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Discussion

Live discussion: From here to there: AβPP as an axonal transport receptor – How could this explain neurodegeneration in AD?[1]

Live Chat held 15 July with Larry Goldstein.
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http://www.alzforum.org/res/for/journal/goldstein/goldsteinβtranscript.asp
Participants: J. Wesson Ashford (*University of Kentucky*), *Jorge Busciglio* (*University of Connecticut Health Center*), *Keith Crutcher* (*University of Cincinnati*), *Larry Goldstein* (*University of California, San Diego*), *Gunnar Gouras* (*Weill Medical College Cornell University*), *June Kinoshita* (*Alzheimer Research Forum*), *Eckhard Mandelkow* (*Max-Planck-Institute Unit for Structural Molecular Biology*), *Marius Sudol* (*Mount Sinai School of Medicine*)

Background text by Larry Goldstein

In neurons, most synthesis and modification of proteins, synthesis of membranes, and biogenesis of organelles occurs in the cell body. Thus, large quantities of material must be moved by the axonal transport machinery to supply the axon and the axonal terminus. Dendrites face analogous logistical problems. Retrograde neurotrophic and damage signals must also be transported over long distances to keep the cell body informed about distant events. In the case of mammalian CNS neurons, it is likely that as an important consequence of their extensive connectivity and complexity their axons, and perhaps dendrites, are very long, branched, and of diminished caliber in their distal regions. These features may predispose axonal and dendritic processes to blockage of transport when vesicles aggregate or become damaged. This would be analogous to what we and others have observed in molecular motor and other mutations that disrupt axonal transport in *Drosophila*.

These observations prompted us to explore potential connections between molecular motor proteins such as kinesins, which generate the forces needed for movement in axons and dendrites, and proteins and vesicles implicated in neurodegeneration, such as amyloid-β protein precursor (AβPP), which plays a key role in the development of Alzheimer's disease (AD).

Recent work in my laboratory has led us propose the following ideas:

1) Anterograde axonal transport of a vesicle population containing BACE, presenilin, the trk receptor, and GAP-43 may require a direct interaction of AβPP and the kinesin light chain subunit of the kinesin-I molecular motor protein.
   This proposal is based on several recent observations:
   a) Kinesin light chain and AβPP exhibit a high-affinity biochemical interaction;
   b) Kinesin light chain is required for normal transport of AβPP in mouse sciatic nerve axons;
   c) Deletion of the AβPP homologue, APPL, in Drosophila causes axonal transport de-

[1] Note: The transcript has been edited for clarity and accuracy.

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fects characterized by vesicle accumulations (clogs) similar to those found in kinesin-I and known axonal transport mutants;

d) Axonal content and transport of kinesin-I, BACE, presenilin, GAP-43, and the trk receptor are diminished in AβPP deletion mutants in the mouse PNS;

e) A vesicle population lacking ER markers but containing kinesin-I, BACE, presenilin, GAP-43, and the trk receptor can be inferred from immuno-isolation experiments using material from sciatic nerve and CNS axons [1–3].

2) Blockage of transport and/or axonal damage leads to increased AβPP proteolysis and amyloid-β (Aβ) production in an axonal vesicle compartment.
We observed that low levels of Aβ are normally present in axonal vesicles containing AβPP, presenilin, and BACE. We also found that AβPP proteolysis can be induced in the axon by nerve ligation *in vivo* or in axonal vesicles *in vitro*. This AβPP proteolysis gives rise to Aβ (confirmed by SELDI mass-spectrometry), a protein fragment with properties characteristic of a free cytoplasmic C-terminus of AβPP, and it causes kinesin-I to let go of the vesicles [2].

3) Disruption of axonal transport in the presence of the Aβ region of AβPP can cause neuronal apoptosis.
This conclusion is based on recent observations in Drosophila [3]:

a) Overexpression of APPL and AβPP in Drosophila neurons causes axonal vesicle accumulations;

b) Formation of axonal clogs and axonal transport of AβPP and APPL require the cytoplasmic C-terminus that contains the proposed kinesin-I binding domain;

c) Overexpression of AβPP, but not APPL, induces neuronal apoptosis. Induction of apoptosis appears to require the presence of the Aβ domain and the formation of axonal clogs mediated by the cytoplasmic C-terminus of AβPP.

d) AβPP-induced axonal blockages and neuronal apoptosis are coordinately enhanced by a 50 percent reduction in kinesin-I dosage but suppressed by a 50 percent reduction in cytoplasmic dynein dosage. We interpret this to mean that overexpression of a motor receptor protein, such as AβPP, titrates kinesin-I motor function away from other critical cargoes in narrow caliber axons. This would lead to transport dysfunction, clogging, and apoptosis if an Aβ region is present.

A transcript of the live discussion follows.

Marius Sudol: Are you aware of a knockout of AβPP in *C. elegans* worms?

Larry Goldstein: I have only heard rumors, but have seen no data – is it lethal?

June Kinoshita: What sort of worm mutants?

Marius Sudol: The phenotype of AβPP deletion in *C. elegans* is a pharyngeal pumping defect. Could you look at vesicle transport in the neurons of the worm mutant?

Larry Goldstein: For worms – one could in principle. Jonathan M. Scholey (University of California, Davis) and Cornelia Bargmann (University of California, San Francisco) and others have done that sort of thing.

Eckhard Mandelkow: What is the percentage distribution, in a differentiated neuron, of AβPP in any given compartment? What is your best guess?

Larry Goldstein: We made one quick estimate based on quantitative immunoblots and came up with somewhere in the one percent range in the sciatic nerve. If the estimate is correct, this is a lot. On the other hand, axons lack nuclei, Golgi, etc., so maybe it could be that abundant. There was also an older estimate from Sangram Sisodia's group (University of Chicago) in either cultured neurons or brain – I cannot remember which. They came up with 0.03 percent total in that population, I think.

Eckhard Mandelkow: Redefining that question, how much of that is in ER, Golgi, axon, or at the synapse?

Larry Goldstein: No clue – our estimate was on sciatic nerve only, which should lack most typical ER, lack Golgi, lack synapses, etc. There will be some contribution from Schwanns and other supporting cells, but these are pretty minor components of mass based on estimates from looking at cross-sections in the microscope.

Eckhard Mandelkow: Because, my feeling is that there is a lot of discussion on AβPP cleavage in ER, Golgi, etc., but your work really emphasizes a later stage – during transport, that is.

Larry Goldstein: I agree that most cleavage may occur in later stages. The evidence at this point from other groups is most convincing for lack of cleavage in ER. This also places focus on later stage compartments. Edward Koo (University of California, San Diego) has argued for endosomes, and there could be some there, as well. We would argue for post-Golgi and probably synapse. Very little work has been done on neurons *in vivo*; most is on cell cultures of various sorts.

Marius Sudol: Larry, how do you connect your cytoplasmic events with nuclear events? To be more specific, I have in mind the Tip60-mediated apoptosis work of Bradley Hyman (Harvard Medical School) and Thomas Südhof (Howard Hughes Medical Institute).

Larry Goldstein: Marius, this is interesting, indeed. We are intrigued by the idea that induction of cleavage in axoplasm could lead to retrograde signals to cell body or to nucleus. The readout, i.e., death or transcriptional changes, could be different depending on neuronal type. We need more data on this point.

Eckhard Mandelkow: If we are talking about most cleavage during post-Golgi and transport, then the questions are: Where does that Aβ go and how would it get out of the cell in order to contribute to plaques?

Jorge Busciglio: Larry, do AβPP knockout mice show some phenotype that you can relate as a kinesin transport defect?

Larry Goldstein: Jorge, we have published data suggesting that AβPP knockout in mouse has transport defects in sciatic nerve and corpus callosum neurons. We have published data in flies suggesting that transport defects are present in segmental nerves. Eckhard, I would argue, but do not know, that secretion or lysis would lead to contribution to plaques. I have two questions for you: What fraction of Aβ that is produced ends up in plaques? Is this the only fate possible?

Eckhard Mandelkow: I thought Brad Hyman's work says that most of what gets out of the cell gets washed away, and only a minority sticks in the plaques.

Jorge Busciglio: In people without AD, most Aβ is degraded and does not accumulate.

Larry Goldstein: Interesting. I will have to follow these up. Perhaps the plaque is not the most important toxic element?

Eckhard Mandelkow: Jorge, is this also true for people with AD, i.e., most is degraded?

Jorge Busciglio: I am not sure this has been proven, but I would estimate that even in AD, most is cleared out. Protofibrils and fibrils may be more toxic than condensed Aβ in senile plaques.

Eckhard Mandelkow: So the minority fraction (whatever it is) contributes to aggregation outside the cell. I know that that is the dogma. I still debate with myself whether any Aβ is toxic, but I dare not say that in public.

Jorge Busciglio: . . . or inside the cell, as Larry Goldstein's data suggest.

Larry Goldstein: Is there any consensus on whether the plaques form at synaptic endings or around cell bodies?

Jorge Busciglio: Within plaques you see a lot of terminals....

Larry Goldstein: So, it could be presynaptic or postsynaptic termini secreting?

Jorge Busciglio: I think so.

Gunnar Gouras: I can say that I think that Aβ can be toxic within neurons and processes/synapses. To Larry Goldstein, what is the function of these post-Golgi vesicles in axons?

Larry Goldstein: Gunnar, I presume that all post-Golgi anterograde vesicles are transport vesicles of one sort or another, ferrying materials to presynaptic termini or to intermediate destinations.

Jorge Busciglio: Larry, how does the apolipoprotein E (ApoE) 4 polymorphism fit in your hypothesis?

Larry Goldstein: Jorge, unclear, other than that ApoE may be taken up by ApoE receptors at termini and perhaps transported back. Deletion of some of these recep-

tors leads to odd tau phosphorylation, which Eckhard and Eva Mandelkow would argue cause kinesin-based transport defects – right, Eckhard?

Eckhard Mandelkow: And the question following from that is – how much AβPP does a synapse need at equilibrium? I think this could be measured by looking at the flow of AβPP-loaded vesicles.

Larry Goldstein: Eckhard, I have no idea how much AβPP is needed at synapse. I have even less of an idea what AβPP is doing at the synapse, but there is reasonable data for an effect on synaptic morphogenesis. I agree that some better quantification and precision on these issues is in order.

Eckhard Mandelkow: When we look at AβPP vesicles in axons, most of them move anterograde, then seem to sit around at the terminal for some time, and then disappear. So I am wondering where the minority of retrograde-moving AβPP vesicles come from.

Larry Goldstein: Endocytic recycling or changes in direction?

Eckhard Mandelkow: I cannot tell for sure, because AβPP vesicles are fast and dim, so any reversals would be hard to detect.

Marius Sudol: Eckhard, Jorge and Larry, I am lobbying for the C. elegans model; I feel that *C. elegans* with an AβPP deletion may have a problem swallowing because of transport defects in selected neurons. It should be easy to check experimentally.

Eckhard Mandelkow: I agree – what would Jon Scholey (University of California, Davis) say? He looked at overall transport in the worm.

Larry Goldstein: Marius, I agree that this would be a good system to investigate. Just not one I am experimentally skilled with.... Eckhard, could you load with an endocytic tracer and ask if the retrograde vesicles have taken up material at the terminus?

Eckhard Mandelkow: To answer these questions, we need to crank up our experimental gear – better detectors, faster detectors – to follow an individual vesicle reliably, just like we can do it with mitochondria, which are bright.

J. Wesson Ashford: There is also a consideration that ApoE modulates the size of the lipid rafts to which the AβPP is connected, and the size of the raft influences whether there would be a greater predisposition to α- or β-secretase processing. What is the relationship between the vesicles and the lipid rafts?

Larry Goldstein: Wes, I do not know the relationship between vesicles and rafts. Is there evidence for rafts in axons?

J. Wesson Ashford: Larry, the raft story just seems to be developing. There was an interesting discussion of the issue by K. Jacobson in the 6/7/2002 issue of Science [4], but I do not know that there is any knowledge of whether the rafts or caveolae would not be present in axons.

June Kinoshita: What is causing the AβPP vesicles to disappear at the terminal? β and γ cleavage?

Larry Goldstein: June, presumably that is so. This would fit with our proposal that β- and γ-secretase are in the vesicular packet delivered to the synapse along with AβPP.

Eckhard Mandelkow: June, I think it could be degradation. Since the AβPP is green at its C-terminus, these could be stubs which then move back retrogradely.

Larry Goldstein: Eckhard, but then why would the fluorescence decrease if they are just stubs?

Eckhard Mandelkow: Not sure which fluorescence decrease you mean – what we observed after inhibiting transport with tau is just the general decrease of vesicles, and also the reversal of overall directionality.

Larry Goldstein: Eckhard, you indicated that the AβPP vesicles go to the synapse, then sit around and disappear. Do you mean disappearance of fluorescence?

Eckhard Mandelkow: Yes, and I am not sure whether the retrograde vesicles we see have ever been near the synapse – they could also turn around midway.

Gunnar Gouras: What about AβPP in dendrites – less has been described about this.

Eckhard Mandelkow: Gunnar, right. It would be interesting to see an individual vesicle transcytosing, thereby proving the concept.

Larry Goldstein: Gunnar, interesting point. We have not done much on dendrites for experimental reasons. It is relatively easy to find bundles of axons to probe biochemically, but I know of no good source of enriched dendrites. Eckhard, do you see AβPP vesicles in dendrites?

Eckhard Mandelkow: Yes, AβPP vesicles are in dendrites, as well, maybe not as dense.

Larry Goldstein: Eckhard, but it sounds as though there are many fewer retrograde than anterograde? Is their brightness distribution the same?

Eckhard Mandelkow: Yes, the brightness distribution seems to be the same in both directions, arguing that the number of fluorescent particles is similar. But the distinction is hard to quantify.

Larry Goldstein: Do dendritic vesicles have the same movement characteristics as axonal vesicles?

Eckhard Mandelkow: Superficially, it seems similar, but we have not analyzed it in detail.

Larry Goldstein: I have heard somebody claim that there is no caveolin in neurons; am I remembering this incorrectly?

Jorge Busciglio: I think that flotillin is more abundant than caveolin in neurons.

Eckhard Mandelkow: General question: If, as Larry suggests, Aβ is continuously being generated along the path of secretion, where, then, does that Aβ become toxic, assuming it is toxic?

Larry Goldstein: I guess the question is that, for proteins that are moving from cell body to synapse and that are thought to be in rafts, do they move in rafts, or move in vesicles prior to assembly into rafts at plasma membrane? *In vivo* studies of AβPP movement sure look vesicular in axons.

Eckhard Mandelkow: Larry, I agree, practically all the fluorescence we see is inside axons, not on the plasma membrane.

Larry Goldstein: Eckhard, if it is toxic, perhaps it becomes so when intracellular, intra-axonal or intradendritic aggregates become large enough to block transport.

Jorge Busciglio: There is no evidence yet of intracellular Aβ toxicity.

Eckhard Mandelkow: Quite so. I guess you are referring to traffic jams, which have bright fluorescence.

June Kinoshita: Gunnar, is there any evidence of such large intracellular aggregates? Intra-axonal, more specifically.

Gunnar Gouras: There is certainly increased suggestion of intracellular toxicity, including Jorge's recent Neuron paper [5]. Yes, several groups have now reported that Aβ can accumulate within neurons.

Larry Goldstein: What is the evidence for extracellular toxicity? By the way, aggregates do not need to be that big to potentially interfere with traffic in narrow axons.

Eckhard Mandelkow: The subcellular aggregates, in my view, are aggregates of organelles, vesicles, etc., and they may be toxic because they jam the axon, but not because there is Aβ around.

Jorge Busciglio: Eckhard, I agree.

Eckhard Mandelkow: Jorge, thanks for telling me that Aβ is not toxic.

Jorge Busciglio: Eckhard, I never said that!

Larry Goldstein: Eckhard, but might a small aggregate, say 0.1–0.5 microns in diameter, nucleate a transport blockage in a 0.2-micron-diameter axon? Could the intracellular Aβ be produced internally as well as taken up by endocytosis?

Eckhard Mandelkow: That is a tough question, but my guess would be yes, considering that even a little tau can block traffic if it sits right on the track.

Larry Goldstein: Eckhard, my point exactly.

Marius Sudol: Going back to the question of caveolins as not being expressed in neurons ... There are solid reports on caveolins being expressed in neurons and astrocytes.

Jorge Busciglio: My recollection is that flotillin, a caveolin homologue, is much more abundant than [caveolin] in neurons.

Larry Goldstein: I am not an expert on caveolins and so have to take others' words for this.

Marius Sudol: Jorge, you are right about dominance of flotillin.

J. Wesson Ashford: Would the vesicles be the transport agent that carries the AβPP to the synapse? Then, does it join with the lipid raft there to meet the secretases? Certain neuronal stimuli, including acetylcholine stimulation, may thus be able to modulate whether processing will be α or β.

Larry Goldstein: How strong is the evidence that a cleavage occurs at presynaptic sites?

Jorge Busciglio: Larry, I sure think that intracellular Aβ is produced internally, and also aggregated intracellularly; that is what we have shown happens in Down's astrocytes.

Eckhard Mandelkow: I would like to repeat, for my own benefit, that perhaps the main consequence of Larry Goldstein's work is that we need to think about the AβPP-motor connection and traffic, rather than about cleavage as such.

Larry Goldstein: Obviously, I agree. I think that ApoE points in this direction, as well, given the tau phosphorylation defects in some of the receptor mutants. By the way, Joachim Herz (University of Texas Southwestern Medical Center, Dallas) has published jip1/2 interactions with ApoE receptors [6]. You may recall that jip1/2 also seems to have kinesin-binding activity required for proper transport.

Eckhard Mandelkow: Jorge, is that intracellular aggregate toxic because it blocks traffic, or because it perturbs some biochemical pathway?

Jorge Busciglio: Larry, we do not know yet. It is associated with energy deficits, but we are not sure what comes first.

June Kinoshita: Eckhard, I thought Larry was also proposing that cleavage has a function in the context of axonal transport by causing kinesin to release the vesicle.

Eckhard Mandelkow: So, the question about cleavage is: Does it exert its effect because it generates Aβ, or does it work by decreasing AβPP and thereby lose some linkage to motors?

Larry Goldstein: June, we did make that suggestion, but it is speculative at present. The idea is that cleavage could play a role in vesicle release at terminus, and might also lead to release following damage.

Eckhard Mandelkow: Talking about discharging kinesin, I think a better candidate would be the kinase connection; see G. Morfini and Scott T. Brady (University of Texas Southwestern Medical Center at Dallas) papers [7–9].

Larry Goldstein: Eckhard, perhaps it is both. An interesting idea is an autocatalytic spiral. Intra-axonal cleavage leads to vesicular Aβ and stalled vesicles because of kinesin release. Both might lead to more stalling, and so on.

Jorge Busciglio: Eckhard, we have evidence that presenilin-1 mutations reduce kinesin binding to vesicles via increased glycogen synthetase kinase 3 activity.

Larry Goldstein: Eckhard, the kinases are interesting and must be important, too. Not clear to me yet just how. It is worth emphasizing that our proposal is simpler than reality is likely to turn out to be because of Ockham's razor. These proteins are almost surely in much bigger complexes with presenilin and kinases.

Eckhard Mandelkow: I still have the impression that this intracellular Aβ is a minor component during traffic, and accumulation becomes noticeable only at the synapse. In this context, kinases control the ins and outs of melanosomes in melanophores.

Larry Goldstein: Eckhard, I do not know of a good measurement of this. If it is generated, at least in part, during transport and also by cleavage at synapse, then major accumulation should be at synapses.

Larry Goldstein: Eckhard, our experience in the fly is that synaptic proteins accumulate at synapses and have low steady-state levels in axons, since they are in transit. If you interfere with transport, then you see accumulations of synaptic proteins in axons.

Eckhard Mandelkow: The point is that there are different mechanisms for regulating the forward and re-

verse transport of vesicles. Go into Pubmed and look for "bidirectional". It can be by having different motors controlled by phosphorylation, or detachment, again possibly controlled by kinases, or perhaps even AβPP.

Larry Goldstein: My read of the literature is that many vesicles alternate between forward and reverse. Cleavage would not be a good way to regulate this. But [maybe] for a vesicle that is highly processive in the forward direction. Eckhard, are your and Dotti's AβPP vesicles like this? Cleavage might be a good way to regulate [these] things.

Eckhard Mandelkow: Larry, yes and no. The problem is that each vesicle probably carries a number of motors, so you would have to do a lot of cleavage. But who knows? Has anyone looked at an axon in a Campenot chamber and tried to look at local effects of Aβ? I am thinking of work by the Carl Cotman group (University of California, Irvine), or by Pierluigi Nicotera (University of Konstanz, Germany), who looked at local signs of apoptosis along an axon.

Larry Goldstein: Eckhard, I do not know – interesting idea.

June Kinoshita: Larry, have you looked at how FAD mutations affect the function of AβPP in axonal transport?

Larry Goldstein: June, we have only looked in the fly, where the mutations appear to be similar to wildtype in overexpression experiments. They may differ in apoptosis induction. We need more experiments on this issue.

J. Wesson Ashford: I am thinking that there may be important differences between dendritic and axonal transport. We did not examine axons. However, this discussion seems to be focusing on AβPP transport, probably involving axons, but I have not seen the direct evidence that the transport of the AβPP is stopped mechanically.

Eckhard Mandelkow: Wes, how do you think the neuropil threads get started?

Larry Goldstein: Wes, there is no such evidence as yet. But I would argue that it is worth testing. I agree that dendrites may be as important, or more important, than the axon for transport blockage by these proteins. We just do not know as much. What are neuropil threads?

Eckhard Mandelkow: These are early aggregates of tau, but in the dendritic compartment, which goes against the dogma of axonal tau.

Larry Goldstein: You are pouring gas on a fire.

Eckhard Mandelkow: Not really, because the distribution of tau is not as predetermined as it might seem. The problem is that a lot of it is difficult to detect, depending on which antibodies one uses. We are waiting for Mark H. Ginsberg's (The Scripps Research Institute) or James Eberwine's (University of Pennsylvania) single-cell determinations!

J. Wesson Ashford: Interesting questions just developing. There does seem to be some tau in the dendrites, probably getting abnormally phosphorylated.

Larry Goldstein: Indeed this has been interesting! The question, of course, is how to test any of this in a human with disease to see if there is a reasonable connection to AD.

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