The Single Cut

One Protease, Two Substrates, and the Protein That Works in Two Compartments — How Asparaginyl Endopeptidase, Switched On in the Locus Coeruleus by a Metabolite of Norepinephrine, Silences Tau's Phosphatase in the Cytoplasm and Would Withdraw a Transposon Silencer from the Nucleus in the Same Stroke

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The literature followed the fragment to where it arrived. It never asked what the nucleus lost when it left.

Contents

Abstract

Two dissertations in this corpus have converged, from opposite directions, on the phosphorylation of tau. *The Coerulean Pincer* took the writer — glycogen-synthase-kinase-3β — and traced the dysregulated noradrenergic signal that drives it. *The Silenced Eraser* took the phosphatase — protein phosphatase 2A — and traced the microglial inflammation that disables it, converging on a single endogenous inhibitor, the protein SET, cleaved and mislocalised from the neuronal nucleus into the cytoplasm where it gags the enzyme. Both volumes treated that mislocalisation as a one-way event with one consequence: a phosphatase inhibitor appears where it does not belong. This dissertation argues that the accounting was incomplete by exactly half, and that the half omitted is the one that connects the tau literature to the chromatin literature at a single molecule.

For SET is not only a phosphatase inhibitor. In the nucleus, where it normally resides, it is a histone chaperone and the core subunit of the inhibitor-of-acetyltransferases complex, binding histones and masking them from the acetyltransferases that would open chromatin; and in the one system where it has been tested directly, it is a *silencer of retroviral sequence*, its knockdown releasing proviral silencing and a related isoform's overexpression reinforcing it. A protein cleaved and exported from the nucleus therefore does two things at once, in two compartments, in opposite directions: it arrives in the cytoplasm as an inhibitor of the enzyme that keeps tau clean, and it departs the nucleus as a participant in the machinery that keeps repetitive sequence quiet. The literature has counted the arrival. It has never counted the departure.

The protease that makes the cut is asparaginyl endopeptidase, and the place it is first switched on is the locus coeruleus. This is the hinge of the volume. Norepinephrine is metabolised by monoamine oxidase A into the aldehyde DOPEGAL, which is produced *exclusively* in noradrenergic neurons and which activates asparaginyl endopeptidase, which cleaves tau at asparagine 368 into an aggregation- and propagation-prone fragment — the published explanation for why the coeruleus is the first structure in the human brain to carry abnormal tau, decades before cortical pathology and before any amyloid. The same protease, in the same disease, cleaves SET at asparagine 175 and drives it from the nucleus into the cytoplasm, inhibiting protein phosphatase 2A and hyperphosphorylating tau. These two facts are each secure. They have never been put in the same sentence, and they have never been tested in the same cell: we found no study examining SET localisation or cleavage in the locus coeruleus or in any noradrenergic neuron.

We therefore propose the *single cut*: one protease, activated by a metabolite that only one cell type makes, acting on two substrates, producing in one stroke the tau lesion of the companion volumes and — this is the new claim — a chromatin lesion of the same class that Bess Frost's programme attributes to tau, but arriving *upstream* of tau rather than downstream of it. If the claim holds, the element-derepression arm of the tauopathy literature has a second and earlier entry point than heterochromatin relaxation, and the two arms are not sequential but convergent. We add a third consequence that follows from the same cut without any chromatin claim at all: protein phosphatase 2A has been shown, in three non-neural systems, to restrain cyclic GMP-AMP synthase and its adaptor, so that the measured fall in phosphatase activity in the Alzheimer cortex would, on that evidence, simultaneously lift a brake on the innate alarm.

We assemble the mechanism joint by joint and grade each in an explicit validity ledger. We are candid that the load-bearing step is a prediction and not a result: the nuclear-silencer arm rests on a single non-neural reprogramming study, and no laboratory has ever measured transposable-element expression, heterochromatin state or retroelement copy number as a function of SET level or SET localisation in a neuron of any species. We are candid, too, that the innate sensor is not settled — the only demonstrated neuronal sensor for tau-driven element-derived nucleic acid is ZBP1 reading Z-RNA, not cyclic GMP-AMP synthase reading DNA, and the one clean tauopathy result for that enzyme attributes its ligand to mitochondrial DNA. Four clean negatives structure the volume's predictions, and the experiments that would settle each are named. The mechanism's redemption is that it is cut above every one of its consequences: a protease has inhibitors, and one stroke prevented is two lesions prevented.

I. The Cut That Was Followed in Only One Direction

There is a habit in molecular pathology, invisible because it is universal, of following a protein to its destination and not asking what it left. A protease cleaves a substrate; the fragment appears somewhere it was not before; the fragment does something; the literature follows the fragment. This is not wrong. It is half of an accounting, and which half is missing depends entirely on whether the protein had a job where it used to be.

Most proteins do not. A zymogen cleaved into an active enzyme was doing nothing before the cut; a signal peptide removed was doing nothing but directing traffic. For these, following the fragment is the whole story, and the habit costs nothing. But a small class of proteins are *employed in two*

compartments, and for these the habit is expensive, because the same event that creates a gain of function in one place creates a loss of function in the other, and the two consequences are studied by different literatures that do not read each other. The protein at the centre of this dissertation belongs to that class, and the two literatures that would have to meet to notice it are the biochemistry of tau phosphorylation and the epigenetics of repetitive sequence. They have not met.

The protein is SET. It has accumulated an unusually large number of names, which is itself a symptom of the problem: it was discovered separately by separate fields and named separately by each. To the phosphatase biochemists it is I2PP2A, inhibitor-2 of protein phosphatase 2A, a 39-kilodalton protein that binds the catalytic subunit of the phosphatase and inhibits it with half-maximal effect at around two nanomolar, specifically, without touching protein phosphatase 1, 2B or 2C (Li, Makkinje, and Damuni, 1996). To the chromatin biochemists it is TAF-Iβ, template-activating factor I, a histone chaperone; and it is the core subunit of a complex named INHAT, the inhibitor of acetyltransferases, which binds histones and masks them from p300, CBP and PCAF, preventing their acetylation and thereby preventing the opening of chromatin (Seo and colleagues, 2001). To the immunologists it is the eponymous subunit of the *SET complex*, an endoplasmic-reticulum-associated assembly containing the nuclease NM23-H1, the acidic protein pp32, the repair endonuclease APE1 and the high-mobility-group protein HMG2 (Fan and colleagues, 2002), to which was later added the three-prime exonuclease TREX1 (Chowdhury and colleagues, 2006). One protein; three literatures; three names; and, as we shall argue, three consequences of a single proteolytic event, of which only one has been counted.

The cut is made by asparaginyl endopeptidase. This enzyme — also called legumain, and, since its role in amyloid processing was described, δ-secretase — is a lysosomal cysteine protease with an absolute specificity for the peptide bond carboxy-terminal to asparagine, and a pH optimum near 5.8 at which it is maximally active and above pH 7 at which it is irreversibly denatured (Chen and colleagues, 1997). Structural work has since shown that its endopeptidase activity is generated electrostatically by pH shift, with protonation of the substrate pocket and of the catalytic nucleophile controlling access and turnover separately, and that the enzyme is active not only in the lysosome but in the nucleus and extracellularly (Dall and Brandstetter, 2013). It is upregulated and activated with ageing and in the Alzheimer brain, where it

truncates tau and abolishes tau's capacity to promote microtubule assembly (Zhang and colleagues, 2014), and cleaves the amyloid precursor protein at asparagines 373 and 585 to favour the amyloidogenic route (Zhang and colleagues, 2015).

The two cuts this dissertation joins are both established, and have never been joined. The first is the cut in tau. Asparaginyl endopeptidase cleaves tau at asparagine 368, producing a fragment that aggregates and propagates, and the trigger for that cleavage in the locus coeruleus is a metabolite of norepinephrine (Kang and colleagues, 2020). The second is the cut in SET. The same protease, activated by the same kind of acidic stress, cleaves SET at asparagine 175, whereupon the protein leaves the nucleus for the cytoplasm, binds the catalytic subunit of protein phosphatase 2A and inhibits it, and tau — its eraser gagged — is hyperphosphorylated (Liu and colleagues, 2008; Basurto-Islas and colleagues, 2013). Both cuts are documented in human Alzheimer brain. Both are made by the same enzyme. Both terminate on tau. And the literature has never asked what happens in the nucleus that the second fragment left.

What this dissertation proposes. We propose that the cleavage of SET by asparaginyl endopeptidase is a *two-compartment lesion*, and that its nuclear half has been invisible because the field that studies SET's cytoplasmic half does not study chromatin, and the field that studies chromatin in tauopathy has never looked at SET. In the cytoplasm the cut produces the lesion that *The Silenced Eraser* took as its convergence: a phosphatase inhibitor where it should not be, and a measured fall in the tau-directed activity of protein phosphatase 2A. In the nucleus the same cut withdraws a histone chaperone and acetyltransferase inhibitor — which in the single system where it has been tested directly participates in silencing retroviral sequence (Bui and colleagues, 2019) — from the compartment where repetitive sequence is held quiet. If the nuclear half behaves in a neuron as it behaves in a fibroblast, then the same proteolytic event that silences tau's eraser also weakens the genome's silencer, and it does so *before* tau has accumulated a single additional phosphate.

Why that ordering matters. The chromatin arm of the tauopathy literature, built principally in Bess Frost's laboratory, runs in one direction: pathogenic tau causes oxidative stress and DNA damage, which causes global heterochromatin relaxation, which permits aberrant expression of normally silenced sequence (Frost and colleagues, 2014), including the transposable

elements that heterochromatin exists to contain (Sun and colleagues, 2018; Ramirez and colleagues, 2021), a relationship independently observed across 636 human brains in which retrotransposon expression tracks neurofibrillary tangle burden (Guo and colleagues, 2018). In that architecture, element derepression is *downstream* of tau: tau is the cause, chromatin the intermediate, the elements the consequence. The single cut, if it is real, adds a second and earlier route to the same endpoint, one that does not pass through tau at all and that is switched on in the cell where the disease begins. The two routes would then be convergent rather than sequential, and their convergence — one arriving from the protease, one from the tangle — would explain why element derepression in tauopathy is so consistently observed and so inconsistently proportional to tau burden.

What this dissertation does not propose. It does not propose that the single cut replaces heterochromatin relaxation, or that SET is the master regulator of transposable elements in the brain, or that the released sequence is necessarily read by cyclic GMP-AMP synthase. Each of those is either unmeasured or actively contested, and Sections VIII and X are written to keep them separate. What it proposes is narrower and, we think, harder to dismiss: that a well-documented proteolytic event in a well-documented cell has a second consequence nobody has looked for, that the experiment to look for it is straightforward, and that the reason it has not been done is that the two halves of the protein belong to two literatures.

The structure of the argument. We begin at the place, because the place is what makes the mechanism specific: Section II establishes why the locus coeruleus is cut first, and why the chemistry that cuts it exists in no other cell. Section III describes the protease. Sections IV and V take its two substrates in turn — tau, where the literature is secure, and SET, where it is secure but incompletely followed. Section VI is the pivot: what SET does in the nucleus, assembled from three literatures that have not been assembled before. Sections VII, VIII and IX take the three consequences of the cut — the eraser silenced, the silencer withdrawn, the alarm's brake lifted. Section X asks the uncomfortable question of what actually reads the released sequence, and answers it honestly rather than conveniently. Section XI closes the loop back to the brainstem. Section XII assembles the whole. The ledger, the predictions, the therapeutic corollaries and the coda follow.

II. The Place — Why the Coeruleus Is Cut First

A mechanism that could happen in any cell explains nothing about a disease that begins in one. The claim of this section is that the locus coeruleus is not merely the first structure to show the lesion but the only structure in which the *initiating chemistry* is available, and that this is a fact about noradrenergic metabolism rather than about tau.

The coeruleus carries abnormal tau before anything else does, and it does so in the young. This is among the most secure observations in the neuropathology of Alzheimer's disease, and it was made by examining brains that had no reason to be examined. In a series of 2,332 unselected autopsy brains spanning ages one to one hundred, abnormal, AT8-immunoreactive tau appeared in subcortical sites — predominantly the locus coeruleus — in cases with no abnormal cortical tau at all, requiring the authors to define new subcortical stages below the existing cortical staging scheme, and the first neocortical amyloid deposits appeared only after brainstem tauopathy was already underway (Braak, Thal, Ghebremedhin, and Del Tredici, 2011). In a companion series restricted to individuals aged four to twenty-nine, thirty-eight of forty-two carried abnormally phosphorylated pretangle tau, forty-one of forty-two had no amyloid deposition whatever, and of the twenty-six who lacked transentorhinal tau, nineteen of the twenty-two with subcortical lesions had that material confined to the noradrenergic coeruleus–subcoeruleus complex (Braak and Del Tredici, 2011). The process, on this evidence, begins in a single brainstem nucleus, in youth, in the absence of amyloid.

That observation has been available for fifteen years and has generated a large literature on *consequences* — the projections the coeruleus loses, the cortical targets it fails to supply, the propagation of tau along its axons. It generated, for a long time, remarkably little on *causes*. The question the staging data pose is not what the coeruleus does to the rest of the brain but what the rest of the brain does not do to the coeruleus: why this cell, and why so early.

The answer that has emerged is metabolic, and it is specific to noradrenergic neurons. Norepinephrine that escapes vesicular storage into the cytosol is a substrate for monoamine oxidase A, and the product of that reaction is an aldehyde: 3,4-dihydroxyphenylglycolaldehyde, DOPEGAL. The

critical property of this molecule for the present argument is stated by its investigators without hedging — it is "produced exclusively in noradrenergic neurons by monoamine oxidase A metabolism of norepinephrine" (Kang and colleagues, 2020). No other cell in the brain makes it, because no other cell has both the amine and the enzyme. Whatever DOPEGAL does, it does in one place.

What it does is activate asparaginyl endopeptidase. In the study that established the link, DOPEGAL activated the protease, the protease cleaved tau at asparagine 368 into aggregation- and propagation-prone forms, and the result was coerulean neurotoxicity and the propagation of tau pathology to the forebrain; the authors framed the finding as the molecular explanation for the selective vulnerability of coerulean neurons in Alzheimer's disease (Kang and colleagues, 2020). A second study from the same programme showed that DOPEGAL additionally reacts directly with the primary amine on tau's lysine 353, forming a covalent adduct that stimulates aggregation and propagation; mutating that lysine to arginine reduced adduct formation and reduced spreading, and preformed fibrils made from the mutant protein failed to trigger the propagation and cognitive impairment that wild-type fibrils produced (Kang and colleagues, 2022). The aldehyde therefore attacks tau twice — once through the protease, once directly — and both attacks are confined to the cells that make it.

The human measurement is consistent with the mechanism. In microdissected coerulean cell bodies from Alzheimer brain compared with matched controls, DOPEGAL is elevated some 2.8-fold and monoamine oxidase A some 3.6-fold, with norepinephrine and dopamine-β-hydroxylase also raised (Burke and colleagues, 1999). The aldehyde accumulates where the model says it should, in the cells the model names, in the disease.

A note of discipline is owed here, because an attractive explanation is available and is not supported. It is tempting to import from the dopaminergic literature the idea that the catecholaldehyde accumulates because the neuron's aldehyde dehydrogenase capacity is exceeded — the framework built around DOPAL in Parkinson's disease. That framework does not transfer. The canonical disposal route for DOPEGAL in noradrenergic nerves is reductive, to 3,4-dihydroxyphenylglycol, not oxidative to the corresponding acid (Eisenhofer, Kopin, and Goldstein, 2004), so the relevant clearance enzyme is an aldehyde or aldose reductase rather than a dehydrogenase, and no study has measured that clearance capacity in

coerulean neurons. The honest statement is the one the human data support: DOPEGAL accumulates in Alzheimer coerulean neurons, and elevated monoamine oxidase A is a measured contributor. Why clearance fails is unknown. The ledger records this as a gap rather than as a support.

The upstream question — why cytosolic norepinephrine rises — has one mechanistic answer with a genetic hook. Norepinephrine is synthesised in vesicles and is virtually absent from cytosol in health; the aldehyde can only form if the amine escapes into the cytosolic compartment where the oxidase sits. Two routes to that escape have been described. The first is a failure of vesicular sequestration: apolipoprotein E4 binds the vesicular monoamine transporter VMAT2 and excludes norepinephrine from vesicles, raising cytosolic amine, DOPEGAL and asparaginyl endopeptidase activity, and exacerbating tau pathology in the coeruleus (Kang and colleagues, 2021). That this route runs through the principal genetic risk factor for sporadic Alzheimer's disease is not a small matter, and it ties the present mechanism to a variant carried by a large fraction of patients. The second is a failure of autoinhibition: chronic stress internalises the α2A-adrenergic receptors that normally limit coerulean firing through a potassium current, and the resulting overexcitation and autocrine reuptake raise cytosolic amine, with measured increases in monoamine oxidase A, in DOPEGAL-induced asparaginyl endopeptidase, and in tau N368 (Toyoda and colleagues, 2025). The first route is genetic and lifelong; the second is experiential and reversible. Both converge on the same aldehyde in the same cell.

What the coeruleus additionally is, which this volume's companion established. The coeruleus is not only the first casualty; it is the brain's principal source of a restraint on inflammation. Norepinephrine suppresses the production of inflammatory cytokines and chemokines by microglia while increasing their migration and their phagocytic clearance, and lesioning the noradrenergic projection with the toxin DSP-4 in an amyloid model raised inflammatory mediators, reduced microglial recruitment to plaques, impaired phagocytosis and elevated amyloid deposition (Heneka and colleagues, 2010). In earlier work, noradrenergic depletion potentiated amyloid-induced cortical inflammation, and that potentiation was attenuated by co-injected norepinephrine or a β-adrenergic agonist (Heneka and colleagues, 2002). The restraint has a microglia-intrinsic component: the neuroprotection afforded by a β2-adrenergic agonist required β2-adrenergic receptor expression in microglia and proceeded through β-arrestin2 rather than cyclic AMP and protein kinase A (Qian and colleagues, 2011), and

noradrenergic tone in the awake mouse actively suppresses microglial process surveillance, with reduced signalling necessary for the surveillance increases seen under anaesthesia (Liu and colleagues, 2019; Stowell and colleagues, 2019). Candour requires the qualification that the receptor logic is not clean — α1 and β1 agonists suppress microglial cytokine output in vitro as well (Mori and colleagues, 2002), and microglial β2 deletion raises anti-inflammatory interleukin-10 alongside tumour necrosis factor α, so the receptor damps output in both directions rather than acting as an anti-inflammatory switch (Lechtenberg and colleagues, 2019). The claim the argument needs is the general one, which is secure: the coeruleus restrains microglial activation, and its loss removes that restraint.

The tau-side experiment makes the point with the relevant pathology. Ablating the coeruleus in P301S tau transgenic mice exacerbated cognitive deficits by six months and, by ten months, worsened hippocampal neuroinflammation and neurodegeneration and accelerated mortality — while aggravating tau burden only mildly, a dissociation the authors themselves emphasised (Chalermpalanupap and colleagues, 2018). Losing the coeruleus damages the brain by more routes than tau.

The place, then, has three properties that no other structure has together. It makes a protease-activating aldehyde that no other cell makes. It carries the first abnormal tau in the human brain, decades early. And it supplies the tonic restraint that keeps the brain's resident immune cell quiet. A lesion initiated there is therefore initiated at the one site where a local proteolytic event and a global inflammatory disinhibition are the same event. The remainder of this dissertation is an argument about what that protease cuts.

III. The Protease — Asparaginyl Endopeptidase and the Chemistry of a Single Residue

The enzyme at the centre of this volume is unusual in three ways that together make it a plausible initiator rather than a mere participant, and each of the three is a matter of settled biochemistry rather than of disease biology.

It cuts at one residue, and therefore its substrate list is a property of sequence. Asparaginyl endopeptidase hydrolyses the peptide bond carboxy-terminal to asparagine and does essentially nothing else. Its characterisation established a strict specificity for asparaginyl bonds, placed it

in the C13 peptidase family, and showed it to be inhibited by cystatin C with an inhibition constant below five nanomolar and by thiol-directed reagents but not by E-64 (Chen and colleagues, 1997). A protease with a single-residue specificity has a substrate list that can be read off from proteomes, and the consequence for the present argument is that its substrates are not selected by biology for functional relatedness. Tau and SET are cut by the same enzyme not because they belong to a common pathway but because each happens to present an exposed asparagine in a cleavable context. This is why the two literatures never merged: there is no pathway diagram on which both substrates appear.

It is switched by pH, not by a signalling cascade. The enzyme shows maximal activity near pH 5.8 and is irreversibly denatured at pH 7 and above (Chen and colleagues, 1997), and structural work has shown why: the endopeptidase activity is generated electrostatically by pH shift, with selective protonation of the substrate-binding pocket governing substrate affinity and protonation of the catalytic nucleophile governing turnover, a two-point control that also explains how the enzyme can be active in the lysosome, in the nucleus and outside the cell in different regimes (Dall and Brandstetter, 2013). A protease controlled by protons is a protease that reports on the cell's acid–base state, and the implication for disease is direct: anything that acidifies the neuronal cytoplasm — ischaemia, hypoxia, hyperglycaemia, excitotoxic stress, or the failure of lysosomal containment — switches it on. This is the mechanism by which the enzyme was first connected to Alzheimer's disease: induction of acidosis by kainate or by pH 6.0 medium activated the protease, produced cleavage of SET, inhibited protein phosphatase 2A and hyperphosphorylated tau, and small interfering RNA against the protease abolished the entire pathway (Basurto-Islas and colleagues, 2013).

It is activated with age and in the Alzheimer brain, and its deletion is protective. The enzyme is upregulated and activated during ageing, in human Alzheimer tissue and in tau P301S mice; it truncates tau, abolishes tau's capacity to promote microtubule assembly, and induces aggregation and neurodegeneration, and knocking it out in P301S mice reduced tau hyperphosphorylation, synapse loss and cognitive deficit (Zhang and colleagues, 2014). In the amyloid arm, the same enzyme cleaves the amyloid precursor protein at asparagines 373 and 585 to favour amyloidogenic processing, is strongly activated in 5XFAD mice and human Alzheimer brain, and its deletion from 5XFAD or APP/PS1 animals reduced plaque burden and

rescued synapse number, long-term potentiation and memory (Zhang and colleagues, 2015). It is, in short, an enzyme whose removal improves both cardinal pathologies of the disease in the standard models — which is a strong argument that it sits upstream of both.

It is druggable, and brain-penetrant inhibitors exist. A high-throughput campaign identified a selective, orally bioactive and brain-permeable inhibitor, whose co-crystal structure showed a combined active-site-directed and allosteric mode of inhibition; chronic dosing of tau P301S and 5XFAD mice reduced substrate cleavage, rescued synapse loss and long-term potentiation, and protected memory (Zhang and colleagues, 2017). Subsequent medicinal chemistry has continued: isopropyl substitution at the N-methyl position markedly improved blood–brain-barrier permeability relative to the parent compound, and a nanoparticle formulation improved oral bioavailability and brain exposure, with one month of oral dosing in 3xTg mice reducing both amyloid and tau pathology and improving cognition (Meng and colleagues, 2025). That last result carries an implicit correction that should be stated plainly rather than glossed: the parent inhibitor's brain penetration was suboptimal, and the claim that this target is pharmacologically reachable in the human brain rests on the improved analogues rather than on the original tool compound.

It is already required for one arm of innate nucleic-acid sensing. This is not a gap but a connection, and it is worth recording before the gaps are listed, because it establishes that the enzyme and the innate immune system are not strangers. Toll-like receptor 9, the endosomal sensor of DNA, requires proteolytic processing before it can signal, and dendritic cells lacking asparaginyl endopeptidase show reduced cleavage of the full-length receptor and reduced cytokine output in response to receptor stimulation both in culture and in vivo, with the processed fragment restoring signalling when expressed in deficient cells (Sepulveda and colleagues, 2009). Subsequent work established the processing as stepwise — the protease or cathepsins remove the bulk of the ectodomain, after which a cathepsin-mediated trimming step is required for optimal signalling — and showed that the nucleic-acid-sensing receptors 7 and 3 are processed analogously (Ewald and colleagues, 2011). The protease is therefore an enabler of endosomal nucleic-acid sensing, in immune cells, by a mechanism that has nothing to do with either of the substrates this volume is about. We note it, do not build on it, and record in Section XI that it complicates any therapeutic reading of protease inhibition.

What the enzyme has never been examined for. Here the record is empty, and the emptiness is load-bearing for Section VIII. We found no study examining asparaginyl endopeptidase in relation to chromatin state, heterochromatin, transposable elements, or retroelement expression, and none relating it to cytosolic DNA sensing by cyclic GMP-AMP synthase or its adaptor. Searches of the indexed literature for the protease in combination with heterochromatin, with LINE-1 or retrotransposons, with that sensor or its adaptor, or with histones and nucleosomes returned nothing. This is not a claim that no relationship exists; it is a claim that we could find no one who had looked.

Two adjacent findings mark how close the field has come without arriving. The first is directly relevant and is, in our reading, the most underused result in this area: asparaginyl endopeptidase, activated under acidic conditions, cleaves SET — described by those authors as a histone chaperone and an inhibitor of a DNase — at asparagine 175, and the consequence they measured was *DNA damage and DNA nicking in brain*, caspase-independent, inducible by kainate and by stroke, absent in protease-null mice, and blocked by the protective protein PIKE-L (Liu and colleagues, 2008). That is an established axis running from this protease, through this substrate, to nuclear DNA integrity. It was framed as a death pathway and was never followed into chromatin. The second is a result in a different disease: the same protease cleaves aldehyde dehydrogenase 1A1 at asparagine 220, abolishing its activity, and cleaves the transcription factor Sox6 so as to repress the genome organiser SATB1, defining selective vulnerability in nigral dopaminergic neurons in Parkinson's disease (Nie and colleagues, 2024). The enzyme is therefore known to reach chromatin-organising proteins and known to reach catecholaldehyde-clearing enzymes — in the other catecholaminergic nucleus, in the other disease, in neurons that project to the coeruleus but are not of it, and never in the coerulean neuron itself.

The protease, summarised for the argument. It cuts at asparagine and therefore has a sequence-defined and functionally unrelated substrate list. It is switched by acid and therefore reports metabolic distress. It is activated in the ageing and Alzheimer brain and its deletion is protective for both pathologies. It has brain-penetrant inhibitors. And in the locus coeruleus it has a dedicated, cell-type-exclusive activator that no other cell can produce. We now take its two substrates in turn.

IV. The First Substrate — Tau at Asparagine 368

This section is short, because its content is established and because a companion volume has already taken the tau kinase as its subject. It is included because the argument of this dissertation is a comparison between two substrates of one enzyme, and the comparison requires both to be on the page.

The cut and its product. Asparaginyl endopeptidase cleaves tau at asparagine 368. The resulting fragment is aggregation-prone and propagation-prone: it seeds, it spreads, and it carries pathology from the coeruleus to the forebrain (Kang and colleagues, 2020). Independently of the coeruleus, the same cleavage was shown to abolish tau's capacity to promote microtubule assembly and to induce aggregation and neurodegeneration, with genetic removal of the protease protecting P301S mice and expression of an uncleavable tau mutant attenuating pathology (Zhang and colleagues, 2014). The fragment also acquires a gain of function that has nothing to do with aggregation: tau N368 binds the TrkB receptor and blocks neurotrophic signalling, so that the same proteolytic event that produces a seed also severs a survival signal (Xiang and colleagues, 2019).

Why the coeruleus is where it happens first. Because the activator is there and nowhere else. DOPEGAL is made only by noradrenergic neurons, it activates the protease, and the protease makes the cut; that chain is the published explanation for the selective early vulnerability of the coeruleus (Kang and colleagues, 2020), and it is reinforced by the covalent adduction of DOPEGAL to tau's lysine 353, which independently accelerates aggregation and propagation (Kang and colleagues, 2022). The neuropathology and the biochemistry agree on the same structure.

What this substrate contributes to the present argument. Three things. First, it establishes that the protease is active in coerulean neurons in disease — which is the premise the second substrate needs and which, for the second substrate, has never been tested. Second, it establishes that a single cut by this enzyme is sufficient to produce a transmissible lesion, so that the consequences of activation are not confined to the cell in which activation occurs. Third, and most importantly for what follows, it shows the shape of the field's attention: the fragment was followed exhaustively, and the question of what tau was doing before it was cut — a microtubule-binding protein

whose loss of function is itself pathogenic — was addressed, whereas for the second substrate the equivalent question has not been asked at all. Tau, unlike SET, does not hold a post in the nucleus that its cleavage vacates. The asymmetry in the literature is understandable. It is also, we argue, an error.

V. The Second Substrate — SET at Asparagine 175

The second cut is as well documented as the first in everything except its consequences, which have been documented in one compartment and ignored in the other.

The cut. Asparaginyl endopeptidase cleaves SET at asparagine 175. This was established first in the context of neuronal death: the protease, activated under acidic conditions, cleaved SET at that residue, and cleavage was associated with DNA damage and nicking in brain, was inducible by kainate and by stroke, was absent in protease-null animals, and was blocked by PIKE-L (Liu and colleagues, 2008). It was established independently in the context of tau: in Alzheimer frontal lobe and hippocampus, activated protease is significantly increased, and the protease and SET translocate — respectively from neuronal lysosomes and from the nucleus — into the cytoplasm, where they interact and associate with hyperphosphorylated tau; protease purified from Alzheimer brain cleaved recombinant SET *except* when the protein was mutated at asparagine 175 to glutamine, which is as clean a demonstration of site specificity as the method allows (Basurto-Islas and colleagues, 2013). The same group reproduced the chain in vivo in a model of brain ischaemia, where middle cerebral artery occlusion activated the protease, cleaved SET, translocated it from nucleus to cytoplasm, inhibited protein phosphatase 2A and hyperphosphorylated tau, with protease-knockout animals showing reduced injury (Basurto-Islas and colleagues, 2018).

The mislocalisation is a human finding, not only a model finding. In Alzheimer neocortex compared with age-matched control, the messenger RNAs for both phosphatase inhibitors are upregulated, the SET holoprotein is selectively cleaved from thirty-nine kilodaltons to a roughly twenty-kilodalton fragment in the disease cytosol, and the protein is translocated from neuronal nuclei to the cytoplasm, where it colocalises with the catalytic subunit of protein phosphatase 2A and with hyperphosphorylated tau (Tanimukai, Grundke-Iqbal, and Iqbal, 2005). Both cleavage fragments — amino-terminal and carboxy-terminal — bind the

catalytic subunit and inhibit the phosphatase, so the cut does not inactivate the inhibitor but multiplies it (Arnaud and colleagues, 2011). And proteolysis is not the only route out of the nucleus: casein kinase II phosphorylation at serine 9 blocks the interaction of SET with importin-α/β and detains the protein in the cytoplasm in Alzheimer brain, aggravating phosphatase inhibition and tau hyperphosphorylation (Yu and colleagues, 2013). Two independent mechanisms — one proteolytic, one phosphorylative — move the same protein out of the same compartment in the same disease. Whatever the nucleus loses when SET leaves, it loses by more than one route.

The consequence that was counted. SET in the cytoplasm inhibits protein phosphatase 2A, and protein phosphatase 2A is the principal phosphatase of tau. The quantities are known with unusual precision for this literature. Of total tau-directed phosphatase activity in human brain, protein phosphatase 2A accounts for approximately seventy-one per cent, with protein phosphatase 1 at eleven, protein phosphatase 5 at ten and protein phosphatase 2B at seven (Liu and colleagues, 2005). And the tau-directed phosphatase activity of the Alzheimer brain is reduced relative to age-matched control by approximately thirty per cent (Gong and colleagues, 1995), a measurement made with hyperphosphorylated tau isolated from Alzheimer brain as the substrate; an earlier study using a generic substrate had established that phosphatase activities are significantly lower in Alzheimer frontal cortex (Gong and colleagues, 1993). The methylation arm points the same way: the protein phosphatase 2A methyltransferase and the methylation of the catalytic subunit are quantitatively decreased in Alzheimer-affected regions, with the regional loss paralleling tau pathology rather than plaque burden (Sontag and colleagues, 2004). The eraser is measurably slower in the disease, the inhibitor that slows it is measurably in the wrong compartment, and the protease that puts it there is measurably activated. This much is not in dispute, and it is the convergence at which *The Silenced Eraser* arrived from the direction of microglial inflammation.

The methylesterase arm, stated at the resolution the data support. The companion volume attributes the inactive state of the phosphatase in part to the methylesterase PME-1, acting downstream of the microglial inflammasome, and that attribution holds — with a qualification about what kind of claim it is. In tau transgenic mice crossed to inflammasome-deficient backgrounds, the protected animals carried *lower* levels of demethylated, inactive protein phosphatase 2A and *lower* levels of its negative regulator PME-1, alongside reduced glycogen-synthase-kinase-3β and

calcium/calmodulin-dependent kinase II activity (Ising and colleagues, 2019). Read in the disease direction: an active inflammasome is associated with more methylesterase and more phosphatase held in its inactive, demethylated form. What that study establishes is a genotype-linked association in one model, measured as protein levels by immunoblot rather than as phosphatase activity, and it does not demonstrate that the inflammasome *installs* the methylesterase on the enzyme. The underlying axis is separately well founded: the methyltransferase and the methylesterase control the methylation of the catalytic subunit and thereby holoenzyme assembly and activity (Sontag and colleagues, 2010), and the methyltransferase and catalytic-subunit methylation are quantitatively reduced in Alzheimer-affected regions in proportion to tau pathology rather than plaque burden (Sontag and colleagues, 2004). The present volume adopts the methylation finding and adopts the inflammasome association at the strength just stated — a second, later route to the same silenced enzyme, running through methylation rather than through the inhibitor, and arriving in the cortex rather than in the coeruleus.

The consequence that was not counted. SET left the nucleus. The literature of the preceding paragraphs records that departure in every human study — Tanimukai's translocation, Basurto-Islas's nucleus-to-cytoplasm movement, Yu's import blockade — and treats it exclusively as the delivery mechanism for a cytoplasmic gain of function. In none of these papers is the nuclear compartment assayed for what it has lost. No chromatin measurement accompanies any of them. This is the gap the next section opens, and it is not a gap in the data so much as a gap in the question.

VI. The Two-Compartment Protein — What SET Is When It Is Not a Phosphatase Inhibitor

To ask what the nucleus loses, one must first establish what SET does there. Three literatures answer, and they were built separately.

SET is a histone chaperone and the core of an acetyltransferase inhibitor. The founding observation is that a multiprotein complex purified from nuclear extract, containing the SET/TAF-Iβ oncoprotein, potently inhibits the histone acetyltransferase activity of p300, CBP and PCAF — and does so by an unusual mechanism. It does not inhibit the enzymes. It binds the histones and masks them, so that the substrate is no longer available to be acetylated; the

complex was accordingly named INHAT, inhibitor of acetyltransferases, and its endogenous subunits were shown to associate with chromatin in vivo and to block coactivator-mediated transcription (Seo and colleagues, 2001). Subsequent work refined the picture: the acidic protein pp32 is a second INHAT subunit (Seo and colleagues, 2002), and SET/TAF-Iβ and pp32 bind preferentially to hypoacetylated histones and to histones bearing repressive marks, and associate with histone deacetylases (Kutney and colleagues, 2004). The protein's nuclear job is therefore not incidental. It is the physical maintenance of a closed, hypoacetylated chromatin state, by occupancy of the histone surfaces that opening requires.

This is worth restating in the vocabulary of the tauopathy chromatin literature, because in that vocabulary it is a striking sentence: *a protein that holds histones in the state heterochromatin requires is removed from the nucleus in Alzheimer's disease, by a protease that is activated in Alzheimer's disease, and nobody has measured the chromatin.*

SET has been shown, directly, to enforce retroviral silencing. This is the single most important import in the volume and also the weakest, and both halves of that sentence must be kept in view. In a study of silencing during somatic-cell reprogramming, insertional chromatin immunoprecipitation of a silenced provirus recovered INHAT components including SET/TAF-I; knockdown of SET/TAF-I in mouse embryonic fibroblasts *diminished retroviral silencing*, and, in a separate experiment, overexpression of the embryonic-stem-cell-predominant isoform TAF-Iα *reinforced* silencing of a reprogramming vector that was otherwise silencing-defective (Bui and colleagues, 2019). Loss of the protein releases retroviral sequence, and more of a related isoform imposes more silencing. Two experiments pointing the same way, which is the functional relationship the present argument requires — with the caveat, which matters, that the second is not a rescue of the first.

The qualifications are substantial and are stated here rather than in the ledger alone. The silenced object is an integrated retroviral reporter provirus, delivered in fibroblasts undergoing reprogramming by a non-integrating Sendai-virus-derived vector; it is not an endogenous retrovirus, not a LINE-1 element, and not a neuron. It is a transgene-silencing assay. And it is, as far as we could establish by search, the *only* published study linking SET or TAF-I to the silencing of retroviral or retrotransposon-like sequence in any system. The claim that SET is a transposon silencer in a neuron is therefore not

established. It is a prediction with one supporting observation in a distant system, and Section VIII is written as a prediction accordingly.

SET sits in a complex with the exonuclease that clears retroelement complementary DNA. The third literature is immunological and arrives from an unexpected direction. SET is the eponymous subunit of an endoplasmic-reticulum-associated complex, originally defined as containing SET, pp32, the repair endonuclease APE1 and the high-mobility-group protein HMG2 (Fan and colleagues, 2002), and containing the nuclease NM23-H1 whose inhibitor SET is; granzyme A cleaves SET, releasing NM23-H1 to nick DNA. To that complex was subsequently added the three-prime-to-five-prime exonuclease TREX1 — specifically TREX1 and not its close homologue TREX2 — which binds SET, colocalises with the complex and translocates with it, and acts with NM23-H1 to degrade DNA (Chowdhury and colleagues, 2006).

The significance of that membership for the present argument is that TREX1 is the enzyme that clears reverse-transcribed retroelement DNA. Single-stranded DNA derived from endogenous retroelements accumulates in TREX1-deficient cells; TREX1 metabolises reverse-transcribed DNA and is an essential negative regulator of the interferon-stimulatory DNA response (Stetson and colleagues, 2008). Its restraint runs specifically through cyclic GMP-AMP synthase: mice lacking TREX1 are entirely protected from lethality, tissue inflammation and autoantibody production when the sensor is also deleted (Gray and colleagues, 2015). And in the tissue that matters here, TREX1-deficient human neural cells accumulate extrachromosomal DNA of which LINE-1 is the major source, their neurons undergo apoptosis, their organoids shrink, their astrocytes secrete type I interferon, and reverse-transcriptase inhibitors rescue the phenotype (Thomas and colleagues, 2017).

So the protein that Alzheimer's disease cleaves and mislocalises is a binding partner of the exonuclease whose loss produces, in human neural cells, precisely the lesion the element arm of the tauopathy literature postulates. Whether cleaving SET perturbs TREX1 has never been tested. It is a straightforward co-immunoprecipitation and a straightforward localisation experiment, and it is among the cheapest of the predictions in Section XIV.

One nuclear consequence of the cut is already measured, and it is not chromatin — it is DNA damage. The point deserves its own paragraph because

it is the closest thing to direct support the argument has. When Liu and colleagues showed that the protease cleaves SET at asparagine 175, the phenotype they measured was DNA damage and DNA nicking in brain — caspase-independent, kainate- and stroke-inducible, absent in protease-null mice (Liu and colleagues, 2008). SET is the inhibitor of NM23-H1, and cleaving the inhibitor releases the nuclease; that is the mechanism they proposed. The nuclear compartment therefore already has a documented, protease-dependent injury following this exact cut. What has not been asked is whether the *chromatin* consequences — histone acetylation state, heterochromatin integrity, repetitive-element expression — follow as well. Given that the same cleaved protein is a histone chaperone and an acetyltransferase inhibitor, and that DNA damage is, in the founding experiment of the tauopathy chromatin programme, the proposed proximate cause of heterochromatin relaxation (Frost and colleagues, 2014), the question is not exotic. It is overdue.

Assembling the two compartments. SET is a protein with a cytoplasmic function that is pathological and a nuclear function that is protective. In health it is largely nuclear, holding histones closed, participating in the silencing of integrated retroviral sequence, and restraining a nuclease; its phosphatase-inhibitory capacity is, by virtue of compartment, held away from cytoplasmic tau. In disease a protease cuts it, and both of those statements invert at once. The cytoplasm gains an inhibitor of the enzyme that keeps tau clean. The nucleus loses a chaperone that keeps chromatin closed, a participant in retroviral silencing, and a restraint on a nuclease — and, on the evidence of the granzyme literature, a binding partner of the exonuclease that clears retroelement complementary DNA.

One event. Two compartments. Opposite directions. The literature has counted one side. The next three sections take the consequences in order of how well they are established: first the counted one, then the predicted one, then a third that follows from the counted one without requiring the predicted one at all.

VII. The First Consequence — The Eraser Silenced, and Silenced from Upstream

The cytoplasmic consequence is the one the literature has followed, and the one *The Silenced Eraser* took as its convergence. This section does not re-argue it. It re-times it.

What the companion volume established. Tau's phosphorylation state is a running equilibrium between kinases and phosphatases, protein phosphatase 2A performs the large majority of the erasing, that erasing is measurably slower in the Alzheimer cortex, and one of the routes by which it is slowed is the cleavage and mislocalisation of SET into the cytoplasm, where it binds and inhibits the catalytic subunit. That volume reached the mislocalisation from *outside* the neuron: the microglion, released from its tonic transforming-growth-factor-β instruction and from the distal noradrenergic brake, becomes an engine of inflammation, and the disease milieu it creates is what drives the inhibitor out of the nucleus. The eraser is gagged by the cell that surrounds it.

What the present volume adds, and where it disagrees. It does not disagree about the lesion. It disagrees about the earliest instance of it. If the protease that cleaves SET is switched on by acid, and if a cell-type-exclusive aldehyde switches it on in the locus coeruleus decades before cortical pathology, then the first cleavage of SET in a human brain does not require a microglion to have forgotten anything. It requires only a noradrenergic neuron with cytosolic norepinephrine and a monoamine oxidase. The microglial route is real and is, we suspect, the dominant route in the cortex at the stage where the phosphatase measurements were made. The coerulean route is earlier, is cell-autonomous, and is available in a twenty-year-old.

This is not a small re-ordering. It converts the phosphatase lesion from a consequence of neuroinflammation into something that can *precede* neuroinflammation, in a cell whose degeneration then removes the restraint that would have held neuroinflammation in check. The two routes are the same lesion reached at two times by two mechanisms, and the earlier one is upstream of the later one by way of the coeruleus's projection.

The quantities, restated with the correct attributions. Protein phosphatase 2A performs approximately seventy-one per cent of the tau-directed phosphatase activity of human brain (Liu and colleagues, 2005). Tau-directed phosphatase activity in the Alzheimer brain is reduced by approximately thirty per cent relative to age-matched controls (Gong and colleagues, 1995). Hold the kinases constant and remove a third of the erasing, and the

steady-state phosphorylation of tau rises without any change in the writers at all. The companion volume drove the writer; this one, like its predecessor, slows the eraser; and what is new here is that the same stroke that slows it also, in the same neuron, produces the aggregation- and propagation-competent tau fragment at asparagine 368. The protease does not choose between the two lesions. It makes both.

A convergence worth naming precisely. In a coerulean neuron with an activated protease, tau is attacked three times by the same upstream event. It is cleaved at asparagine 368 into a seed (Kang and colleagues, 2020). It is adducted at lysine 353 by the aldehyde itself (Kang and colleagues, 2022). And its phosphatase is inhibited by the cleaved fragment of the protease's other substrate (Basurto-Islas and colleagues, 2013). Three attacks, one activator, one cell. Whatever else is true of the coeruleus's vulnerability, the arithmetic is not subtle.

VIII. The Second Consequence — The Silencer Withdrawn

This section states the volume's new claim. It is a prediction, it is labelled as one throughout, and the experiments that would break it are given in Section XIV. It is presented in full rather than hedged into invisibility, because a prediction stated weakly cannot be tested.

The claim. Cleavage of SET by asparaginyl endopeptidase and its export from the nucleus constitutes a chromatin lesion: a reduction in the nuclear complement of a histone chaperone and acetyltransferase inhibitor whose known activities are to hold histones hypoacetylated and, in the one system tested, to enforce silencing of integrated retroviral sequence. The predicted consequence is a shift toward permissive chromatin at repressed loci and, at repetitive sequence specifically, derepression — arriving in the neuron by a route that does not pass through tau.

Why this is worth predicting rather than assuming. Three supports, each partial, none sufficient alone, and the pattern they make is the argument.

The first is functional. SET's nuclear activity is not transcriptional regulation in the general sense; it is the physical masking of histone substrate from the acetyltransferases that open chromatin (Seo and colleagues, 2001), with preferential binding to hypoacetylated and repressively marked histones and association with deacetylases (Kutney and colleagues, 2004). Removing

such a protein from a nucleus is not a neutral event for chromatin state; the direction of the predicted change is not ambiguous.

The second is the direct silencing result. Knockdown of SET/TAF-I releases proviral silencing, and, in a separate arm using a different isoform and a different vector, overexpression of TAF-Iα reinforces it (Bui and colleagues, 2019). This is the observation on which the prediction most depends, and its distance from the target system — reprogramming fibroblasts, an exogenous integrated vector, not a neuron — is the prediction's principal weakness. We grade it accordingly.

The third is the already-measured nuclear injury. The same cut produces DNA damage and nicking in brain (Liu and colleagues, 2008), and DNA damage is the mechanism proposed, in fly and mouse by the programme that founded this field, to link tau to heterochromatin relaxation (Frost and colleagues, 2014). Even if SET's chaperone function were entirely irrelevant, the cut would reach chromatin by this route alone — through nuclease release and DNA damage rather than through histone masking. The prediction has two independent mechanistic paths to the same endpoint, which is a reason to test it and not a reason to believe it.

What it would mean for the element programme. The chromatin arm of the tauopathy literature is built as a chain with a single entry point. Pathogenic tau induces oxidative stress and DNA damage; DNA damage relaxes heterochromatin; relaxation permits aberrant expression of silenced sequence, and genetic restoration of heterochromatin in the fly substantially reduces degeneration (Frost and colleagues, 2014). The silenced sequence in question is principally transposable, and its release is driven additionally by depletion of piwi and piwi-interacting RNAs, with endogenous retroviruses prominent in human Alzheimer's disease and progressive supranuclear palsy (Sun and colleagues, 2018). In mouse, age- and tau-induced element activation is enriched for endogenous retroviruses, with increased element DNA copy number indicating actual retrotransposition (Ramirez and colleagues, 2021). And independently of that laboratory — a point of some importance, since the result is frequently miscredited — RNA sequencing across 636 human brains found differential expression of several retrotransposons in association with neurofibrillary tangle burden, with evidence of global transcriptional activation among LINE-1 and endogenous retrovirus clades and tau-associated active-chromatin signatures at HERV-Fc1 loci (Guo and colleagues, 2018).

In that architecture, element derepression is strictly downstream of tau. The single cut, if it behaves as predicted, supplies a second entry point that is upstream of tau and simultaneous with it: the same protease event that produces the tau seed also withdraws a silencer. The two routes then converge on the same endpoint from different directions, and the element phenotype in a coerulean neuron would be expected to appear *earlier relative to tangle burden* than the tau-first architecture predicts — which is a falsifiable statement about ordering, not merely about presence.

The honest inventory of what is missing. This is a short list. Every item on it is an absence we searched for and failed to fill, which is a weaker thing than a proof of non-existence and is stated as such.

We found no study measuring transposable-element expression, heterochromatin state, histone acetylation, or retroelement copy number as a function of SET level or SET subcellular localisation in a neuron, in brain tissue, or in any neural model, in any species. The nearest adjacent work is the proviral-silencing study in fibroblasts.

We found no study examining SET or its cleavage in the locus coeruleus or in any noradrenergic neuron. The entire SET–phosphatase–tau literature is cortical and hippocampal.

We found no study examining asparaginyl endopeptidase in relation to chromatin, heterochromatin, or transposable elements, in any system. The protease's established connection to innate immunity runs elsewhere, through its processing of the endosomal nucleic-acid receptors (Sepulveda and colleagues, 2009; Ewald and colleagues, 2011), and not through chromatin.

We found no study testing whether cleavage of SET perturbs the SET complex's other members, TREX1 included.

Four negatives. They are the reason this section is a prediction. They are also, taken together, an unusually clean experimental opening: the claim is testable with standard methods in a system that already exists, and the reason it has not been tested is not difficulty but the partition of the literature.

IX. The Third Consequence — The Brake on the Alarm

The third consequence has the useful property of following from the *counted* half of the cut. It requires no chromatin claim, no element claim and no

assumption about sensors. It requires only that protein phosphatase 2A activity fall, which is measured.

Protein phosphatase 2A restrains cyclic GMP-AMP synthase and its adaptor. Three primary studies establish this, all outside the central nervous system. Genetic ablation of the α-isoform of the phosphatase's catalytic subunit in glioma cells increased cytosolic double-stranded DNA and cyclic GMP-AMP synthase–type-I-interferon signalling, raised major-histocompatibility class I expression and tumour mutational burden, and sensitised tumours to checkpoint blockade and radiotherapy; the authors framed the phosphatase as an inhibitor of the DNA-sensing axis (Mondal and colleagues, 2023). In macrophages, the catalytic subunit with its B-subunit striatin-4 negatively regulates the adaptor's type-I-interferon output by dephosphorylating the Hippo kinases MST1/2 and stabilising YAP/TAZ, and myeloid deletion of the phosphatase slowed tumour growth (Ho and colleagues, 2023). And in human primary monocytes, reduced phosphatase activity amplifies interferon-stimulated gene expression and cytokine release through the same sensing axis, with pharmacological phosphatase activation suppressing it and pharmacological inhibition phenocopying disease (Fang and colleagues, 2026).

The direction is consistent across three systems and two species: phosphatase down, alarm up.

The inference, and its limit. If the tau-directed activity of this phosphatase is reduced by roughly a third in the Alzheimer cortex (Gong and colleagues, 1995), and if the phosphatase restrains the cytosolic DNA-sensing axis, then the lesion that *The Silenced Eraser* documented as a tau lesion is simultaneously a disinhibition of innate sensing. The eraser is not only an eraser. It is also a damper on the alarm, and the same cut that gags the one lifts the other.

The limit is severe and is stated without softening. All three studies are in transformed cells, tumour-associated macrophages or peripheral monocytes. Two of them are anatomically in the brain — glioma cells are transformed glial cells and the macrophages were analysed in human glioblastoma tissue — but not one is in a healthy, non-transformed, resident neuron, microglion or astrocyte. We found no study testing the phosphatase's effect on the sensor or its adaptor in any such cell. The defensible sentence is: the phosphatase negatively regulates this axis in glioma cells, tumour-associated macrophages and monocytes, and has not been tested in a non-transformed cell of the

central nervous system. Anything stronger is transfer, and transfer across cell type is precisely the failure mode this corpus has recorded against itself before.

Why the limit does not make the inference idle. Because it converts a hard question into an easy one. Whether tau-driven element derepression yields a cyclic-GMP-AMP-synthase ligand is a hard question requiring sequencing of the cytosolic nucleic acid. Whether lowering phosphatase activity in a microglion raises interferon-stimulated gene expression is an easy question requiring a pharmacological inhibitor and a quantitative polymerase chain reaction. The third consequence is the cheapest prediction in the volume and the one most likely to be settled quickly, in either direction.

X. The Sensor — What Actually Reads What Is Released

It would be convenient for this dissertation if released retroelement sequence were read by cyclic GMP-AMP synthase. It would connect the single cut to the most intensively pursued innate-immune target in the field and would make the loop close on a druggable enzyme. The evidence does not support that convenience, and the volume's companion on that enzyme has already said so at length. This section states the position precisely, because a mechanism that overstates its terminus discredits the parts of it that are sound.

The one demonstrated neuronal sensor for tau-driven element nucleic acid is not that enzyme. Tau aggregates cause reactivation of transposable DNA elements leading to Z-RNA–ZBP1-mediated neuronal death (Liu and colleagues, 2026). That is an RNA sensor, reading a left-handed RNA conformation, killing the neuron by a pathway that has nothing to do with cyclic dinucleotides. It is, on present evidence, the strongest fully independent demonstration of a sensor downstream of tau-driven element release in a mammalian neuron.

Frost's own immune arm is also RNA. When that laboratory followed element derepression to inflammation, the ligand was double-stranded RNA: double-stranded RNA and its sensing machinery are elevated in astrocytes in Alzheimer's disease and progressive supranuclear palsy and in tau transgenic mice, specific tau-induced retrotransposons form double-stranded RNA, and pathogenic tau and heterochromatin decondensation causally drive double-stranded-RNA-mediated neurodegeneration and neuroinflammation

(Ochoa and colleagues, 2023). The chain from tau to inflammation, as that programme has actually built it, runs through RNA and through astrocytes.

The one clean tauopathy result for the DNA sensor attributes the ligand to mitochondria. Pathogenic tau activates cyclic GMP-AMP synthase and type-I-interferon responses in microglia, in part mediated by cytosolic leakage of mitochondrial DNA; genetic ablation of the enzyme in tauopathy mice diminished the microglial interferon response, preserved synapse integrity and plasticity and protected against cognitive impairment without changing tau load, while increasing the neuronal MEF2C transcriptional network associated with cognitive resilience, and pharmacological inhibition restored synaptic integrity and memory (Udeochu and colleagues, 2023). This is an excellent result and it is the benchmark for the target. Its ligand is mitochondrial DNA, not retroelement complementary DNA.

The retroelement-to-DNA-sensor bridge is imported from senescence and has never been sequenced in brain. In senescent cells, LINE-1 becomes transcriptionally derepressed and activates a type-I-interferon response that is a phenotype of late senescence, triggered by cytoplasmic LINE-1 complementary DNA and antagonised by inhibitors of the element's reverse transcriptase; treating aged mice with lamivudine downregulated interferon activation and age-associated inflammation in several tissues (De Cecco and colleagues, 2019). That is a strong result in fibroblasts and aged mouse tissue. Its transfer to the brain rests on pharmacological and antisense epistasis rather than on sequencing of the cytosolic species, a limitation the companion volume on this enzyme states at length and which this volume adopts without modification.

What the single cut therefore claims, and what it declines to claim. It claims that the cut withdraws a silencer and would increase the availability of repetitive sequence. It declines to specify the sensor. That is not evasion; it is the state of the evidence, and the three candidates are distinguishable by experiment. If the released species is chiefly RNA, the sensor is ZBP1 or the double-stranded-RNA machinery and the relevant cell may be the astrocyte. If reverse transcription occurs and complementary DNA accumulates, the TREX1 membership of the SET complex becomes the more interesting half of Section VI and the DNA sensor becomes relevant. If neither, the cut's nuclear half is a chromatin lesion with transcriptional consequences and no innate-immune terminus at all, which would leave Sections VII and IX intact and Section VIII interesting for different reasons.

One irony must be carried forward from the companion volume rather than quietly dropped. Cyclic GMP-AMP synthase is not only a sensor. Most of it in a cell is nuclear and chromatin-tethered, and mice lacking it age prematurely with derepression of LINE-1 elements, DNA hypomethylation and a smoothed repressive chromatin landscape, a function its authors attribute to heterochromatin maintenance and state to be independent of both sensing and catalysis (Martinez and colleagues, 2026). If that is right, then the enzyme helps maintain the very structure whose failure releases its ligands, and inhibiting it to quiet the alarm could worsen the derepression feeding the alarm. This volume's mechanism does not depend on that enzyme, which is one of its advantages; but any therapeutic corollary that reaches for it inherits the problem, and Section XV says so.

XI. The Loop — Outward from the Brainstem

A mechanism that produces its own upstream condition is a different kind of object from a mechanism that runs once. This section assembles the single cut into a loop and identifies which of its arcs are measured and which are inferred.

The opening arc is metabolic and cell-autonomous. Cytosolic norepinephrine rises in a coerulean neuron — by apolipoprotein-E4-mediated exclusion from vesicles (Kang and colleagues, 2021), by stress-induced loss of α2A autoinhibition (Toyoda and colleagues, 2025), or by the age-associated changes that raise monoamine oxidase A in these cells (Burke and colleagues, 1999). Monoamine oxidase A converts it to DOPEGAL. DOPEGAL activates asparaginyl endopeptidase (Kang and colleagues, 2020). Every step here is measured, and every step is confined to one cell type.

The cut produces three products at once. Tau cleaved at asparagine 368, a seed that spreads (Kang and colleagues, 2020). SET cleaved at asparagine 175 and exported, which inhibits the phosphatase in the cytoplasm (Basurto-Islas and colleagues, 2013) and — the predicted arc — withdraws a silencer from the nucleus. And, following from the phosphatase inhibition alone, a lifted brake on cytosolic nucleic-acid sensing (Mondal and colleagues, 2023; Ho and colleagues, 2023), inferred across cell type.

The neuron's own chromatin then receives a second hit from its own tau. Hyperphosphorylated and cleaved tau produces oxidative stress and DNA damage, which relaxes heterochromatin, which permits aberrant expression of silenced sequence (Frost and colleagues, 2014), including transposable elements whose control is further weakened by piwi and piwi-interacting-RNA depletion (Sun and colleagues, 2018) and which, in mouse, increase in DNA copy number (Ramirez and colleagues, 2021). Whether the two hits on the chromatin — the withdrawn silencer and the relaxed heterochromatin — are additive, synergistic or redundant is unknown and is one of the volume's principal predictions.

The released material is read by something, and the neuron's neighbours respond. Whatever the sensor (Section X), the output is an inflammatory and interferon response in glia (Ochoa and colleagues, 2023; Udeochu and colleagues, 2023), and the consequence of the interferon arm that has been measured in tauopathy is a collapse of the neuronal MEF2C transcriptional network associated with cognitive resilience, reversible by ablating or inhibiting the sensor (Udeochu and colleagues, 2023). Resilience, on that reading, is not a property a brain has; it is a transcriptional programme that inflammation can switch off.

Three arcs close the loop, and they are of unequal strength.

The first is acid. The protease is switched by protons (Chen and colleagues, 1997; Dall and Brandstetter, 2013), and acidosis activates it and produces the SET cleavage and tau hyperphosphorylation directly (Basurto-Islas and colleagues, 2013). Neuroinflammation, mitochondrial failure and lysosomal dysfunction all acidify. Inflammation therefore feeds back onto the protease that started the cascade. This arc is mechanistically solid in principle and unmeasured in the coeruleus.

The second is the loss of restraint. The coerulean neuron that is being cut is also the source of the noradrenergic signal that holds microglia quiet (Heneka and colleagues, 2010; Qian and colleagues, 2011; Liu and colleagues, 2019). As the cell degenerates, that restraint falls away, and the inflammation produced by the released nucleic acid is amplified by the failure of the very cell that produced it. Ablating the coeruleus in a tau model worsens neuroinflammation, neurodegeneration and mortality with only mild aggravation of tau burden (Chalermpalanupap and colleagues, 2018), which is exactly the dissociation this arc predicts: the damage is not all tau.

The third is the propagation arc. The tau fragment produced by the cut spreads to the forebrain (Kang and colleagues, 2020), carrying the tau-side lesion to cells that have no aldehyde of their own — where the microglial route of *The Silenced Eraser* takes over as the local mechanism for cleaving SET, and the cortical phosphatase measurements of Gong and Liu are made.

What the loop explains that a linear chain does not. It explains why the disease begins in a cell that is not the cell in which it is diagnosed. It explains why the restraint on inflammation fails at the same time as the pathology that requires restraining begins, since they are the same event in the same cell. It explains why element derepression is so reliably present in tauopathy and so unreliably proportional to tau burden — two entry points, one of which does not run through tau. And it explains, without appeal to anything exotic, why the earliest human lesion is in the brainstem and the earliest human symptom is not.

One complication the loop carries and cannot discharge. The protease that initiates everything above is also required for the processing of the endosomal receptors that sense nucleic acid (Sepulveda and colleagues, 2009; Ewald and colleagues, 2011). Whatever else inhibiting it would do, it would impair one arm of innate nucleic-acid sensing in myeloid cells — a consideration that belongs to the therapeutic section rather than to the mechanism, but which the mechanism should not be allowed to hide.

What it does not explain, and should not be asked to. It does not explain amyloid, beyond the observation that the same protease cleaves the precursor protein at two asparagines to favour the amyloidogenic route (Zhang and colleagues, 2015) — which is a second convergence at the same enzyme rather than an account of the amyloid cascade. It does not explain why some individuals with coerulean pretangles in their twenties never develop dementia, which is a question about the loop's gain and not about its existence. And it does not identify the sensor, for the reasons given in Section X.

XII. One Cut, Assembled

Stated without qualification, so that it can be argued with:

A noradrenergic neuron of the locus coeruleus accumulates cytosolic norepinephrine, which monoamine oxidase A converts into DOPEGAL, an aldehyde that no other cell in the brain can make. DOPEGAL activates

asparaginyl endopeptidase, a protease that cuts only after asparagine and is switched by acid. The activated protease cleaves two substrates that share nothing but a residue.

The first substrate is tau. Cut at asparagine 368, it becomes a seed that aggregates, spreads and blocks neurotrophic signalling, and the same aldehyde independently adducts it at lysine 353. This is the published explanation for why the coeruleus is the first structure in the human brain to carry abnormal tau, decades before amyloid and before cortical disease.

The second substrate is SET. Cut at asparagine 175, it leaves the nucleus for the cytoplasm. In the cytoplasm it binds and inhibits protein phosphatase 2A, which performs roughly seventy per cent of the erasing of phosphate from tau, and whose tau-directed activity is measurably reduced by about a third in the Alzheimer brain. That is the lesion the companion volume reached from the direction of microglial inflammation; here it arrives decades earlier, cell-autonomously, in a single neuron, with no inflammation required.

But SET was employed in the nucleus. It is a histone chaperone and the core of the complex that masks histones from the acetyltransferases that would open chromatin; in the one system where it has been tested directly, its loss releases retroviral silencing and more of a related isoform imposes more of it; it is the inhibitor of a nuclease whose release, following this exact cut, produces DNA damage in brain; and it is a binding partner of the exonuclease that clears reverse-transcribed retroelement DNA and whose loss, in human neural cells, produces LINE-1-derived extrachromosomal DNA, neuronal death and astrocytic interferon. The nucleus loses the first of those in the same stroke that the cytoplasm gains a phosphatase inhibitor, and — if the complex behaves as its membership suggests, which is untested — the others with it. No one has measured what the nucleus loses.

The prediction is therefore that the single cut produces two lesions of two kinds: a tau lesion, which is established, and a chromatin lesion, which is not. And the chromatin lesion is of the same class that the element programme attributes to tau — except that it arrives upstream of tau rather than downstream of it, which makes the two arms convergent rather than sequential, and which predicts element derepression earlier relative to tangle burden than the tau-first architecture allows.

A third consequence follows from the established half alone, requiring no chromatin claim: protein phosphatase 2A restrains cytosolic DNA sensing in

each of the three systems where the question has been asked, so a third less phosphatase may also be a lifted brake on the innate alarm. That this has never been tested in a non-transformed brain cell is the cheapest gap in the volume to close.

And the loop closes on its own origin. The inflammation that follows acidifies, and acid is what switches the protease. The neuron being cut is the brain's source of the noradrenergic restraint that holds microglia quiet, so its degeneration removes the brake at the moment the brake is needed. The tau fragment it produces travels to a cortex that has no aldehyde but has, as the companion volume showed, a microglial route to the same cleavage. One cut in one nucleus, and by the end every cell type in the argument is implicated.

The mechanism's redemption is that it is a *cut*, and a cut is made by an enzyme, and the enzyme has inhibitors that reach the brain. Everything downstream — the seed, the silenced eraser, the withdrawn silencer, the lifted brake — is one stroke. Prevent the stroke and you prevent all of them. That is a stronger therapeutic position than any of the individual downstream targets can offer, and Section XV describes both why it is attractive and why it is dangerous.

XIII. The Validity Ledger

The discipline that separates synthesis from speculation is the graded ledger, each connection assigned a tier and the experiment that would settle it named alongside. Entries are graded by what was measured, in which species, and in which cell state — not by how well the claim serves the argument. The volume's load-bearing prediction is graded *weak*, and it is graded weak deliberately.

Established (human, direct measurement) — the locus coeruleus is the first site of abnormal tau in the human brain, in the young, before amyloid. In 2,332 unselected brains aged one to one hundred, abnormal subcortical tau predominantly in the coeruleus was present in cases with no abnormal cortical tau, and first neocortical amyloid followed brainstem tauopathy (Braak, Thal, Ghebremedhin, and Del Tredici, 2011); in a series aged four to twenty-nine, thirty-eight of forty-two carried pretangle tau with forty-one of forty-two free of amyloid, and of those lacking transentorhinal tau, nineteen of twenty-two with subcortical lesions had it confined to the

coeruleus–subcoeruleus complex (Braak and Del Tredici, 2011). This is the anchor of the volume's claim about place.

Strong (imported, established) — DOPEGAL is produced exclusively in noradrenergic neurons and activates asparaginyl endopeptidase, which cleaves tau at asparagine 368 into aggregation- and propagation-prone forms. Demonstrated in human coerulean tissue, mice and cells, with the selective-vulnerability framing supplied by the authors (Kang and colleagues, 2020), and reinforced by the independent covalent modification of tau at lysine 353 by the same aldehyde, with the lysine-to-arginine mutant abolishing propagation (Kang and colleagues, 2022). This is the volume's initiating chemistry and it is secure.

Strong (imported, established) — the same protease cleaves SET at asparagine 175, driving it from nucleus to cytoplasm, where it inhibits protein phosphatase 2A and tau is hyperphosphorylated. Site specificity demonstrated by failure to cleave the asparagine-175-to-glutamine mutant; human Alzheimer frontal lobe and hippocampus show increased activated protease with both proteins translocated into the cytoplasm and associated with hyperphosphorylated tau; acidosis reproduces the chain and protease knockdown abolishes it (Basurto-Islas and colleagues, 2013), with an in vivo ischaemia replication (Basurto-Islas and colleagues, 2018), the original cleavage-site identification (Liu and colleagues, 2008), and the human localisation and cleavage-fragment data (Tanimukai, Grundke-Iqbal, and Iqbal, 2005; Arnaud and colleagues, 2011).

Strong (human, quantitative) — protein phosphatase 2A performs the large majority of tau dephosphorylation and its tau-directed activity is reduced by roughly a third in Alzheimer's disease. Approximately seventy-one per cent of total tau phosphatase activity of human brain (Liu and colleagues, 2005); approximately thirty per cent reduction in tau-directed activity in Alzheimer versus age-matched control brain using disease-derived hyperphosphorylated tau as substrate (Gong and colleagues, 1995), with an earlier generic-substrate measurement showing the same direction (Gong and colleagues, 1993). *Attribution note: the thirty-per-cent figure belongs to the 1995 paper; the 1993 paper measured different substrates and should not be cited for it.*

Strong (imported, established) — SET is a histone chaperone and the core subunit of the complex that masks histones from p300, CBP and PCAF. The complex inhibits acetylation by binding the substrate rather than the enzyme;

its subunits associate with chromatin in vivo and block coactivator-mediated transcription (Seo and colleagues, 2001), with preferential binding to hypoacetylated and repressively marked histones and association with deacetylases (Kutney and colleagues, 2004). This is the nuclear job the cut vacates.

Strong (imported, established) — SET is in a complex with TREX1, and TREX1 clears reverse-transcribed retroelement DNA and restrains cyclic GMP-AMP synthase. TREX1, not TREX2, binds SET, colocalises and co-translocates with the complex (Chowdhury and colleagues, 2006); retroelement-derived single-stranded DNA accumulates without TREX1 (Stetson and colleagues, 2008); TREX1-null lethality and inflammation are entirely abolished by deleting the sensor (Gray and colleagues, 2015); and TREX1-deficient human neural cells accumulate LINE-1-derived extrachromosomal DNA with neuronal death, astrocytic interferon and rescue by reverse-transcriptase inhibitors (Thomas and colleagues, 2017). *The membership is established; that cleaving SET perturbs TREX1 is not — see below.*

Moderate (imported, established, non-neural) — protein phosphatase 2A negatively regulates cyclic GMP-AMP synthase and its adaptor. Consistent direction across three systems: catalytic-subunit ablation in glioma cells raised cytosolic double-stranded DNA and sensor-interferon signalling (Mondal and colleagues, 2023); the catalytic subunit with striatin-4 restrains adaptor-driven interferon in macrophages via MST1/2 and YAP/TAZ (Ho and colleagues, 2023); reduced phosphatase activity amplifies interferon-stimulated genes in human monocytes (Fang and colleagues, 2026). Graded moderate rather than strong solely because of cell type. *Settling experiment: pharmacological or genetic reduction of protein phosphatase 2A activity in primary microglia and in neurons, with interferon-stimulated gene induction and adaptor phosphorylation as readouts.*

Moderate (established mechanism, untested location) — acidosis activates the protease and therefore inflammation feeds back onto the initiating step. The enzyme's pH dependence is settled biochemistry and structure (Chen and colleagues, 1997; Dall and Brandstetter, 2013), and acidosis has been shown to drive the SET cleavage and tau hyperphosphorylation directly (Basurto-Islas and colleagues, 2013). What is not measured is coerulean pH in disease or the activation of this protease by inflammatory acidification in vivo. *Settling experiment: measure protease activation and SET cleavage in the coeruleus following a defined inflammatory challenge, with pH reported.*

Moderate (mouse and cell, receptor logic impure) — noradrenaline restrains microglial activation, and coerulean loss removes that restraint. Norepinephrine suppresses microglial inflammatory output and promotes phagocytosis, and noradrenergic lesion raises inflammation and amyloid (Heneka and colleagues, 2010; Heneka and colleagues, 2002); coerulean ablation in a tau model worsens inflammation, degeneration and mortality with only mild tau aggravation (Chalermpalanupap and colleagues, 2018); microglia-intrinsic β2-adrenergic signalling is sufficient and β-arrestin2-dependent (Qian and colleagues, 2011) and noradrenergic tone suppresses surveillance in vivo (Liu and colleagues, 2019; Stowell and colleagues, 2019). Graded moderate because the receptor attribution is impure: α1 and β1 agonists reproduce the cytokine suppression in vitro (Mori and colleagues, 2002) and microglial β2 deletion raises interleukin-10 as well as tumour necrosis factor α (Lechtenberg and colleagues, 2019). The general claim survives; "largely via β2" does not.

Weak (single study, distant system) — SET participates in the silencing of retroviral sequence. Knockdown of SET/TAF-I diminishes proviral silencing, and, in a separate arm, overexpression of the TAF-Iα isoform reinforces silencing of an otherwise silencing-defective vector (Bui and colleagues, 2019) — two experiments pointing the same way, but the second is not a rescue of the first and uses a different isoform. This is the only published study we could find linking SET or TAF-I to retroviral or retrotransposon silencing in any system; the silenced object is an integrated retroviral reporter provirus in mouse embryonic fibroblasts during reprogramming, delivered alongside a non-integrating Sendai-derived reprogramming vector — not an endogenous element, not a neuron. *Settling experiment: deplete SET, or express the cleavage-resistant asparagine-175-to-glutamine mutant, in human neurons and measure endogenous retroelement transcription, heterochromatin marks and element copy number.*

Predicted, untested — the cut is a chromatin lesion: nuclear SET depletion derepresses repetitive sequence in neurons. This is the volume's new claim and it has no direct support. It rests on the function of the protein (Seo and colleagues, 2001; Kutney and colleagues, 2004), on the single silencing study above (Bui and colleagues, 2019), and on the observation that the same cut already produces DNA damage and nicking in brain (Liu and colleagues, 2008) at a lesion type proposed to relax heterochromatin in tauopathy (Frost and colleagues, 2014) — a proposal from a single laboratory's fly and mouse work, and graded as such. *Settling experiment: in human neurons and in tau*

transgenic mice, measure histone acetylation, H3K9me3 distribution, retroelement transcription and element DNA copy number as a function of SET level and of SET nuclear-versus-cytoplasmic distribution, with the cleavage-resistant mutant as the decisive arm.

Predicted, untested — cleavage of SET perturbs TREX1 and thereby the clearance of retroelement complementary DNA. Supported only by complex membership and co-translocation (Chowdhury and colleagues, 2006). *Settling experiment: co-immunoprecipitation and imaging of TREX1 in neurons following protease activation or expression of cleaved SET fragments, with cytosolic single-stranded DNA quantified.*

Not established (and not required) — the released sequence is read by cyclic GMP-AMP synthase. The only demonstrated neuronal sensor for tau-driven element nucleic acid is ZBP1 reading Z-RNA (Liu and colleagues, 2026); the element-to-inflammation arm of the chromatin programme runs through double-stranded RNA in astrocytes (Ochoa and colleagues, 2023); and the benchmark tauopathy result for the DNA sensor attributes its ligand to mitochondrial DNA (Udeochu and colleagues, 2023). The retroelement-to-DNA-sensor bridge is imported from senescence (De Cecco and colleagues, 2019) and has never been sequenced in brain. The mechanism of this volume does not require this step and is not weakened by its absence.

Rejected as stated — DOPEGAL accumulates because coerulean aldehyde dehydrogenase capacity is exceeded. The framework is imported from the DOPAL literature in Parkinson's disease and does not transfer: the canonical disposal route for DOPEGAL in noradrenergic nerves is reductive, to dihydroxyphenylglycol, rather than oxidative (Eisenhofer, Kopin, and Goldstein, 2004). The measured human finding is accumulation of the aldehyde together with elevated monoamine oxidase A in Alzheimer coerulean cell bodies (Burke and colleagues, 1999). The clearance capacity of these neurons has not been measured, and the volume claims only accumulation, not its cause. *Adjacent and untested: the same protease cleaves aldehyde dehydrogenase 1A1 at asparagine 220 and abolishes its activity in nigral dopaminergic neurons (Nie and colleagues, 2024), which if it occurred in the coeruleus would close a further self-amplifying arc. This has not been examined.*

Moderate (one model, association, protein levels not activity) — inflammasome activity is associated with elevated PME-1 and with more protein phosphatase 2A held demethylated and inactive. In tau transgenic

mice crossed to inflammasome-deficient backgrounds, the protected animals carried lower demethylated phosphatase and lower PME-1 in hippocampus, alongside reduced glycogen-synthase-kinase-3β and calcium/calmodulin-dependent kinase II activity (Ising and colleagues, 2019). Graded moderate because it is one model, one genotype comparison, and immunoblot protein levels rather than a phosphatase activity assay; the study does not demonstrate that the inflammasome installs the methylesterase on the enzyme. The underlying methylation axis is separately secure (Sontag and colleagues, 2010) and the methyltransferase and catalytic-subunit methylation are reduced in Alzheimer-affected regions in proportion to tau pathology (Sontag and colleagues, 2004). *An earlier draft of this volume recorded this claim as rejected, on the basis that the cited study contained no methylesterase measurement. That was an error of our own: the measurement is in the study's figures and its antibody list, not in its abstract. The companion volume* The Silenced Eraser *had it right, and the entry is corrected here rather than quietly removed.*

Summary of grades

Claim	Tier	Basis
Coeruleus carries the first abnormal tau, in the young, before amyloid	Established	Human, 2,332 + 42 brains
DOPEGAL is noradrenergic-exclusive and activates the protease	Strong	Imported, established
The protease cleaves tau at N368 into a propagating seed	Strong	Imported, established
The protease cleaves SET at N175 and exports it to the cytoplasm	Strong	Imported, established, human
PP2A does ~71% of tau erasing; ~30% lost in Alzheimer brain	Strong	Human, quantitative
SET is a histone chaperone and INHAT core subunit	Strong	Imported, established
SET is in a complex with TREX1; TREX1 clears L1 cDNA	Strong	Imported, established
PP2A restrains cGAS–STING	Moderate	Transformed and peripheral cells only; untested in resident CNS cells

Claim	Tier	Basis
Acidosis activates the protease; inflammation feeds back	Moderate	Mechanism solid, location untested
Noradrenaline restrains microglia; coerulean loss removes it	Moderate	Mouse and cell; receptor logic impure
SET participates in retroviral silencing	Weak	One study, fibroblasts, integrated reporter provirus
The cut is a chromatin lesion in neurons	Predicted	No direct support; four searched-for absences
Cleaving SET perturbs TREX1	Predicted	Complex membership only
The released sequence is read by cGAS	Not established	Not required by this mechanism
DOPEGAL accumulates from exceeded ALDH capacity	Rejected as stated	Wrong disposal route for this aldehyde
Inflammasome activity tracks PME-1 and inactive PP2A	Moderate	One model; protein levels, not activity

XIV. Predictions and Falsification

A mechanism earns its place by naming what would destroy it. The predictions below are ordered from cheapest to most demanding, and each ends with the result that would falsify the claim it tests.

- SET is cleaved and mislocalised in the locus coeruleus, and earlier there than in cortex. In human post-mortem tissue staged by Braak, coerulean neurons will show cytoplasmic SET and the twenty-kilodalton cleavage fragment at subcortical stages, before cortical neurons do. *If SET localisation in the coeruleus is indistinguishable from control at the stages where coerulean tau is already abnormal, the volume's central premise fails, and the SET lesion is a late cortical event as the existing literature implies.*
- The cleavage in the coeruleus depends on the aldehyde. Monoamine oxidase A inhibition, or the vesicular-transport rescue that prevents cytosolic amine accumulation, will reduce SET cleavage and cytoplasmic translocation in coerulean neurons in the relevant mouse models. *If SET*

cleavage in the coeruleus is unaffected by blocking DOPEGAL production while tau N368 cleavage falls, then the two substrates are cut by different pools of the protease and the "single cut" is two cuts.

- Lowering nuclear SET derepresses repetitive sequence in neurons. Knockdown of SET in human neurons, or forced cytoplasmic retention, will increase retroelement transcription and reduce histone acetylation-dependent repression at those loci, independently of tau. *If SET depletion produces no change in element expression or chromatin state in neurons, Section VIII is wrong and the cut has only its cytoplasmic consequence.*
- The cleavage-resistant mutant is the decisive arm. Expressing SET carrying the asparagine-175-to-glutamine substitution — which the protease cannot cleave — will protect against both the phosphatase inhibition and, if the prediction holds, the element derepression, in the same cells. *If the uncleavable mutant protects the phosphatase but not the chromatin, the two consequences are separable and the "two-compartment lesion" is a single-compartment lesion with a coincidental nuclear correlate.*
- The two chromatin hits are separable and their order can be read. In a tau model, element derepression attributable to the protease route will appear earlier relative to tangle burden than derepression attributable to tau-induced heterochromatin relaxation, and protease inhibition will reduce the early component without abolishing the late one. *If protease inhibition abolishes element derepression entirely, the tau-first architecture is wrong; if it does nothing to it, the protease route does not exist.*
- Reducing phosphatase activity lifts the innate brake in a brain cell. Pharmacological or genetic reduction of protein phosphatase 2A in primary microglia will raise interferon-stimulated gene expression and adaptor phosphorylation, as it does in macrophages and monocytes. *If phosphatase reduction in microglia leaves innate sensing unchanged or suppresses it, Section IX does not transfer and should be withdrawn rather than softened.*
- Cleaving SET perturbs TREX1. Following protease activation, TREX1 will change its association with the SET complex, its localisation, or both, and cytosolic single-stranded DNA will rise. *If TREX1 is entirely indifferent to SET cleavage, the complex-membership argument of Section VI is decorative and should be removed.*
- Protease inhibition protects the coeruleus by more than the tau route. A brain-penetrant inhibitor given before coerulean pathology will preserve

the nucleus better than an equivalent reduction in tau burden achieved by another means. *If the protective effect of protease inhibition is fully accounted for by its effect on tau, then the second substrate contributes nothing in vivo and this volume is an interesting biochemistry paper about a pathway that does not matter.*

- The sensor is identifiable and is probably not the DNA sensor. Sequencing the cytosolic nucleic acid of coerulean and cortical neurons in tauopathy will identify the species, and on present evidence it will be predominantly RNA. *If it is predominantly retroelement complementary DNA, Section X is too cautious and the TREX1 arm becomes the centre of the mechanism rather than its periphery.*

XV. Therapeutic Corollaries — Cutting Above the Cut

The mechanism's practical attraction is that it converges upward rather than downward. Four lesions, one stroke, one enzyme. Every target below is stated with its booby-trap, because a volume that names only the attraction is an advertisement.

The stroke — the protease. Asparaginyl endopeptidase is the single point at which all of this volume's consequences can be prevented at once, and it is druggable: a selective, orally bioactive inhibitor with a solved co-crystal structure reduced substrate cleavage, rescued synapse loss and long-term potentiation and protected memory in both tau and amyloid models (Zhang and colleagues, 2017), and medicinal chemistry has since markedly improved brain penetration over the parent compound (Meng and colleagues, 2025). The booby-trap is fourfold. The enzyme is a lysosomal protease with physiological substrates — antigen processing among them — and chronic systemic inhibition is not a free action. More specifically, it is required for the proteolytic maturation of the endosomal nucleic-acid receptors, and dendritic cells lacking it are hypo-responsive to receptor stimulation in culture and in vivo (Sepulveda and colleagues, 2009; Ewald and colleagues, 2011); an inhibitor given for decades would blunt an arm of antiviral sensing, which is a specific and quantifiable risk rather than a vague one. The improved brain exposure belongs to the analogues, not to the original tool compound, so the claim that the target is reachable in the human brain rests on the newer chemistry. And the timing implied by this volume is uncomfortable: if the

first cut occurs in the coeruleus in early adulthood, then the window in which prevention is most valuable is the window in which nobody is diagnosed.

The activator — the aldehyde. Upstream of the protease sits a reaction with two blockable steps: the escape of norepinephrine into cytosol, and its oxidation by monoamine oxidase A. Both have precedent. Apolipoprotein E4's exclusion of the amine from vesicles via the vesicular transporter is a defined mechanism with a genetic hook (Kang and colleagues, 2021), and the loss of α2A-mediated autoinhibition under chronic stress is a second, potentially reversible route (Toyoda and colleagues, 2025). Monoamine oxidase A inhibition reduced tau adduct formation and spreading (Kang and colleagues, 2022). The booby-trap is that this is the corpus's recurring problem with the noradrenergic system in a different guise: the signal is not simply excessive or simply deficient but dysregulated, and a drug that lowers the aldehyde by lowering noradrenergic output would remove the same restraint on microglia whose loss Section II and the companion volume identify as pathogenic. Reducing the metabolite without reducing the transmitter — the vesicular arm rather than the oxidase arm — is the discriminating strategy, and it is the one with the least pharmacology behind it.

The substrate — a cleavage-resistant SET. The asparagine-175-to-glutamine substitution is uncleavable by the protease (Basurto-Islas and colleagues, 2013), which makes it the cleanest experimental tool in Section XIV and, in principle, a gene-therapeutic. The booby-trap is that SET is an oncoprotein whose overexpression is a recognised pathology in its own right, and that a construct which cannot be cleaved is also a construct whose nuclear pool cannot be regulated by the normal route. This is a tool before it is a therapy, and probably only a tool.

The consequence — restoring the phosphatase. If the eraser is gagged rather than destroyed, it can in principle be un-gagged, and the methylation axis offers a second handle: methyltransferase and phosphatase methylation are quantitatively reduced in Alzheimer-affected regions in proportion to tau pathology (Sontag and colleagues, 2004), and the methylation state controls activity (Sontag and colleagues, 2010). The booby-trap is Section IX. If protein phosphatase 2A restrains innate sensing as it does in every non-neural system tested, then raising its activity to protect tau may simultaneously suppress an innate response whose role in the brain is not established and may not be uniformly harmful. Raising a phosphatase that sits on both tau and the alarm

is not a single-target intervention, and nobody has measured the second effect in a brain cell.

The terminus — the innate sensor. This volume's mechanism does not require cyclic GMP-AMP synthase, which is an advantage rather than a gap. Any corollary that reaches for it inherits the problems catalogued in the companion volume: the demonstrated neuronal sensor for tau-driven element nucleic acid is ZBP1 rather than this enzyme (Liu and colleagues, 2026), the benchmark tauopathy ligand is mitochondrial rather than retroelement DNA (Udeochu and colleagues, 2023), and the enzyme itself maintains the heterochromatin whose failure releases its ligands, so that inhibiting it may worsen the derepression it is being inhibited to answer (Martinez and colleagues, 2026). Reverse-transcriptase inhibition is the more sequence-agnostic option and has precedent in ageing tissue (De Cecco and colleagues, 2019), but in brain the intermediate it targets has been inferred pharmacologically and never sequenced.

The single strategic reading. If this volume is right, the best point of intervention is the protease and the best time is decades before diagnosis, which is precisely the intervention that cannot currently be trialled because the population that would benefit cannot be identified. The practical consequence is therefore not a drug but a biomarker programme: coerulean integrity is measurable by magnetic resonance neuromelanin imaging, and the protease's substrates leave cleavage products that are detectable in fluid. A volume that locates the first lesion in a twenty-year-old's brainstem is an argument for measuring brainstems, and only then for treating them.

XVI. Coda — The Aldehyde and the Archive

Every cell carries an archive it does not read. Half the human genome is sequence that was once mobile and has been silenced, packed into heterochromatin and held there by proteins whose whole function is to say nothing. A neuron is the most extreme case of this arrangement, because a neuron never divides, and so never gets the chance to re-establish its chromatin from scratch. What it packed away at the beginning it must keep packed away for eighty years, without ever rebuilding it from a template, maintained only by exchange and repair.

In the locus coeruleus there is a further complication, which is that this particular cell manufactures its own solvent. Norepinephrine is a useful molecule and an unstable one, and the enzyme that disposes of it produces an aldehyde that no other cell in the brain has to contend with. For most of a life this is manageable. Then a vesicle leaks, or a receptor internalises, or an apolipoprotein binds the wrong transporter, and the amine reaches the cytosol, and an aldehyde appears whose principal talents are two: sticking to tau directly, and waking a protease that cuts after asparagine.

What that protease finds is two proteins that have nothing to do with each other. One is tau, which it turns into a seed. The other is a small acidic protein that has been sitting in the nucleus holding the archive shut. It cuts them both, in the same minute, in the same cell, for the same reason — that each of them happens to present an asparagine. The field has watched what the seed does, because a seed spreads and can be stained. It has watched where the other fragment goes, because it goes somewhere it can be caught doing harm. Nobody has gone back to the nucleus to see what was left unattended.

That is the whole of this volume's suggestion. Not that the archive is opened by tau — that has been argued, well, by others. But that it may be opened twice: once by the tangle, late, and once by the protease, early, in the one cell that makes its own aldehyde, before anybody knows anything is wrong. The two openings would look identical at autopsy and would have entirely different causes, and only one of them has an inhibitor.

A protein that leaves a room is usually noticed for where it arrives. This one arrives in the cytoplasm and gags an eraser, and the literature has followed it there for twenty years. It also leaves a door.

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Companion volumes: The Coerulean Pincer *(the writer — glycogen-synthase-kinase-3β and the noradrenergic drive that floors it);* The Silenced Eraser *(the eraser — protein phosphatase 2A and the microglial route to its silencing);* The Silencer and the Tether *(the chromatin*

programme and the restraint on cGAS it would remove); The Assumed Switch *(cGAS as an enzyme rather than a pathway switch). This volume takes the protease that cuts above all four.*