organelles, two lipids, two ions, and two literatures meet; and it may be where the longest disease of the mind begins.

Acknowledgments and Methodological Note

This dissertation was prepared under the Organic Network Synthesis (ONS) methodology of AdultCognitiveDisease.com. It is a work of synthesis: it advances no primary experimental data of its own but assembles, grades, and interprets the published record, welding two lines of that record — the ceramide/sphingolipid literature treated in *The Janus Lipid* and the mitochondrial/bioenergetic literature treated in *The Bioenergetic Collapse* — around a single organelle contact. Every mechanistic claim is tied to a specific, individually verified primary source; the validity of each major connection is graded explicitly in Section 9, including the identification of one retracted foundational paper that the argument was deliberately built to avoid depending upon. Bibliographic details were verified against PubMed. The synthesis is offered as a heuristic that generates falsifiable predictions — chief among them that the pre-plaque metabolic phase of the disease is generated by C99 and ceramide at the MAM, upstream of amyloid deposition — and not as a settled account.

References

Agrawal, R. R., Larrea, D., Xu, Y., Shi, L., Zirpoli, H., Cummins, L. G., Emmanuele, V., Song, D., Yun, T. D., Macaluso, F. P., Min, W., Kernie, S. G., Deckelbaum, R. J., & Area-Gomez, E. (2022). Alzheimer's-associated upregulation of mitochondria-associated ER membranes after traumatic brain injury. *Cellular and Molecular Neurobiology*, 43(5), 2219–2241. https://doi.org/10.1007/s10571-022-01299-0

Area-Gomez, E., de Groof, A. J. C., Boldogh, I., Bird, T. D., Gibson, G. E., Koehler, C. M., Yu, W. H., Duff, K. E., Yaffe, M. P., Pon, L. A., & Schon, E. A. (2009). Presenilins are enriched in endoplasmic reticulum membranes associated with mitochondria. *The American Journal of Pathology*, 175(5), 1810–1816. https://doi.org/10.2353/ajpath.2009.090219

Area-Gomez, E., del Carmen Lara Castillo, M., Tambini, M. D., Guardia-Laguarta, C., de Groof, A. J. C., Madra, M., Ikenouchi, J., Umeda, M., Bird, T. D., Sturley, S. L., & Schon, E. A. (2012). Upregulated function of mitochondria-associated ER membranes in Alzheimer disease. *The EMBO Journal*, 31(21), 4106–4123. https://doi.org/10.1038/emboj.2012.202

Area-Gomez, E., & Schon, E. A. (2017). On the pathogenesis of Alzheimer's disease: The MAM hypothesis. *FASEB Journal*, 31(3), 864–867. https://doi.org/10.1096/fj.201601309

Bionda, C., Portoukalian, J., Schmitt, D., Rodriguez-Lafrasse, C., & Ardail, D. (2004). Subcellular compartmentalization of ceramide metabolism: MAM (mitochondria-associated membrane) and/or mitochondria? *The Biochemical Journal*, 382(Pt 2), 527–533. https://doi.org/10.1042/BJ20031819

Colombini, M. (2013). Membrane channels formed by ceramide. *Handbook of Experimental Pharmacology*, (215), 109–126. https://doi.org/10.1007/978-3-7091-1368-4_6

Cutler, R. G., Kelly, J., Storie, K., Pedersen, W. A., Tammara, A., Hatanpaa, K., Troncoso, J. C., & Mattson, M. P. (2004). Involvement of oxidative stress-induced abnormalities in ceramide and cholesterol metabolism in brain aging and Alzheimer's disease. *Proceedings of the National Academy of Sciences of the United States of America*, 101(7), 2070–2075. https://doi.org/10.1073/pnas.0305799101

de Brito, O. M., & Scorrano, L. (2008). Mitofusin 2 tethers endoplasmic reticulum to mitochondria. *Nature*, 456(7222), 605–610. https://doi.org/10.1038/nature07534

Del Prete, D., Suski, J. M., Oulès, B., Debayle, D., Gay, A. S., Lacas-Gervais, S., Bussiere, R., Bauer, C., Pinton, P., Paterlini-Bréchot, P., Wieckowski, M. R., Checler, F., & Chami, M. (2017). Localization and processing of the amyloid-β protein precursor in mitochondria-associated membranes. *Journal of Alzheimer's Disease*, 55(4), 1549–1570. https://doi.org/10.3233/JAD-160953

Ganesan, V., Perera, M. N., Colombini, D., Datskovskiy, D., Chadha, K., & Colombini, M. (2010). Ceramide and activated Bax act synergistically to permeabilize the mitochondrial outer membrane. *Apoptosis*, 15(5), 553–562. https://doi.org/10.1007/s10495-009-0449-0

Hedskog, L., Pinho, C. M., Filadi, R., Rönnbäck, A., Hertwig, L., Wiehager, B., Larssen, P., Gellhaar, S., Sandebring, A., Westerlund, M., Graff, C., Winblad, B., Galter, D., Behbahani, H., Pizzo, P., Glaser, E., & Ankarcrona, M. (2013). Modulation of the endoplasmic reticulum-mitochondria interface in Alzheimer's disease and related models. *Proceedings of the National Academy of Sciences of the United States of America*, 110(19), 7916–7921. https://doi.org/10.1073/pnas.1300677110

Montesinos, J., Pera, M., Larrea, D., Guardia-Laguarta, C., Agrawal, R. R., Velasco, K. R., Yun, T. D., Stavrovskaya, I. G., Xu, Y., Koo, S. Y., Snead, A. M., Sproul, A. A., & Area-Gomez, E. (2020). The Alzheimer's disease-associated C99 fragment of APP regulates cellular cholesterol trafficking. *The EMBO Journal*, 39(20), e103791. https://doi.org/10.15252/embj.2019103791

Pera, M., Larrea, D., Guardia-Laguarta, C., Montesinos, J., Velasco, K. R., Agrawal, R. R., Xu, Y., Chan, R. B., Di Paolo, G., Mehler, M. F., Perumal, G. S., Macaluso, F. P., Freyberg, Z. Z., Acin-Perez, R., Enriquez, J. A., Schon, E. A., & Area-Gomez, E. (2017). Increased localization of APP-C99 in mitochondria-associated ER membranes causes mitochondrial dysfunction in Alzheimer disease. *The EMBO Journal*, 36(22), 3356–3371. https://doi.org/10.15252/embj.201796797

Proulx, J., Park, I. W., & Borgmann, K. (2021). Cal'MAM'ity at the endoplasmic reticulum-mitochondrial interface: A potential therapeutic target for neurodegeneration and human immunodeficiency virus-associated neurocognitive disorders. *Frontiers in Neuroscience*, 15, 715945. https://doi.org/10.3389/fnins.2021.715945

Schon, E. A., & Area-Gomez, E. (2010). Is Alzheimer's disease a disorder of mitochondria-associated membranes? *Journal of Alzheimer's Disease*, 20(Suppl 2), S281–S292. https://doi.org/10.3233/JAD-2010-100495

Schon, E. A., & Area-Gomez, E. (2012). Mitochondria-associated ER membranes in Alzheimer disease. *Molecular and Cellular Neurosciences*, 55, 26–36. https://doi.org/10.1016/j.mcn.2012.07.011

Siskind, L. J., Kolesnick, R. N., & Colombini, M. (2002). Ceramide channels increase the permeability of the mitochondrial outer membrane to small proteins. *The Journal of Biological Chemistry*, 277(30), 26796–26803. https://doi.org/10.1074/jbc.M200754200

Stoica, R., De Vos, K. J., Paillusson, S., Mueller, S., Sancho, R. M., Lau, K. F., Vizcay-Barrena, G., Lin, W. L., Xu, Y. F., Lewis, J., Dickson, D. W., Petrucelli, L., Mitchell, J. C., Shaw, C. E., & Miller, C. C. J. (2014). ER-mitochondria associations are regulated by the VAPB-PTPIP51 interaction and are disrupted by ALS/FTD-associated TDP-43. *Nature Communications*, 5, 3996. https://doi.org/10.1038/ncomms4996

Szabadkai, G., Bianchi, K., Várnai, P., De Stefani, D., Wieckowski, M. R., Cavagna, D., Nagy, A. I., Balla, T., & Rizzuto, R. (2006). Chaperone-mediated coupling of endoplasmic reticulum and mitochondrial Ca2+ channels. *The Journal of Cell Biology*, 175(6), 901–911. https://doi.org/10.1083/jcb.200608073

Vance, J. E. (1990). Phospholipid synthesis in a membrane fraction associated with mitochondria. *The Journal of Biological Chemistry*, 265(13), 7248–7256.

Williamson, C. D., Wong, D. S., Bozidis, P., Zhang, A., & Colberg-Poley, A. M. (2015). Isolation of endoplasmic reticulum, mitochondria, and mitochondria-associated membrane and detergent resistant membrane fractions from transfected cells and from human cytomegalovirus-infected primary fibroblasts. *Current Protocols in Cell Biology*, 68, 3.27.1–3.27.33. https://doi.org/10.1002/0471143030.cb0327s68

Zampese, E., Fasolato, C., Kipanyula, M. J., Bortolozzi, M., Pozzan, T., & Pizzo, P. (2011). Presenilin 2 modulates endoplasmic reticulum (ER)-mitochondria interactions and Ca2+ cross-talk. *Proceedings of the National Academy of Sciences of the United States of America*, 108(7), 2777–2782. https://doi.org/10.1073/pnas.1100735108

Retraction note: Puglielli, L., Ellis, B. C., Saunders, A. J., & Kovacs, D. M. (2003). Ceramide stabilizes β-site amyloid precursor protein-cleaving enzyme 1 and promotes amyloid β-peptide biogenesis. J. Biol. Chem. 278(22), 19777–19783 — has since been **retracted***, and is cited here solely to document that this dissertation does not rely upon it (see §9.4).*