The Unmade Eraser

UBE3A, PTPA and Ephexin5 — Whether the Alzheimer Neuron Fails to Build Its Principal Tau Phosphatase, and What One Ubiquitin Ligase Does to Two Diseases That Share It

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Before an enzyme can be switched off, it has to be built. One ligase governs the brake and the eraser both — and the two diseases that lose it disagree about which way the eraser moves.

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Abstract

Protein phosphatase 2A removes roughly seventy per cent of the phosphate that comes off tau in the human brain, and its activity is reduced in Alzheimer's disease. The field has explained that reduction almost entirely as a failure of *regulation*: an inhibitor that leaves the nucleus, a methyl group that is not placed, a protease that cuts, a kinase that writes. This volume asks a question that sits one step earlier and has not been asked of the Alzheimer neuron at all. **Before an enzyme can be switched off, it has to be built.** What, in a neuron, actually produces this phosphatase — and is any part of that production line damaged in the disease?

The parts list is longer than the enzyme's name suggests. The working enzyme is a three-part complex: a catalytic subunit from one of two genes, a scaffolding subunit from one of two more, and one regulatory subunit drawn from four unrelated families spanning about fifteen genes, which between them decide what the enzyme will dephosphorylate and where it will sit. Only one of those regulatory subunits makes the enzyme a tau phosphatase. The catalytic subunit is not born active: it is held in a restrained state, and it must be opened by a dedicated activation chaperone, **PTPA**, in a reaction that consumes ATP and installs the catalytic metals, and then carboxy-methylated by a dedicated methyltransferase before the tau-directed regulatory subunit will bind. Five accessory proteins exist for no other purpose than to prevent this enzyme from working before it is finished. The catalytic subunit's own abundance is held constant by a translational autoregulatory loop that defeats attempts to make more of it. And the regulatory subunits are not interchangeable: deleting one is embryonically lethal, de novo variants in another cause a human neurodevelopmental disorder, and deleting a third gives mice a tauopathy without any loss of catalytic activity at all. This is a production line with a fixed output and several single points of failure, and the field has audited almost none of it in human disease tissue.

One of those points of failure is damaged in Alzheimer's brain, and the finding has gone almost unremarked. PTPA — the activation chaperone without which the catalytic subunit cannot acquire its serine/threonine specificity — is reduced in Alzheimer cortex and in two independent transgenic mouse models. Knocking it down in cells inhibits the phosphatase and hyperphosphorylates tau. If that result holds, the Alzheimer deficit is partly a deficit of *manufacture*, not only of regulation, and it is upstream of every lock the field has described.

But the same protein is a ubiquitin ligase substrate, and that is where the account breaks. PTPA is destroyed by UBE3A, the ligase whose maternal loss causes Angelman syndrome. In Angelman model mice, losing UBE3A raises PTPA, increases holoenzyme assembly and **raises** phosphatase activity — enough that a phosphatase *inhibitor* rescues the synaptic and motor phenotype. UBE3A also destroys Ephexin5, the exchange factor that restrains excitatory synapse formation, and Arc, the protein that drives AMPA receptors off the surface. And UBE3A function is **lost in Alzheimer's disease too**: it is depleted in amyloid-bearing mice before the behavioural deficits appear, driven out of solution by a tyrosine kinase activated downstream of amyloid-β, and its loss there raises Ephexin5 and Arc exactly as the Angelman model predicts.

So two diseases lose the same ligase, and two of its three substrates behave identically in both — while the third behaves in opposite directions. Ephexin5 rises in Angelman and in Alzheimer's. Arc rises in both. PTPA rises in Angelman and *falls* in Alzheimer's. The arithmetic does not close, and no paper in either literature cites the other. We set out five ways it could be resolved and grade them: a second, ligase-independent route of PTPA loss that overwhelms the stabilisation; a partial rather than complete loss of ligase function in Alzheimer's that spares the PTPA arm; a bulk-tissue measurement confounded by the loss of neurons themselves; a difference in what the two fields mean by "activity"; or an error in one of the two measurements. The first is the most likely and the easiest to test, and no experiment currently distinguishes them. A sixth candidate explanation — that the shared proteasome is simply degrading less of everything — turns out to make the contradiction worse rather than better, because a capacity deficit would raise all three substrates and PTPA falls.

A second, weaker inference falls out of the Alzheimer evidence and is recorded because it is cheap to test. The two studies that establish the ligase lesion point in opposite causal directions: in one, amyloid-β disables the ligase through a tyrosine kinase; in the other, removing the ligase *reduces* amyloid production while worsening cognition. Read together they describe a negative feedback loop on amyloid — and a loop of that shape is self-limiting on the plaque arm and not on the synaptic arm, which would predict plaque load saturating while synaptic loss continues. That dissociation is the most robust discrepancy in the neuropathology of this disease and the original amyloid cascade does not account for it. We grade the loop as the weakest inference in this volume and name the single cross that would test it, because it predicts something unusual: that restoring the ligase should improve synapses while *increasing* amyloid, which is an inversion a trial-stage programme would want to learn on purpose rather than by accident.

Two subtractions follow, and both are forced by reagents rather than by failures of replication. The published mechanism by which PTPA is said to activate the phosphatase in the Alzheimer context runs through dephosphorylation of a tyrosine at residue 307 of the catalytic subunit. Three studies from two independent laboratories have since shown that the antibodies used to report that modification are not phosphosensors at all — they are blocked by a methyl group at an adjacent residue — and the genuine tyrosine sites have been relocated to residues 127 and 284. The PTPA *abundance* finding in Alzheimer brain does not depend on those reagents and survives. The *mechanism* attached to it does not, and should not be carried forward. Separately, the widely repeated claim that this phosphatase strips the phosphate from the AMPA receptor's serine 845 is not supported by any primary study we could locate; the experiment usually cited measures a combined phosphatase activity pharmacologically and does not resolve which enzyme acts. We decline the attribution rather than repeat it, along with two others — that the enzyme destabilises PSD-95, and that cytoskeletal collapse releases an actin-anchored pool of it.

One positive inference is new and resolves a problem of sign. The tyrosine whose phosphorylation biases Ephexin5 toward RhoA is the same tyrosine whose phosphorylation licenses the ligase to destroy it. A neuron that loses the *ligase* therefore accumulates the phosphorylated, RhoA-biased, spine-eliminating form — which is the form the disease requires. A neuron that loses the *receptor kinase* upstream would accumulate the dephosphorylated, Cdc42-biased,

growth-promoting form, which is not. The ligase lesion fits the observed Alzheimer phenotype where the receptor lesion inverts it, and one measurement — phospho-tyrosine-361 fraction rather than total protein, in human tissue — would decide between them with reagents that already exist.

What survives is a coherent and testable proposition that nobody has stated. If the Alzheimer neuron's phosphatase problem is partly a manufacturing problem, then the therapeutic logic of the field is pointed at the wrong stage: compounds that bias an existing holoenzyme are being asked to compensate for a shortage of finished enzyme. And the two diseases that share this ligase currently imply **opposite drugs** — a phosphatase inhibitor for Angelman, a phosphatase activator for Alzheimer's — which is either a profound dissociation worth understanding or a sign that one of the two activity measurements is wrong.

The experiment. PTPA, UBE3A and the tau-directed holoenzyme measured in the same human cortical tissue, resolved to neurons rather than to homogenate, with the activity assay run on synaptic and non-synaptic fractions separately instead of averaged across them; and the decisive counterpart in culture: in a neuron exposed to amyloid-β, does lowering UBE3A raise PTPA, as it does in the Angelman brain, or does it fail to? One answer says the ligase is the controller and Alzheimer's has a second lesion stacked on it. The other says the ligase's grip on this substrate is broken in the disease. The reagents exist, the tissue exists, and the question has not been put.

Part I

The Production Line

Four genes, fifteen regulatory choices, two chaperones and a methyl group — what has to go right before a neuron owns a working tau phosphatase.

I. The Question That Sits One Step Earlier

There is a habit of thought in disease biology that treats an enzyme as a fixture. The enzyme is assumed to be present, in the right amount, in the right place, and the only interesting question is what turns it up or down. For most enzymes the habit is harmless. For protein phosphatase 2A it is not, because this enzyme is not a fixture. It is an assembly, built from parts encoded by separate genes, finished by dedicated chaperones, and licensed for its substrate by a chemical modification placed at the last step. The assembly can fail at several points, and failure at any of them looks, from the outside, exactly like inhibition.

The disease context makes the distinction consequential. Tau's phosphorylation state is a running balance between the kinases that add phosphate and the phosphatases that remove it, and the phosphatase side of that balance is dominated by one enzyme. Partitioned across the four serine/threonine phosphatases that act on tau in human brain, protein phosphatase 2A performs approximately seventy-one per cent of the total activity, with protein phosphatase 1 at eleven per cent, protein phosphatase 5 at ten per cent, and calcineurin at seven per cent.1 Total phosphatase activity toward tau is decreased in Alzheimer brain, and the decrease is carried by protein phosphatase 2A and protein phosphatase 5, while calcineurin activity on the same measurement is increased.1,2 The deficit is real, it is specific, and it has been replicated for three decades.3,4

What the field has done with that deficit is explain it as a problem of regulation. The explanations are good ones and we rehearse them only to say what they have in common. The catalytic subunit's carboxy-terminal methyl ester at leucine 309 — placed by leucine carboxyl methyltransferase 1, removed by phosphatase methylesterase 1 — is required for recruitment of the regulatory subunit that makes the enzyme a tau phosphatase, and both the methyl group and the methyltransferase are reduced in Alzheimer cortex in a pattern that tracks neurofibrillary pathology.5,6,7 The catalytic subunit is bound and inhibited by an endogenous inhibitor that is nuclear in health and cytoplasmic in disease. A tyrosine kinase mistargeted to the postsynaptic density in a tau-dependent manner is held to write an inactivating phosphotyrosine.8 Each of these is a statement about an enzyme that exists and has been interfered with.

None of them asks whether the enzyme exists in the first place. That question has a specific and checkable form, and in one place it has already been answered in a direction the field did not pursue: messenger RNA for the phosphatase's catalytic subunit is quantitatively decreased in Alzheimer hippocampus.9 A transcript measurement is not an enzyme measurement, and the usual confounds apply — bulk tissue, neuronal loss, post-mortem RNA integrity — but it is the only published observation that speaks to supply rather than to control, and it has not been followed up at the level of the assembly machinery. This volume follows it.

We begin with the parts.

II. What Actually Produces the Enzyme in a Neuron

The working enzyme is a trimer, and it is the trimer that has a function. The noun "PP2A" names a family, not a molecule. A functional holoenzyme consists of a catalytic subunit, a scaffolding subunit, and one regulatory subunit, and the regulatory subunit is what supplies the substrate specificity, the subcellular address, and the fine-tuning of activity.10 Two genes encode the catalytic subunit — *PPP2CA* and *PPP2CB*, giving the Cα and Cβ isoforms, of which Cα is the dominant species in brain. Two more encode the scaffold, *PPP2R1A* and *PPP2R1B*, giving Aα and Aβ. Together these produce the core dimer, which has catalytic competence and almost no specificity.

Specificity arrives with the third subunit, and there are about fifteen candidates from four unrelated families. The B family proper (B55) is encoded by *PPP2R2A–D*; the B′ family (B56) by *PPP2R5A–E*; the B″family (PR72 and relatives) by *PPP2R3A–C*; and the B‴family by the three striatin genes, which in neurons assemble into larger signalling complexes at dendritic spines. The families share no sequence homology with one another. They bind the same surface of the core dimer and they direct the enzyme to entirely different substrates.

This matters for the disease because **only one of these subunits makes the enzyme a tau phosphatase.** The B55α subunit binds tau directly, at a motif that is also recognised by a kinase of the Src family, and the structure of the B55-containing holoenzyme shows how the subunit positions tau for dephosphorylation.11,12,13 The ABαC holoenzyme — scaffold, B55α, catalytic subunit — is the species whose levels are altered in Alzheimer brain.5 A measurement of "total PP2A activity" in a brain homogenate does not report on this species. It reports on a population of many possible trimers of which this is one.

The catalytic subunit is not born working. This is the part of the story that the word "expression" conceals. Newly synthesised catalytic subunit exists in a conformationally restrained, poorly specific state, and a set of accessory proteins exists for the express purpose of keeping it that way until assembly is complete. Five such modulators are described: the α4 protein, which binds and stabilises the unassembled catalytic subunit; **PTPA**, the activation chaperone; leucine carboxyl methyltransferase 1, which places the carboxy-terminal methyl ester; phosphatase methylesterase 1, which removes it and which also holds the catalytic subunit in an inactive complex; and TIPRL1.10 The review that assembled this scheme stated its logic plainly: these proteins serve to prevent promiscuous phosphatase activity until the holoenzyme is completely assembled.10 A phosphatase that could act before it was finished would be a liability to the cell, and the cell has invested five gene products in making sure it cannot.

PTPA opens the enzyme, and it does so by hydrolysing ATP. The crystal structure of the activation chaperone bound to the phosphatase with a non-hydrolysable ATP analogue shows

the mechanism directly: PTPA makes broad contacts with the structural elements surrounding the active site and with the adenine moiety of ATP; its binding stabilises the protein fold of the apo-enzyme required for activation; and it orients the ATP phosphoryl groups into the active site itself, so that the phosphatase and the chaperone together constitute a composite ATPase.14,15 The functional consequence is the one that matters here. ATP binding and hydrolysis selectively and drastically enhance the binding of the endogenous catalytic metal ions, and that metal loading is what confers phosphoserine/phosphothreonine-directed activity.14 Without this reaction, there is no serine/threonine phosphatase. There is a folded protein with the wrong metals in it.

The methyl group comes last, and it is what admits the tau-directed subunit. Carboxy-methylation of the catalytic subunit at leucine 309 by leucine carboxyl methyltransferase 1 is required for the association of the B55α subunit, and the requirement is subunit-selective: methylation is essential for Bα binding but not for the binding of striatin or SG2NA.16 The structural basis of demethylation and the role of the carboxy-terminus in regulatory-subunit selection have both been worked out.17,18 The consequence is that demethylation does not simply reduce the enzyme's activity. **It redirects the enzyme**, taking it off the tau-directed pathway while leaving other pathways supplied — which is why "reduced PP2A activity" and "reduced tau phosphatase activity" are not synonyms, and why bulk activity assays have been a poor instrument for this disease.

And the supply is capped. The catalytic subunit is subject to a translational autoregulatory mechanism that adjusts its protein level to a constant, independent of transcription or RNA processing; the mechanism was discovered because forced overexpression in fibroblasts simply failed to raise the protein.19 The experiment is old, it was done in a mouse fibroblast line, and it establishes only that the cell resists an *increase*. Whether a neuron can defend against a *decrease*, and whether the loop operates at all in a post-mitotic cell under amyloid stress, is unknown. But the finding has a consequence the therapeutic literature has not absorbed: if the lesion is a shortage of catalytic subunit, transcriptional strategies to make more of it are arguing with a homeostat.

III. The Regulatory Subunits Have Their Own Diseases

If the regulatory subunit is what makes the enzyme do one thing rather than another, then a mistake in *which* subunit is installed should produce disease by itself, independently of how much catalytic activity the cell has. It does, and the demonstrations are among the most useful evidence that this enzyme should be read as an assembly rather than as an activity.

The B55 family. Deletion of *Ppp2r2a*, the gene encoding B55α — the subunit that binds tau — is not survivable. Knockout mice reveal B55α as an essential regulator of neuronal and epidermal embryonic development, with the animals failing during development rather than exhibiting a late

phenotype.20 The subunit that makes this a tau phosphatase in the adult brain is also the subunit the embryo cannot do without, which puts a hard ceiling on any strategy proposing to manipulate it globally.

The B56 family, and a human neurodevelopmental disorder. De novo single-nucleotide changes in *PPP2R5D*, which encodes the B′56δ subunit, cause a dominant early-childhood condition characterised by intellectual disability, hypotonia, autism-spectrum features, macrocephaly and dysmorphic features.21 The variants are heterozygous and missense — one copy of one regulatory subunit, altered by one base — and the molecular consequence has been traced: a clinically observed E420K variant, introduced into human cells by single-base editing, produced a previously unrecognised interaction with AKT, constitutively active AKT–mTOR signalling, increased cell size and uncoordinated growth.21 Nothing about this phenotype is a generic loss of phosphatase activity. It is a *misdirection* of a phosphatase onto a pathway it should not be governing.

And the same family produces tauopathy in mice by subunit identity alone. Mice lacking the B′δ subunit develop a spatially restricted tauopathy, through deregulation of the kinases CDK5 and GSK3β.22 This is worth stating precisely, because it is the cleanest available demonstration of the argument this volume is making. These animals have not lost catalytic subunit, have not lost scaffold, and have not been given an inhibitor. They have lost one regulatory subunit out of fifteen, and they develop tau pathology. **Tau pathology can be produced by an assembly error.**

The striatins, and the subunit that does not need the methyl group. The B‴family — the three striatins — assemble in neurons into larger signalling complexes concentrated at dendritic spines. Their relevance here is a negative result with large consequences: carboxy-methylation of the catalytic subunit at leucine 309 is essential for the association of B55α but **not** for the association of striatin or SG2NA.16 Loss of the methyl group therefore does not take the enzyme out of service. It takes the enzyme off tau while leaving it available to the striatin-directed pathways. The disease-relevant quantity is not how much enzyme is working but how the available enzyme has been allocated, and every bulk activity assay in this literature is blind to allocation.

One human genetic observation touches the pathway from an unexpected direction. The protective effect of the *APOE* ε2 allele against Alzheimer's disease has been linked, in a large genetics consortium analysis, to protein phosphatase 2A together with complement component 4.23 We carry this at low weight and name its limits: it is an association study, the mechanistic interpretation is not established, and the phosphatase appears as a pathway rather than as an identified subunit. But it is the only place where human genetics and this enzyme meet in Alzheimer's disease, and it meets on the protective side.

Taken together, these results support a specific reading. **The fifteen regulatory subunits are not interchangeable parts drawn from a common pool; they are fifteen different enzymes sharing one catalytic engine.** Two of them have human or murine diseases of their own. One of them is required for the embryo. One of them binds tau, and one of them does not need the modification that the Alzheimer brain has lost. An account of this enzyme in disease that does not say which subunit it means has not yet said anything checkable.

IV. What This Means for Reading the Disease Literature

Three readings follow from the parts list, and each of them changes how an Alzheimer measurement should be interpreted.

First, "PP2A activity is reduced" is at least four different claims. It could mean less catalytic subunit; or the same catalytic subunit incompletely activated for want of chaperone; or a fully active catalytic subunit assembled with the wrong regulatory subunit; or a correctly assembled holoenzyme occupied by an inhibitor. These have different causes, different time courses and different remedies. A homogenate assay against a generic substrate cannot distinguish them, and most of the canonical human numbers come from such assays.

Second, the tau-relevant question is narrower than the enzyme. What matters for tau is the abundance of the ABαC species. The two upstream requirements for that species are the activation reaction and the methylation reaction. The methylation arm has been examined in human tissue with care and has produced the strongest result in this field.5›6›7 The activation arm has been examined once.

Third, a failure of manufacture would be invisible to every assay the field has used to look for inhibition. An enzyme that was never properly loaded with its metals is not an inhibited enzyme; it is an absent one. Add an inhibitor-displacing compound and nothing happens. Measure "inhibition" and you find none. This is the specific reason the manufacturing question is worth asking separately rather than folding into the existing accounts.

Part II

The Ligase

One enzyme destroys the activation chaperone, the synaptic brake and the receptor-removal protein — and it is lost in two diseases that do not agree about what happens next.

V. UBE3A and Its Three Relevant Substrates

UBE3A, also called E6-AP, is an E3 ubiquitin ligase: the component of the ubiquitin–proteasome system that selects which protein is to be destroyed. Maternal loss of function in the gene causes Angelman syndrome; duplication of the chromosomal region containing it is among the more reproducible genetic findings in autism. Three of its substrates are relevant here, and the three were identified by different laboratories for different reasons.

Ephexin5 is the synaptic brake. Ephexin5, encoded by *ARHGEF15*, is a guanine nucleotide exchange factor that was identified as a negative regulator of excitatory synapse number. The developmental logic was worked out in a single paper: the receptor EphB2, engaged by its ligand, phosphorylates Ephexin5; the phosphorylated form is recognised by UBE3A, ubiquitinated, and degraded; and the removal of Ephexin5 relieves a brake on excitatory synapse formation.24 The model made Ephexin5 abundance a direct readout of ligase function.

Arc is the receptor-removal protein. Arc is an immediate-early gene product that binds dynamin and specific endophilin isoforms, assembles an endocytic complex at the postsynaptic membrane, and accelerates the internalisation of AMPA-type glutamate receptors; neurons lacking Arc show markedly reduced endocytosis and increased steady-state surface receptor levels.25 Arc is also a UBE3A substrate, and its accumulation is one of the better-established consequences of ligase loss.

PTPA is the activation chaperone, and this is the recent and least-absorbed finding. Using stable-isotope labelling in mammals together with ubiquitination assays, PTPA was identified as a bona fide UBE3A substrate.26 The experiment is clean and the direction is unambiguous: maternal loss of *Ube3a* increased PTPA levels, promoted holoenzyme assembly and elevated phosphatase activity, while maternal duplication of the 15q11-13 region containing *UBE3A* down-regulated PTPA and lowered phosphatase activity.26 The authors then did what the model demanded. Reducing PTPA in vivo restored the defects in dendritic spine maturation in Angelman model mice, and pharmacological **inhibition** of the phosphatase with the small molecule LB-100 alleviated both the reduction in excitatory synaptic transmission and the motor impairment.26

Read together, these three results say something simple. **UBE3A sets, simultaneously, how hard the synapse's structural brake is applied, how fast its receptors are removed, and how much finished phosphatase the neuron owns.** It is an unusual degree of convergence for one ligase, and it is the reason the ligase is worth putting at the centre of an account rather than listing among the mechanisms.

VI. What the Angelman Brain Says

The Angelman arithmetic is internally consistent and it is the cleaner of the two cases, because the genetic lesion is unambiguous and the direction of every arrow has been measured in the same animals.

Losing the ligase stabilises all three substrates. Ephexin5 accumulates and over-applies the structural brake through RhoA, suppressing excitatory synapse development.24 Arc accumulates and strips AMPA receptors from the surface.25 PTPA accumulates, more holoenzyme is assembled, and phosphatase activity rises.26 The synaptic phenotype — impaired spine maturation, reduced excitatory transmission — is the sum of three deficits pulling in the same direction, and two independent interventions correct it: lowering Ephexin5 genetically,24 and lowering phosphatase activity pharmacologically.26

The phosphatase arm of that result deserves emphasis because it runs against the intuition the Alzheimer field has built. In Angelman syndrome, this enzyme is a problem because there is *too much* of it working. The therapeutic move is to inhibit it. Nothing about the enzyme's biology makes that paradoxical — a phosphatase with the wrong set-point is as damaging as a kinase with the wrong set-point — but it is the first time in this literature that the direction has been fixed by a rescue experiment rather than by correlation.

VII. The Dosage Mirror

One further result in the Angelman study deserves its own heading, because it establishes that the ligase behaves as a rheostat rather than a switch, and because its direction is the one the Alzheimer field should find arresting.

Maternal **duplication** of the 15q11-13 region containing *UBE3A* — more ligase, not less — down-regulated PTPA and **lowered** phosphatase activity.26 The gene-dosage series is therefore complete and monotonic: less ligase, more activation chaperone, more finished phosphatase; more ligase, less chaperone, less finished phosphatase.

The consequence is a mirror that nobody has held up. On the phosphatase axis, **the duplication condition — which is a genetic cause of autism — sits on the same side as Alzheimer's disease, and Angelman syndrome sits on the other.** Three conditions, one enzyme, and the deletion syndrome is the outlier.

We do not propose that this means anything mechanistically about autism and Alzheimer's disease sharing a lesion, and we would resist that inference strongly: the duplication result is a measurement in mouse brain, the Alzheimer result is a measurement in human cortex, the ages

differ by seventy years, and the causes are unrelated. What the mirror does establish is narrower and methodologically useful. **A low level of finished phosphatase is reachable from either direction of ligase dosage**, because the ligase sets the chaperone's destruction rate and the chaperone sets the enzyme's activation rate. That is a two-step control system, and two-step control systems are the ones in which an observed output tells you least about the input. It is the structural reason the contradiction set out below is not easily argued away.

VIII. What the Alzheimer Brain Says About the Same Ligase

The Alzheimer result arrived from an entirely different direction and was not framed as a statement about Angelman syndrome at all.

The ligase is lost in amyloid-bearing brain, and it is lost early. In the Tg2576 mouse, which overexpresses human amyloid precursor protein carrying the Swedish mutation and accumulates amyloid-β, UBE3A is reduced; the depletion *precedes* both the age-dependent behavioural deficits and the loss of dendritic spines.27 The mechanism is a solubility change rather than a transcriptional one: following exposure to amyloid-β, the ligase is phosphorylated by the kinase c-Abl and comes out of solution.27 And the downstream consequences are exactly the ones the Angelman model predicts for two of the three substrates. Loss of the ligase triggers accumulation of Arc and of Ephexin5, driving internalisation of the GluR1 receptor subunit and activation of RhoA respectively, culminating in the pruning of synapses — and restoring the ligase blocks it.27

A second, independent group reached the same place in a different model. In APPswe/PS1ΔE9 mice, the soluble pool of the ligase decreased age-dependently relative to wild-type controls, and brain-specific ligase deficiency on that background accelerated cognitive and motor deficits.28 That study also reported something the amyloid field will find awkward and which we flag rather than adopt: the ligase-deficient animals generated *less* amyloid-β and formed fewer plaques, apparently through increased activity of the α-secretase ADAM10 shifting precursor processing toward the non-amyloidogenic route.28 Worse cognition, less plaque, same animals.

The convergent human claim about the brake is strong. Independently of the ligase work, Ephexin5 was shown to be acutely induced by amyloid-β in mature hippocampal neurons and in human amyloid precursor protein mice; its expression is **highly elevated in the hippocampi of human Alzheimer patients**; removing it eliminated hippocampal spine loss and rescued the behavioural deficits in those mice; and selectively reducing it in the dentate gyrus of presymptomatic adolescent animals was sufficient to protect them from developing cognitive impairment.29,30

So for two of the three substrates, the two diseases agree. Ligase down, Ephexin5 up, Arc up, synapses pruned — in Angelman syndrome and in Alzheimer's disease alike.

IX. The Ligase and the Amyloid Loop

Before turning to the contradiction, one structural feature of the Alzheimer evidence deserves to be drawn out, because the two studies that establish the ligase lesion point in opposite causal directions and nobody has put them together.

The first study makes the ligase a consequence of amyloid. In the Tg2576 mouse, amyloid-β exposure activates c-Abl, c-Abl phosphorylates the ligase, and the ligase leaves solution.27 Amyloid is upstream; the ligase is downstream; the synaptic consequences follow. That is a conventional cascade and it reads cleanly.

The second study makes amyloid a consequence of the ligase. In APPswe/PS1ΔE9 animals, brain-specific ligase deficiency *reduced* amyloid-β generation and plaque formation, apparently by raising α-secretase activity and shifting precursor processing toward the non-amyloidogenic route — while simultaneously accelerating cognitive and motor decline.28 Amyloid is downstream; the ligase is upstream; and the sign is protective for plaque and harmful for cognition.

Each study is internally coherent. Taken together, they describe a **negative feedback loop**: amyloid disables the ligase, and a disabled ligase suppresses amyloid production. We state what that would and would not mean, because a loop of this shape has consequences that are easy to over-read.

What it would mean. A negative feedback loop on amyloid production is self-limiting by construction. The amyloid arm would tend toward a plateau, because the more amyloid accumulates, the more it disables the ligase, and the more the ligase is disabled the less amyloid is made. The synaptic arm has no such property: ligase loss raises Ephexin5 and Arc, prunes synapses, and nothing in the loop feeds back to restore the ligase. **The structure therefore predicts a dissociation in which plaque load saturates while synaptic loss continues** — which is, as it happens, the single most robust discrepancy in the clinical and neuropathological literature on this disease, and one that the amyloid cascade in its original form does not explain.

What it would not mean. It would not make amyloid irrelevant, and it would not make the loop a therapeutic target. Both halves are single studies in different transgenic models from different laboratories, neither cites the other, and the two were not measured in the same animals. The α-secretase arm in particular carries the standard objection to any overexpression model: in animals producing supraphysiological quantities of precursor protein, a shift in processing route is easier to produce and harder to interpret than it would be in a knock-in. **We grade the loop as inference, and as the weakest of the inferences in this volume** — but we record it because it is cheap to test and because the prediction it makes is unusual. Most proposals in this field predict

that an intervention will help. This one predicts a specific pattern of *non*-correspondence between two measurements that are already being made in the same cohorts.

The test is a single cross. Measure amyloid burden, ligase level and solubility, and synaptic density across a time course in one amyloid model with and without ligase restoration. The loop predicts that restoring the ligase will *increase* amyloid production while *improving* synapses — an inversion of the field's usual expectation, and one that a trial-stage programme would want to know about before it discovers it by accident. The ligase-restoration construct already exists and has already been shown to block synapse loss in this model.27 What has not been done is to look at what it does to the plaque.

One further observation belongs here, because it bears on timing rather than on direction. The ligase depletion *precedes* both the behavioural deficits and the spine loss in the Tg2576 animals.27 If that ordering holds, then the ligase lesion is not a late consequence of a failing neuron but an early event — and the manufacturing question raised in Part I becomes a question about the pre-symptomatic period, where the quantity to measure is not how much phosphatase a dying cell has left but how much a stressed cell is still able to build. Nothing in this literature has been measured at that stage, in either disease, in any species.

X. The Arithmetic That Does Not Close

Now the third substrate.

If UBE3A destroys PTPA, and UBE3A function is lost in Alzheimer's disease, then PTPA should be **elevated** in Alzheimer tissue, holoenzyme assembly should be increased, and phosphatase activity should be **higher**, exactly as it is in the Angelman brain.

The measurement says the opposite. **PTPA protein is decreased in the brains of Alzheimer's disease patients, and in two independent transgenic mouse models** — one expressing mutant human amyloid precursor protein, one expressing the longest human tau isoform.31 Knocking PTPA down inhibits the phosphatase and enhances tau phosphorylation; overexpressing it activates the phosphatase and reduces tau phosphorylation.31 A second study from the same group found PTPA localised to the mitochondrial membrane, and that its knockdown induced apoptosis through the mitochondrial pathway, with simultaneous tau hyperphosphorylation attenuating that cell death.32 And the disease direction of phosphatase activity itself is not in doubt: it is reduced.1,3,4

Two diseases lose the same ligase. Two of its substrates rise in both. The third rises in one and falls in the other. The reason the contradiction has gone unnoticed is partly a matter of literature size. Searched by subject, the entire intersection of this activation chaperone

with this disease is **three papers** — the two that report the deficit,31,32 and one that mentions the chaperone incidentally in the course of correcting the tyrosine sites42 — and none of them postdates the ligase result. The intersection of the ligase, the phosphatase and the disease is **empty**. The Angelman study does not cite the Alzheimer measurement; no Alzheimer study has had the opportunity to cite the Angelman one, because none has been published since. As far as we can establish, the contradiction has never been stated.

It is worth being precise about what is and is not in conflict, because the two literatures are not symmetric in kind. The Angelman result is a mechanism study: a substrate identified by two orthogonal methods, directions established in both loss-of-function and gain-of-function genetic conditions, and a rescue in vivo.26 The Alzheimer result is an abundance measurement in human tissue plus a cell-culture manipulation.31 Neither is weak; they are different kinds of claim. The conflict is specifically between **the predicted direction of PTPA in a ligase-depleted neuron** and **the observed direction of PTPA in Alzheimer brain**.

Five resolutions are available.

Resolution one: a second route of PTPA loss, stronger than the stabilisation. The ligase sets the rate at which PTPA is destroyed; it does not set the rate at which PTPA is made. If transcription or translation of PTPA falls in the Alzheimer neuron — or if a second, ligase-independent degradation route opens — the net level can fall even as one of its destruction routes is removed. This resolution has a direct precedent in the same enzyme system: messenger RNA for the catalytic subunit is itself decreased in Alzheimer hippocampus,9 which is a supply-side change of exactly the kind required. It is also the only resolution consistent with both measurements being correct as reported. **We grade it the most likely.**

Resolution two: a partial loss of ligase function that spares the PTPA arm. The Alzheimer lesion is not a deletion. It is a solubility shift driven by a tyrosine kinase,27 which is a different thing from the absence of a gene product. A ligase driven out of solution might lose access to substrates in one compartment while retaining access in another. Ephexin5 and Arc are synaptic; PTPA has been localised to mitochondrial membrane.32 If the insoluble ligase is lost from the spine but not from the perinuclear or mitochondrial compartment, the substrates would dissociate exactly as observed. **We grade this plausible and untested.** It is also the most interesting of the five, because it would make compartment, not abundance, the operative variable.

Resolution three: a bulk-tissue artefact. Every human PTPA measurement in this literature is an immunoblot of homogenate.31 Alzheimer cortex has lost neurons and gained glia. If PTPA is enriched in neurons, the measured decrease could track the disappearance of the cells rather than a change within surviving ones. This objection applies with equal force to most of the proteins in this field and is rarely controlled; we raise it not to dismiss the finding but because it has

never been excluded, and the exclusion is a single-cell experiment, not a difficult one. **We grade it a live confound.**

Resolution four: the two fields do not mean the same thing by activity. The Angelman study measured phosphatase activity in a context where it had also measured holoenzyme assembly, and the two moved together.26 The Alzheimer numbers are activities against tau or against a generic phosphopeptide in homogenate.1,3,4 Given that demethylation *redirects* rather than abolishes the enzyme,16 it is formally possible for total assembled holoenzyme to rise while the tau-directed fraction of it falls. **We grade this a genuine possibility that no study has addressed**, and note that it would be the most deflationary resolution: the contradiction would be an artefact of two fields using one word for two quantities.

Resolution five: one of the measurements is wrong. We consider this the least likely and say why in Part III — but with an important qualification, because part of the Alzheimer PTPA paper does not survive scrutiny, and the part that does not survive is not the part that generates the contradiction.

XI. The Proteasome, Not Only the Ligase

There is a second way for a ubiquitin ligase substrate to accumulate, and it does not involve the ligase at all. A protein tagged for destruction still has to be destroyed, and the machine that does the destroying is also damaged in this disease.

Proteasome function is impaired in Alzheimer brain, measured directly in human tissue and reported more than two decades ago.33 The finding has been extended and complicated since, but the direction has not been overturned. And neurons possess a proteasome species that other cells do not: a nervous-system-specific plasma-membrane proteasome complex that degrades nascent polypeptides in an activity-dependent manner and modulates neuronal function, including circuit activity in vivo and learning-induced behavioural plasticity.34,35,36 The neuron's degradative capacity is therefore not a single quantity, and at least one pool of it is located exactly where the substrates in this volume act.

This matters for the argument in two opposite ways, and both are worth stating.

It weakens the attribution of substrate accumulation to the ligase. If Arc and Ephexin5 rise in Alzheimer tissue, the rise is consistent with reduced tagging *or* with reduced degrading, and the two have been distinguished in only one of the relevant studies — the one in which restoring the ligase reversed the accumulation, which does argue that the tagging step is limiting in that model.27 Elsewhere the attribution is an inference. This is not a small point: an

entire class of synaptic-protein accumulation in this disease could be a capacity problem rather than a specificity problem, and the two imply completely different interventions.

But it sharpens the contradiction rather than dissolving it. A proteasome deficit is substrate-indiscriminate. If the Alzheimer neuron is degrading less of everything the ligase marks, then PTPA — a ligase substrate — should rise along with Arc and Ephexin5. It falls.31 So the most obvious alternative explanation for the accumulation of the other two substrates makes the behaviour of the third *harder* to account for, not easier. Whatever lowers PTPA in the Alzheimer neuron is acting against both a reduced tagging rate and a reduced degradation rate, which means it is either a substantial loss of synthesis or a destruction route that bypasses the proteasome entirely.

We note, finally, that the ligase and the neuronal proteasome reach the synaptic literature through overlapping groups and the same compartment, and that the two have never been measured together in the same tissue. A single set of blots carrying ligase level, proteasome activity and all three substrate levels from one series of human samples would settle more than any of the individual studies has.

Part III

The Reagent Beneath the Mechanism

What survives of the PTPA result once the antibody it depends on is withdrawn, what the activation chaperone actually does to specificity, and the one residue that licenses two things at once.

XII. The Antibody Problem, and What It Does and Does Not Reach

The published account of *how* PTPA activates the phosphatase in the Alzheimer context runs as follows: PTPA raises the level of protein tyrosine phosphatase 1B, which removes an inhibitory phosphate from tyrosine 307 of the catalytic subunit, and the dephosphorylated enzyme is active.31 Every step of that chain was read out with antibodies against phospho-tyrosine-307.

Those antibodies are not phosphosensors. In 2020, two laboratories published back-to-back in the same issue of *Cell Reports* and showed, independently, that commercial anti-phospho-Tyr307 reagents bind unphosphorylated catalytic subunit with comparable affinity and are instead sensitive to other modifications of the catalytic subunit — in particular, their binding is blocked by the methyl ester at leucine 309.37,38 A companion paper extended the finding to the broader class of antibodies raised against the catalytic subunit's carboxy-terminus and concluded that they are unsuitable for evaluating phosphatase activity or holoenzyme composition.39 The original assignment of residue 307 dates to 1992 and rested on inference from a truncation mutant rather than on sequencing or mass spectrometry.40,41 The genuine tyrosine phosphorylation sites have since been relocated to tyrosines 127 and 284, with phosphorylation at 284 promoting dissociation of the Bα subunit and thereby altering substrate specificity.42

The consequence for the present argument has to be stated carefully, because it is narrower than it first appears.

What falls: the mechanism. The claim that PTPA works by lowering phospho-tyrosine-307, and the claim that protein tyrosine phosphatase 1B is the intermediary, are reported through a reagent that cannot support them. A signal read as "less phospho-tyrosine-307" is, on the corrected understanding, as consistent with *more methylation at leucine 309* as with any change in phosphorylation — and more methylation at leucine 309 would independently increase tau-directed holoenzyme assembly.16 That alternative reading is not merely possible; it is mechanistically tidier, since PTPA and the methyltransferase act in the same assembly pathway and a single experiment could not have told them apart. The chain would need repeating with mass spectrometry, or with the corrected-site reagents, before any mechanism could be asserted.

What stands: the abundance. That PTPA protein is reduced in Alzheimer brain and in two transgenic models is an immunoblot against PTPA itself, not against the catalytic subunit's carboxy-terminus. It is untouched by the reagent problem. The same holds for the functional direction: PTPA knockdown inhibits the phosphatase and hyperphosphorylates tau, PTPA overexpression does the reverse.31 Those are activity and phospho-tau measurements, independent of the discredited antibody.

So the contradiction in §X survives the subtraction intact. The finding that generates it — PTPA down in Alzheimer brain — is the part of the paper that holds. We record the asymmetry because it is a recurring hazard in this literature: a paper can be simultaneously right about a quantity and wrong about the reason for it, and a field that discards the whole paper loses the measurement along with the explanation.

XIII. What the Activation Chaperone Actually Does to Specificity

There is a second, older complication in the PTPA literature that neither disease field has reckoned with, and it bears directly on what a loss of PTPA would mean.

The first structural study of the chaperone reported that its binding to the core dimer produces a **dramatic alteration of substrate specificity, with enhanced phosphotyrosine phosphatase activity and decreased phosphoserine phosphatase activity**, strictly dependent on the composite ATPase activity.15 Read literally, that says PTPA converts this enzyme *away* from the serine/threonine activity that tau requires — in which case losing PTPA in Alzheimer's disease should, if anything, favour tau dephosphorylation.

The later structure, with the ATP analogue bound, supports a different account: ATP binding and hydrolysis selectively enhance the loading of the endogenous catalytic metal ions, and that loading is what is *crucial for acquisition of phosphoserine/threonine-specific phosphatase activity*.14 On this reading PTPA is what makes the enzyme a serine/threonine phosphatase in the first place, and its loss is straightforwardly a loss of tau phosphatase.

These are not the same claim, and the field has quietly adopted the second without retiring the first. The likeliest reconciliation is that the apparent phosphotyrosine activity in the earlier work reflects a transition state of the enzyme with non-physiological metals bound — the later study's central point is precisely that which metals occupy the active site determines which chemistry the enzyme does, and the earlier work was performed before that was known. But that reconciliation is an inference, not a published result, and it matters here: the entire interpretation of "PTPA is reduced in Alzheimer's disease" as a *tau-relevant* finding depends on it. We carry the second reading, note the first explicitly, and grade the dependency in the ledger.

A further point follows from the metal argument and has not, to our knowledge, been made. If the acquisition of serine/threonine specificity is a **metal-loading event requiring ATP hydrolysis**, then this step is energy-dependent in a cell whose energetics are compromised. The amount of finished tau phosphatase a neuron owns would then be partly a function of its ATP status — a coupling between bioenergetic failure and tau phosphorylation that requires no intermediary signalling cascade at all, only a shortage of substrate for one reaction. The prediction is specific and

unusual: the coupling should be to ATP concentration rather than to any signalling event, and it should therefore be reversible in vitro by restoring ATP to a lysate. We raise it as a hypothesis and claim nothing for it: no study has measured holoenzyme activation state against ATP availability in neurons, and the in vitro reversibility test has not been attempted.

XIV. Ephexin5, Briefly, and Why the Brake Is Not Only a Brake

The second substrate requires a correction of its own, because the therapeutic prescription that the Alzheimer literature attached to it has been overtaken.

Ephexin5 entered the field as a RhoA-selective exchange factor and a pure negative regulator of spine density.24 On that model, its elevation in Alzheimer tissue is unambiguously bad and the remedy is to lower it.29,30 Two subsequent results complicate both halves.

It is not RhoA-selective, and it is not only a brake. Ephexin5 activates both RhoA and Cdc42 in the brain, and live imaging of GTPase biosensors shows that at single synapses it regulates activity-dependent Cdc42 signalling rather than RhoA. The selectivity between the two is set by tyrosine phosphorylation, which is itself regulated by neuronal activity. And downstream of activity, Ephexin5 **positively** regulates synaptic growth and stabilisation — the opposite sign from its developmental role.43 An intervention that removes the protein removes the growth arm along with the brake.

Chronic loss has a human phenotype, and it is not benign. Heterozygous loss-of-function mutations in *ARHGEF15* cause autosomal-dominant hereditary cerebral small vessel disease with osteoporotic fracture, through inactivation of RhoA/ROCK2 signalling in vascular cells.44 Lowering Ephexin5 systemically is therefore a phenocopy of a human vasculopathy, in a patient population whose small vessels are already a liability.

Neither point touches the disease observation — the protein is elevated in human Alzheimer hippocampus and its removal rescues mice29 — but both touch what should be done about it. The object of intervention is better framed as the *state* of the protein than its amount, and the state is set by a phosphorylation that no one has measured in human disease tissue. The next section is about that phosphorylation, because it turns out to carry more weight than its own literature has asked of it.

XV. The Residue That Licenses Both, and Why the Ligase Fits Better Than the Receptor

The Ephexin5 arm contains a coincidence that has not been drawn out, and once drawn out it resolves a problem of sign in the Alzheimer model and favours one upstream lesion over another.

The same tyrosine does two things. The residue at which the RhoA/Cdc42 selectivity switch is thrown is tyrosine 361, and the selectivity has been established with a phosphomutant: Y361F, which cannot be phosphorylated, enhances Cdc42 activation while leaving RhoA activation unchanged, and rescues activity-driven spine growth in knockout neurons in a manner that requires exchange-factor catalytic activity rather than the mere presence of the protein.43 That same tyrosine, within the same conserved regulatory motif, is the residue whose phosphorylation recruits the ligase and consigns the protein to the proteasome.24

So phosphorylation at this one residue does two things at once: it biases the exchange factor toward RhoA, and it marks the exchange factor for destruction. Dephosphorylation likewise does two things: it releases the Cdc42-directed activity, and it protects the protein from ligase-dependent turnover. **Abundance and activity are not independent variables in this system. They are two readings of one covalent modification.** Each half of that statement is established separately; the coupling itself has not been tested, and we grade it as inference.

Three consequences follow, and the third is the useful one.

First, the phenotype "Ephexin5 is elevated" is ambiguous on its own. A cell with elevated Ephexin5 may have lost the kinase input that marks it for destruction, in which case the accumulated protein is hypo-phosphorylated at tyrosine 361 and Cdc42-biased; or it may have gained a phosphorylation input not coupled to ligase recruitment, in which case the accumulated protein is RhoA-biased. These two states have opposite structural consequences for the spine, and current measurements do not distinguish them, because the assays used to report Ephexin5 levels report total protein.

Second, the two upstream lesions proposed for the Alzheimer neuron therefore make **opposite predictions**. The original account is that amyloid depletes the receptor EphB2 — and EphB2 is the kinase that phosphorylates tyrosine 361. Losing the kinase should *lower* the phosphorylated fraction. That explains an accumulation, since the phospho-form is the degraded form, but it predicts a pool that is dephosphorylated and therefore **Cdc42-biased**, which is to say growth-promoting. The observed phenotype is spine loss. On the receptor account, the sign comes out wrong.

Third — and this is the inference the two literatures have not drawn — **the ligase account does not have this problem.** If the lesion is loss of the ligase rather than loss of the kinase, then the protein accumulates *because the step after phosphorylation has failed*, and what accumulates is the phosphorylated, RhoA-biased form. That is the form the disease phenotype requires. The ligase route predicts spine loss where the receptor route predicts spine growth, and spine loss is what is

observed in both the mouse and the human tissue.29›27 **The ligase is the better-fitting upstream lesion, and the reason is a residue, not a correlation.**

We want to be careful about how much weight this carries. It is an argument from consistency, not a measurement, and there is at least one way the receptor account can be rescued: a different tyrosine kinase could phosphorylate residue 361 without licensing ligase recruitment, if the ligase requires a co-incident receptor-dependent signal that the disease has removed. Protein kinase C epsilon phosphorylates and activates Ephexin5 in a manner that suppresses spines,45 and Src-family kinases are mistargeted to the postsynaptic density in a tau-dependent fashion in these models,8 so candidate kinases are available. In that case the neuron would hold a pool of Ephexin5 that is simultaneously abundant and RhoA-locked for a reason unrelated to the ligase. The two accounts are then distinguishable only by measurement.

The discriminating measurement is the same in either case and has not been made: the phospho-tyrosine-361 fraction of Ephexin5, not total Ephexin5, in human Alzheimer hippocampus stratified by stage, with active RhoA and active Cdc42 measured in the same tissue. An elevated phospho-fraction with unchanged Cdc42 activity leaves the disease model standing and moves the target to whichever enzyme is responsible. A reduced phospho-fraction with elevated Cdc42 activity inverts the model's sign and would make Ephexin5 accumulation a growth-directed, plausibly compensatory response to synaptic loss rather than its cause — in which case the genetic rescue was removing a failed repair attempt whose collateral cost exceeded its benefit.

It is worth recording that the reagent for this measurement is not a limitation. Site-specific antibodies against phospho-tyrosine-361 were generated and validated by the laboratory that identified the residue, against both the phosphomutant and knockout tissue,24 which places them above most commercial phospho-reagents in this field. What is unknown is whether they have ever been applied to human post-mortem cortex, where phospho-epitope lability is the real obstacle, and whether usable serum survives. The measurement is unattempted rather than impossible, and that distinction is the difference between a question one can put to a laboratory and a programme one has to fund.

Part IV

The Downstream, Audited

Which consequences attributed to this pathway rest on a primary experiment, which are inferences that have acquired the grammar of findings, and where the measurement is being made in the wrong place.

XVI. The Receptor, and a Residue Nobody Assigned

The pathway as usually drawn ends at the AMPA receptor, and a specific claim recurs: that this phosphatase dephosphorylates the GluA1 subunit at serine 845, destabilising the receptor and driving it from the surface.

We could not locate a primary study that makes this assignment. Serine 845 is a protein kinase A site, and its dephosphorylation is a well-replicated event in long-term depression and in receptor internalisation; that much is not in question. What is in question is which phosphatase performs it. The experiment most readily reached for — in which impaired dephosphorylation of GluA1 serine 845 and serine 831 accompanies disrupted receptor internalisation — attributes the deficit to decreased activity of "protein phosphatases 1/2A" measured together, pharmacologically, and does not resolve the two enzymes or assign either to a specific residue.46 That is a combined-activity measurement, not an enzyme assignment.

We therefore decline the attribution rather than repeat it. This is not a trivial point of bookkeeping. If the proposition on the table is that one ligase controls both the structural brake and the phosphatase, then the specific residue at which the phosphatase is supposed to act on the receptor is load-bearing — it is the junction at which the two arms are claimed to meet on a single molecule. An unsourced residue at a junction is the place where a diagram stops being a mechanism.

The same verdict applies to the claim that this phosphatase dephosphorylates and destabilises PSD-95. We found no primary demonstration and do not carry it.

What does hold at the receptor is the Arc arm, and it holds well. Arc binds dynamin and specific endophilin isoforms, assembles an endocytic complex, and accelerates AMPA receptor internalisation; the knockout shows the expected mirror phenotype of reduced endocytosis and raised surface receptor.25 Arc accumulates when the ligase is lost, in both diseases.26,27 **The route from ligase loss to receptor loss is therefore intact and does not require the phosphatase at all** — which is worth saying plainly, because the attraction of the phosphatase arm was that it appeared to explain the receptor loss, and it is not needed for that purpose. The phosphatase arm earns its place in this account through tau, not through the receptor.

XVII. The Actin Loop, and Whether It Closes

A tidy feedback loop is sometimes proposed: Ephexin5 drives RhoA and ROCK, ROCK inactivates cofilin, the actin cytoskeleton depolymerises, and the depolymerisation releases

actin-anchored phosphatase into the cytoplasm, where it acts on nearby synaptic substrates — turning a local structural lesion into a local enzymatic one.

The individual arrows in the first half are standard. The RhoA–ROCK–cofilin axis is a well-characterised determinant of spine structure, and pharmacological ROCK inhibition has been pursued in Alzheimer models, where it suppresses amyloid-β production.47 Cofilin dysregulation is among the better-supported effectors of amyloid toxicity at the synapse, and the synaptotoxic species has been narrowed to soluble oligomers isolated directly from human brain.48

The closing arm — that cytoskeletal collapse releases a pool of this phosphatase and thereby raises its local activity — we could not source. It is the step that converts two parallel pathways into one loop, and it is the step without a primary demonstration. We state it as a hypothesis worth testing and not as a finding, and we note what testing it would require: a measurement of holoenzyme localisation and activity in a dendritic spine before and after cytoskeletal disruption, which is within reach of existing methods and has not been reported.

There is an additional reason for caution that the loop's proponents have not addressed. The proposed release mechanism is indiscriminate about holoenzyme composition — it releases whatever was anchored — whereas everything in Part I says that what the enzyme then does depends entirely on which regulatory subunit it is carrying. A loop that cannot say which trimer is released has not yet predicted an outcome.

XVIII. The Microglial Step, and Why It Is Not Downstream

Synapses destabilised by any of the above are physically removed by microglia, and the mechanism is established: complement component C1q tags vulnerable synapses, microglia engulf them through complement receptor 3, and in amyloid-bearing mice this pathway mediates early synapse loss before plaques appear.49

It is worth being exact about the relationship, because the ordering is usually asserted rather than shown. **No study links the intracellular state described in this volume — ligase level, chaperone level, holoenzyme composition, phosphatase activity — to whether a given synapse is tagged by complement.** The two accounts are compatible and they predict the same outcome, but the arrow between them has not been drawn by an experiment. A synapse might be tagged because it has been electrically silenced by receptor loss; it might be tagged for reasons entirely independent of the neuron's phosphatase status; the complement pathway might select synapses by a criterion the neuron does not control at all. Treating the complement step as the downstream terminus of this pathway is an inference, and we mark it as one.

The experiment that would draw the arrow is not out of reach, and it is worth naming because it would discipline a large amount of loose talk about synaptic "tagging": complement deposition scored per synapse against a per-synapse readout of the intracellular state, in the same tissue, by array tomography. The per-synapse resolution is the whole point. Bulk correlations between complement burden and phosphatase activity across cases would be uninformative, because both change with disease stage for reasons that have nothing to do with each other.

XIX. Tau, the Axon, and the Compartment Problem

The sharpest unresolved issue in this scope is not about any single molecule. It is about where in the cell the measurement is being made.

The account that the Alzheimer field has assembled requires this phosphatase to be doing two opposite things at once. In the axon and cell body it must be **suppressed**, because that is what permits tau hyperphosphorylation, microtubule disassembly, and the transport failure that follows.1,5,6 At the postsynaptic density, the version of the story that runs through the ligase requires it to be **elevated**, because that is what the Angelman rescue establishes for a ligase-depleted neuron.26 And hyperphosphorylated tau mislocalises from axon to dendritic spine, where it mediates synaptic dysfunction independently of neurodegeneration50 — so the two compartments are not even independent, and tau itself is the thing that crosses between them.

Nobody has measured this enzyme's activity by compartment in human tissue. Every canonical human number is a homogenate. Synaptosomal preparations exist and are routine in this field for other purposes; they have not been used for this question. The result is that a model requiring opposite signs in two compartments of one cell is being tested against a measurement that averages them, and an average of plus and minus is a null. It is not merely that the compartmental question is unanswered. It is that the existing measurements are the wrong instrument for it, and a null result from them would carry no information.

Two further observations sharpen the problem.

The subunit that makes the enzyme a tau phosphatase and the subunits enriched at the spine are not the same subunits. B55α binds tau and MAP2;11,12 the striatins concentrate in larger complexes at dendritic spines. If the holoenzyme populations differ by compartment — and the subunit distributions say they do — then "PP2A activity" in a synaptosome and "PP2A activity" in a cytosolic fraction are not even measurements of the same enzyme. The compartmental model may not require a paradox at all. It may require two different trimers whose levels move independently, which is a far less exotic proposition and a directly testable one.

And the methylation requirement differs between those two populations. The methyl ester at leucine 309 is needed for B55α to bind, and not needed for striatin.16 A neuron losing methylation capacity — which is what the human methylation data describe6 — would therefore lose the tau-directed, axonal population preferentially while leaving the spine-directed population supplied. **That is the compartmental dissociation the model needs, and it emerges from the assembly chemistry rather than from any additional assumption.** We advance it as the most parsimonious available explanation of how one enzyme can be down in one compartment and up in another in the same cell, and we grade it as inference: the subunit distributions and the methylation requirement are each established, their conjunction has not been measured in neurons, and no study has resolved either by compartment in human tissue.

XX. What the Seventy-One Per Cent Does Not License

A single number organises this field, and it is worth examining what it will and will not bear. The partitioning study that assigned approximately seventy-one per cent of tau phosphatase activity to this enzyme also reported the Michaelis constants: for the first three of the four phosphatases they lie between eight and twelve micromolar, which is close to the intraneuronal concentration of tau in human brain, while calcineurin's is fivefold higher.1 The enzymes are operating in the régime where their kinetics are sensitive to substrate concentration rather than saturated and indifferent to it.

Three things follow, and two of them cut against the way the number is usually deployed.

It licenses the claim that this enzyme is the right object of study. If one enzyme is to be held responsible for the erasing side of the tau balance, this is the one, and no competing candidate is close. That is a real result and it justifies the attention the enzyme has received.

It does not license the claim that restoring the enzyme would restore the balance. A measurement made in homogenate against a defined substrate under defined conditions reports the *capacity* of the extracted enzyme population, not the throughput achieved in a living compartment where the substrate is partly bound to microtubules, where the enzyme is partly sequestered by inhibitors, and where the relevant trimer is a minority of what the assay measures. The gap between capacity and throughput is exactly where the assembly argument of this volume lives.

And it does not license reading the Alzheimer deficit as a proportional loss. Because the enzymes are working below saturation, a fractional change in enzyme concentration produces a roughly proportional change in rate — but the quantity that matters for tau is the concentration of the *tau-directed trimer*, and a loss concentrated in that species would produce a disproportionate effect on tau for a modest effect on total measured activity. This is a testable asymmetry and it has not been tested: it predicts that the reduction in ABαC holoenzyme should

exceed the reduction in total activity, which is a comparison the existing human studies contain the material to make and have not reported.

Part V

The Ledger and the Experiment

What is established, what is inferred, what is contradicted — the direction problem the two diseases create, and the single set of measurements that would adjudicate it.

XXI. The Validity Ledger

Grades: **Established** — replicated in human tissue, or demonstrated by independent laboratories with reagents that have survived scrutiny. **Moderate** — a sound primary result not yet replicated, or replicated outside the relevant system. **Weak** — single result, model-system only, or reagent-dependent. **Inference** — assembled here from separately established parts, not itself measured. **Contradicted** — the primary evidence does not support the claim as stated. **Open** — no measurement exists.

Claim	Grade	Basis
PP2A performs ~71% of tau phosphatase activity in human brain	Established	Partitioned enzymology across four phosphatases1
Tau phosphatase activity is decreased in Alzheimer brain	Established	Replicated across three decades1,3,4
The working enzyme is a trimer whose B-type subunit sets specificity	Established	Structural and biochemical consensus10,13,18
~15 regulatory-subunit genes across four unrelated families	Established	Gene family enumeration10
Only the B55α-containing holoenzyme is the tau-directed species	Established	Direct binding, structure, and human tissue11,12,13,5
Five accessory proteins prevent premature phosphatase activity	Established	Biogenesis review synthesising primary work10
PTPA activates the catalytic subunit by an ATP-dependent reaction	Established	Crystal structure with ATP analogue; composite ATPase14,15
ATP-dependent metal loading confers Ser/Thr specificity	Moderate	Single structural study, in vitro14
PTPA binding instead favours pTyr and reduces pSer activity	Contradicted-in-part	Reported in the earlier structure15; superseded in practice, never retracted

Claim	Grade	Basis
Methylation at Leu309 is required for Bα but not striatin binding	Established	Subunit-selective requirement demonstrated16
PP2A catalytic subunit abundance is translationally autoregulated	Weak	Single study, mouse fibroblasts, 1998; increase only19
PP2A catalytic subunit mRNA is decreased in Alzheimer hippocampus	Moderate	Human tissue, single study, bulk9
Ppp2r2a **(B55α) deletion is embryonically lethal in mice**	Established	Knockout developmental phenotype20
De novo *PPP2R5D* **variants cause a human neurodevelopmental disorder**	Established	Human genetics plus base-edited cellular model21
The *PPP2R5D* **E420K variant drives constitutive AKT–mTOR signalling**	Moderate	Single-base-edited human cells, one laboratory21
Ppp2r5d **deletion alone produces tauopathy in mice**	Established	In vivo phenotype via CDK5/GSK3β deregulation22
APOE **ε2 protection is linked to PP2A and complement component 4**	Weak	Association study; mechanism not established; pathway-level23
EphB2 phosphorylation licenses UBE3A-dependent Ephexin5 degradation	Established	Original mechanism paper with validated reagents24
Arc accelerates AMPA receptor endocytosis via endophilin and dynamin	Established	Interaction plus knockout mirror phenotype25
PTPA is a bona fide UBE3A substrate	Established	Orthogonal methods; loss- and gain-of-function directions26
UBE3A loss raises PTPA, holoenzyme assembly and PP2A activity	Established	Angelman model, in vivo, with rescue26
A PP2A inhibitor rescues synaptic and motor deficits in Angelman mice	Established	In vivo pharmacological rescue26

Claim	Grade	Basis
15q duplication lowers PTPA and lowers PP2A activity	Moderate	Same study, opposite dosage arm26
UBE3A is depleted in amyloid-bearing mouse brain before deficits appear	Moderate	Single laboratory, one model, with mechanism27
The depletion is a c-Abl-dependent solubility shift, not transcriptional	Moderate	Single study27
Soluble UBE3A declines age-dependently in a second amyloid model	Moderate	Independent laboratory, second model28
UBE3A loss in amyloid models raises Arc and Ephexin5 and prunes synapses	Moderate	Single study, reversed by ligase restoration27
Ligase deficiency *reduces* **plaque burden while worsening cognition**	Weak	Single study; flagged, not adopted28
Ephexin5 is elevated in human Alzheimer hippocampus	Moderate	Human tissue, single laboratory29
Removing Ephexin5 rescues spine loss and cognition in APP mice	Established	Genetic removal plus regional knockdown29
Ephexin5 activates Cdc42 as well as RhoA and promotes spine growth	Established	Biosensor imaging at single synapses43
Substrate selectivity is set by phosphorylation at Tyr361	Established	Phosphomutant rescue with catalytically dead control43
Tyr361 is both the selectivity switch and the degradation licence	Inference (ours)	Each half established separately24,43; coupling untested
A knockout-validated phospho-Tyr361 antibody exists	Established	Validated against phosphomutant and null tissue24

Claim	Grade	Basis
ARHGEF15 **loss of function causes human cerebral small-vessel disease**	Established	Autosomal-dominant human genetics44
PP2A dephosphorylates GluA1 at Ser845	Open	No primary assignment located; cited study measures PP1/2A jointly46
PP2A dephosphorylates and destabilises PSD-95	Open	No primary demonstration located
Cytoskeletal collapse releases actin-anchored PP2A and raises local activity	Open	No primary demonstration located
Proteasome function is impaired in Alzheimer brain	Established	Direct measurement in human tissue33
Neurons carry a distinct plasma-membrane proteasome	Established	Biochemistry, in vivo circuit and behavioural requirement34,35,36
Genuine PP2Ac tyrosine sites are Tyr127 and Tyr284	Established	Phosphomutants plus site-specific reagents42
PTPA activates PP2A by lowering phospho-Tyr307 via PTP1B	Contradicted	Reagent shown not to be a phosphosensor37,38,39
PTPA protein is decreased in Alzheimer brain and two mouse models	Moderate	Human immunoblot plus two models, one laboratory31
PTPA knockdown inhibits PP2A and hyperphosphorylates tau	Moderate	Cell lines; direction consistent across two studies31,32
C1q/CR3 mediates early synapse loss in amyloid models	Established	Mouse genetics plus blockade49
Complement tagging is downstream of the neuron's phosphatase state	Open	Compatible but never tested
Mislocalised tau impairs synapses independently of neurodegeneration	Established	Dendritic tau model50

Claim	Grade	Basis
PP2A activity differs in sign between axon and spine in Alzheimer's	Open	Required by the model; never measured by compartment
LCMT1 overexpression protects against impairment in an amyloid model	Moderate	Single in vivo study; the only assembly-stage intervention tried51,52
Amyloid-β disables the ligase, and the disabled ligase suppresses amyloid production	Inference (ours)	Two single studies in different models, neither citing the other27,28
The resulting loop predicts plaque saturation with continuing synaptic loss	Inference (ours)	Follows from the loop's asymmetry; weakest inference here
PTPA direction conflicts between Angelman and Alzheimer's	**Inference (ours)**	**Both measurements sound; neither literature cites the other**
A second, ligase-independent route lowers PTPA in Alzheimer's	Inference (ours)	Most parsimonious resolution; supported by the mRNA result9
Compartmental loss of ligase function dissociates its substrates	Inference (ours)	Consistent with the solubility mechanism27; untested
A proteasome-capacity deficit cannot explain the PTPA fall	Inference (ours)	Capacity deficits are substrate-indiscriminate33
The ligase lesion predicts the observed spine phenotype; the receptor lesion inverts it	Inference (ours)	Follows from the Tyr361 coupling24,43; not measured
Subunit-specific methylation requirements can produce the compartmental dissociation	Inference (ours)	Follows from the methylation selectivity16; not measured in neurons
Energy status gates finished tau phosphatase via ATP-dependent activation	Inference (ours)	Follows from the activation chemistry14; never measured in neurons

XXII. Predictions and Falsification

Each prediction is stated so that a single experiment can defeat it.

1. PTPA is reduced in surviving Alzheimer neurons, not merely in Alzheimer homogenate. Neuron-resolved measurement in human cortex, stratified by Braak stage. *Falsified by* a normal per-neuron level alongside a reduced bulk signal, which would make the finding a cell-composition artefact and dissolve the contradiction entirely.

2. Lowering UBE3A in an amyloid-exposed neuron fails to raise PTPA, although it raises Ephexin5 and Arc in the same cells. *Falsified by* a normal PTPA rise, which would place the Alzheimer PTPA deficit downstream of a second, independent lesion and exonerate the ligase for that substrate.

3. The Alzheimer reduction in assembled ABαC holoenzyme exceeds the reduction in total catalytic subunit and in total measured activity. *Falsified by* proportional reductions, which would indicate a pure supply lesion with no assembly or allocation component.

4. Phosphatase activity in Alzheimer tissue differs in sign between synaptosomal and non-synaptosomal fractions. *Falsified by* the same sign in both, which would retire the compartment hypothesis and force a single-sign model — and would make the Angelman and Alzheimer results genuinely irreconcilable rather than merely unreconciled.

5. The holoenzyme populations of the two compartments differ in subunit composition, with B55α enriched in the non-synaptic fraction and striatins in the synaptic. *Falsified by* equivalent composition, which would remove the parsimonious explanation offered in §XIX and leave the compartmental paradox standing as a paradox.

6. The phospho-tyrosine-361 fraction of Ephexin5 is elevated in Alzheimer hippocampus relative to total Ephexin5. *Falsified by* a reduced or unchanged phospho-fraction with elevated total protein, which would invert the sign of the synaptic model and recast the accumulation as compensatory.

7. Restoring the ligase in an amyloid model improves synaptic density while *increasing* **amyloid production.** A time course in one model with and without the ligase-restoration construct already used to block synapse loss.27 *Falsified by* amyloid falling or holding constant under ligase restoration, which would retire the loop in §IX and leave the two Alzheimer ligase studies simply unreconciled.

8. Activation-state markers of the catalytic subunit covary with neuronal ATP status, and the covariance is reversible by restoring ATP to a lysate. *Falsified by* independence, which would sever the bioenergetic coupling proposed in §XIII.

9. Re-run with corrected reagents, the PTPA-dependent change in the catalytic subunit is a change in leucine-309 methylation rather than in tyrosine phosphorylation. *Falsified by* a mass-spectrometric demonstration of genuine tyrosine-site change, which would rehabilitate a version of the original mechanism at the corrected residues.

XXIII. Therapeutic Corollaries The Direction Problem

The practical consequence of this volume is a problem of sign, and it should be stated bluntly because it is not currently acknowledged anywhere.

Two diseases that lose the same ubiquitin ligase currently imply opposite drugs against the same enzyme. In Angelman syndrome, the phosphatase is over-supplied and a phosphatase inhibitor, LB-100, rescues synaptic transmission and motor function.26 In Alzheimer's disease, the phosphatase is under-supplied and the field's therapeutic programmes — methyltransferase overexpression, small-molecule activators — are all directed at raising its activity.51,52 Both cannot be straightforwardly correct about a shared controller unless something distinguishes the two neurons, and the candidates for that something are precisely the five resolutions in §X. **The direction problem is not an obstacle to the science; it is the most informative fact in it**, because whichever resolution is true is also the thing that would have to be exploited therapeutically.

Four further corollaries follow.

If the lesion is manufacture, activator compounds are aimed at the wrong stage. A small molecule that stabilises or biases an assembled holoenzyme cannot compensate for holoenzyme that was never assembled. The distinguishing experiment is prediction 3, and it should precede rather than follow a programme built on activator chemistry. This is not an argument against such compounds; it is an argument that the stage of the lesion is currently unknown and is cheap to determine.

The autoregulatory cap bounds one whole class of strategy. If the catalytic subunit's level is defended translationally,19 then approaches that work by raising its transcription are arguing with a homeostat, and the tractable targets are the assembly factors — the chaperone and the methyltransferase — rather than the enzyme itself. Methyltransferase overexpression has already been shown to protect against cognitive and electrophysiological impairment in an amyloid model,52 and methylation state governs sensitivity to amyloid-β in both directions,51 which is

consistent with exactly this reading and is, as far as we can tell, the only assembly-stage intervention that has been tried in vivo. **The chaperone has never been tried.** Given that it is the component actually measured as reduced in human tissue,31 that is a conspicuous gap, and the reagents for closing it — viral overexpression in an amyloid model, with tau phosphorylation and holoenzyme composition as readouts — are entirely standard.

Subunit-selectivity is the property any serious candidate needs, and nothing has it. The regulatory subunits are not interchangeable: one is embryonically essential,20 one causes a neurodevelopmental disorder when altered by a single base,21 and one produces tauopathy when deleted.22 A compound that raises or lowers total activity acts on all of them. The assembly-stage targets are attractive partly because they are the only points in the system where subunit selectivity is naturally available — the methyl ester is required by B55α and not by striatin,16 which means a methylation-directed intervention is intrinsically more selective than an activity-directed one.

Lowering Ephexin5 systemically is contraindicated by human genetics. The prescription implied by the mouse rescue29 is a phenocopy of an autosomal-dominant cerebral small-vessel disease44 in patients whose cerebrovasculature is already compromised, and it would additionally remove the activity-dependent growth arm that the biosensor work established.43 Neuron-restricted delivery solves the first problem and not the second. If the Tyr361 analysis in §XV is right, the object of intervention is the *state* of the protein rather than its amount, and the agent required is one that biases substrate selection toward Cdc42 without depleting the enzyme — functionally, a small-molecule mimic of the Y361F state. No such agent exists, and the structural work that would start it has at least been localised to a defined interface by the phosphomutant studies.43

XXIV. The Experiment

One set of measurements adjudicates the contradiction, and it is not expensive.

The human arm. Take cortical tissue, Alzheimer and control, and measure in the same material: UBE3A (total and soluble, since the proposed lesion is a solubility shift), PTPA, the catalytic subunit, the methylation state of the catalytic subunit, and the assembled ABαC holoenzyme. Resolve the measurements to identified neurons rather than to homogenate. Run the phosphatase activity assay on synaptosomal and non-synaptosomal fractions separately rather than on whole homogenate. The reagents are standard, the fractionation is routine, the tissue is banked, and nothing in the protocol is novel — which is the reason the gap is worth pointing out rather than excusing.

The outcome space is small and every branch of it is informative. If PTPA is normal per neuron, the contradiction was a composition artefact and §X resolution three stands. If PTPA is low

per neuron while UBE3A is also low, a second route of loss exists and resolution one stands. If the two track together when separated by compartment, resolution two stands and compartment becomes the governing variable. If activity differs in sign between the fractions while total activity is unchanged, resolution four stands and the two fields have been using one word for two quantities.

The culture arm, which is the decisive half. In mature hippocampal neurons exposed to amyloid-β, knock down UBE3A and measure all three substrates. Ephexin5 and Arc should rise — that is the published expectation and serves as the positive control.27 The question is PTPA. If it rises in the amyloid-exposed neuron, the ligase still governs it and Alzheimer's disease has a second, stronger lesion stacked on top of the ligase lesion. If it does not rise, the ligase's grip on this one substrate is broken in the disease, and the reason it is broken is the next question worth asking.

Two cheap additions would extend the reach considerably. First, score the same cultures for phospho-tyrosine-361 Ephexin5 as a fraction of total, using the validated antibody,24 which tests prediction 6 in a system where the epitope has not been through post-mortem delay. Second, repeat the phosphatase activity assay on lysates with and without added ATP, which tests prediction 8 and costs one additional condition.

Neither arm has been done. The two laboratories best placed to do them have the reagents, and the citation record between them is empty in both directions.

XXV. Coda Switched Off, or Never Built

The vocabulary of this field is a vocabulary of interference. Enzymes are inhibited, silenced, suppressed, locked. Every one of those words presumes a working object that something has been done to, and the presumption is so natural that it has organised three decades of experiments around a single kind of question.

The parts list does not support the presumption. This enzyme is a thing that gets made, by a process with its own machinery, its own energy cost, its own chaperones and its own ways of going wrong — and the one component of that machinery anybody has measured in Alzheimer brain turns out to be missing. That measurement has sat in the literature for more than a decade with a mechanism attached to it that has since been withdrawn from under it, in a journal the synapse people do not read, contradicted by a ligase result in a journal the tau people do not read, and nobody has put the two on the same page.

What the contradiction suggests, if it survives the first experiment above, is a disease in which one neuron suffers two insults to one enzyme at once: a ligase that stops marking its substrates for destruction, which should leave the neuron with *more* finished phosphatase, and a second lesion in supply that takes more away than the first adds. A cell can be failing in both directions at the same

site. That is an uncomfortable kind of model, because it predicts that a single intervention will look ineffective while two in combination look transformative, and drug development is poorly shaped for finding such things.

Whether the Alzheimer neuron's eraser is switched off or never built is not a rhetorical distinction. It is the difference between a drug that releases an enzyme and a drug that supplies one, and no experiment currently in the literature tells us which we need.

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