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Modeling proteasome dynamics in Parkinson's disease

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Abstract
In Parkinson's disease (PD), there is evidence that α-synuclein (αSN) aggregation is coupled to dysfunctional or overburdened protein quality control systems, in particular the ubiquitin–proteasome system. Here, we develop a simple dynamical model for the on-going conflict between αSN aggregation and the maintenance of a functional proteasome in the healthy cell, based on the premise that proteasomal activity can be titrated out by mature αSN fibrils and their protofilament precursors. In the presence of excess proteasomes the cell easily maintains homeostasis. However, when the ratio between the available proteasome and the αSN protofilaments is reduced below a threshold level, we predict a collapse of homeostasis and onset of oscillations in the proteasome concentration. Depleted proteasome opens for accumulation of oligomers. Our analysis suggests that the onset of PD is associated with a proteasome population that becomes occupied in periodic degradation of aggregates. This behavior is found to be the general state of a proteasome/chaperone system under pressure, and suggests new interpretations of other diseases where protein aggregation could stress elements of the protein quality control system.

S This article has associated online supplementary data files

Introduction

Parkinson's disease (PD) is characterized by the loss of dopaminergic neurons in the *substantia nigra* region of the brain, which leads to movement disorders such as stiffness, slowness of movement and tremors [1]. Many dopaminergic neurons in the PD brain accumulate intracellular aggregates or inclusion bodies known as Lewy bodies (LB), which predominantly consist of fibrils of the 140-residue protein α-synuclein (αSN) which is natively unfolded in aqueous solution [2]. αSN fibrillates readily in solution over a period of hours to weeks, depending on pH [3, 4], additives such as polyamines [5], salts, detergents [6, 7] and heparin [8], and the extent of mechanical shaking. LB as such are not likely to be the causative agent in PD; they are also found in healthy age-matched controls [9], do not occur in some familial PDs involving mutant αSN [10] or in mouse models of PD [11, 12] and may even have a protective function [9]. Rather the cytotoxic species is more likely to be a soluble oligomer formed at an early stage in the aggregation process, which can assume an annular shape [13] and may lead to cell death through perturbation of organellar and cellular membranes in affected neurons [14, 15].

It is not completely clear whether this cytotoxic oligomer is an essential fibril precursor or an off-pathway species. Some αSN oligomers have been reported to convert to fibrils (directly or indirectly) in the presence of monomers [13]. Oligomers form readily upon incubation of αSN well before fibrillation starts [13]. However, there is growing evidence for an off-pathway scenario. Chaperones such as Hsp70 can prevent fibrillation by targeting the prefibrillar species [16]. More importantly, chemical modification of αSN by methionine oxidation (either directly [17] or through αSN's sacrificial

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ability to protect lipids against oxidation [18]), nitration [19] or reactions with small molecules such as dopamine [20], baicalein [21] and dequalinium [16] inhibits fibrillation and leads to the accumulation of oligomers. In support of this, Danzer *et al* [22] recently demonstrated that it is possible to prepare at least three different types of αSN oligomers, of which the cytotoxic class does not stimulate aggregation in contrast to the non-cytotoxic classes. This suggests that oligomerization and fibrillation are competing pathways.

Most cases of PD are sporadic. However, within the last decade, numerous examples of heritable PD associated with mutations in different proteins have come to light, shedding more light on the rather complex etiology of the disease. In addition to mutations in αSN itself [23], variations in the parkin and UCH-L1 genes have been identified, which all code for proteins involved in the degradation of aggregated αSN, primarily in the ubiquitin–proteasome system (UPS). The proteasome is responsible for degrading more than 70% of all intracellular proteins [24] particularly those who have become corrupted by oxidation or other covalent and therefore are prone to misfolding and aggregation [25]. It is a multicatalytic system containing the 20S core proteasome and several regulatory components which mediate the recognition of ubiquitinylated substrates [26], though the free 20S proteasome is the predominant form [27] and has some activity of its own [28]. The UPS system can be inhibited by numerous different aggregates [29, 30], and UPS impairment has been linked to Huntington's disease and cystic fibrosis [31]. Over-expression of ubiquitin can partially compensate for these effects [32]. Although these and other data[33–36] provide substantial evidence for the role of proteasomal impairment in PD, there are also numerous indications for lysosome involvement in aggregate degradation [37–39].

A reduction in proteasome activity may allow oligomers to accumulate and bind to organellar membranes where they can destroy chemical gradients and compromise functionality, e.g. in the mitochondria, while presumably remaining impervious to proteasome attack. Mitochondrial dysfunction, in turn, leads to the leakage of reactive oxygen species from the respiratory chain which can oxidize side chains and cleave the polypeptide backbone [40]. In general, oxidative modification of proteins in the cell can lead to the formation of aggregates, further cross-linked by cellular metabolites and oxidants [40]. These aggregates can usually not be rescued by repair enzymes such as methionine sulfoxide reductase, and therefore have to be removed or metabolized; however, they can bind to and inhibit proteasomes [29, 30].

There is substantial evidence for an interplay between different etiologies in the development of PD. Thus, sporadic PD brain samples show a reduction in proteasome activity and reduced levels of the α subunit of the 20S proteasome as well as increased oxidative stress [41]. Proteasome inhibitors favor αSN aggregation [42], and mitochondrial inhibitors such as MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) and rotenone only induce PD symptoms in the presence of αSN [43]. Inducible [44] or mutants of the protein parkin inhibits proteasome activity and is counterbalanced by an up-regulation of one of the components of the UPS system [9].

All these observations identify αSN as a key player in the development of PD, whose aggregation is linked to decreased protein quality control, increased mitochondrial dysfunction, oxidative stress and chemical modification of αSN, leading to a positive feedback cycle that exacerbates the effects of aggregation [45]. Often macromolecular networks form feedback loops whose dynamics depend on the coordinated behavior of many parts. For example, negative feedback systems are often associated with homeostasis [46, 47] or oscillations [48], whereas positive feedback loops generate bistability [49] or ultrasensitivity [50, 51]. In spite of incompleteness of data for most biological systems, mathematical modeling may lead to quantitative predictions [49, 46, 52] which can outline reasons for the system function or mal-function [53]. Overall, such modeling builds on simplification of cellular processes, [54] by focusing on the key components and their interplay in a particular biological context. In any of such case studies there will, of course, be interference from other components, and thus an essential part of model building is to check their robustness against variations.

To analyze the interplay between key players development of PD, we develop in this paper an *in silico model* of the central feedback mechanisms. In addition to providing further insight into the molecular etiology of the disease, such a model can be used to predict the effect of perturbing the molecular network influencing the disease and thereby provide inspiration for developing targeted drugs.

The central tenet of our model is that PD develops when the accumulation of cytotoxic αSN oligomers cannot be suppressed by available proteasome. While oligomer formation will be encouraged by the chemical modification of αSN due to, e.g., oxidation [55] or formation of dopamine adducts [20, 56], the proteasome capacity in parallel is overwhelmed by the accumulation of intractable fibrillar aggregates which sequester the proteasome molecules as observed *in situ* [57, 58] and prevent them from degrading oligomers. There is as yet no direct biological evidence for the role of proteasomes in oligomer degradation, but this most likely reflects the difficulty in detecting the metabolism of a very elusive species *in vivo*. Our main prediction is that when protofilament production exceeds a threshold value set by proteasome production capacity, then there is a sharp onset of oscillations. The period of these oscillations depends on the model parameters and is always longer than the proteasome average lifetime. We explore the hypothesis that these oscillations are linked to the development of PD.

Figure 1 shows the molecular network around the path leading from free αSN to LB in the cell. This network also includes the alternative path leading to the formation of cytotoxic oligomers which is promoted by the chemical modifications of the protein (for example, oxidation, nitration or reaction with dopamines).

The proteasome will bind to several species of αSN, including early-stage aggregates (here termed pfa for protofilaments) which represent a 'mixed bag' of fibril precursors spanning a wide range of sizes from dimers [59] to small protofilament [60] fibrils. The proteasome will also bind

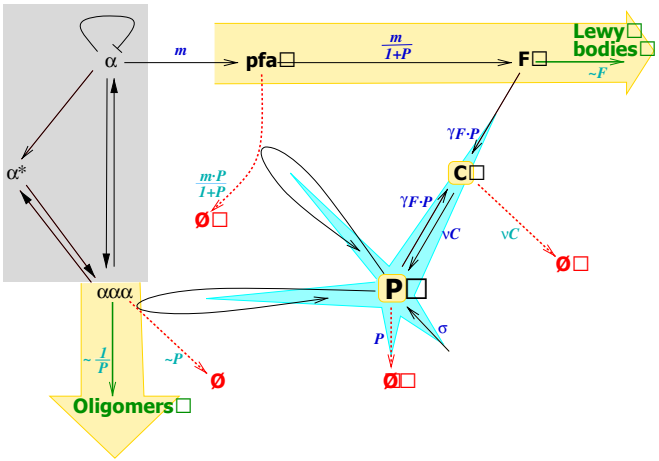

Figure 1. Minimal model leading from αSN to oligomer formation, respectively, fibril formation and degradation. The gray area indicates the processes that lead to the formation of critical seeds ($\alpha\alpha\alpha$) for oligomer formation. The dashed arrows refer to degradation processes ($\varnothing$ stands for elimination). The dark blue labels refer to 'terms' that are included in the core model (equations (1)–(3)) whereas the light blue labels refer to fluxes that are not essential in the feedback loop. The gray area is not explicitly modeled here, as it only provides a constant influx of $\alpha\alpha\alpha$ and m. The light blue area surrounding P highlights the actions taken by the proteasome in our description.

to the mature fibrils (F) themselves as well as to precursors or seeds of the cytotoxic oligomer which is treated as an off-pathway species. We distinguish between oligomer seeds ($\alpha\alpha\alpha$) and oligomers in order to emphasize that once the 'mature' oligomer forms, it can bind to membranes and be protected from proteasomal activity.

For simplicity and due to the lack of quantitative data, we represent the UPS system by the proteasome (P) alone, without specifying the subunit composition of the proteasome and the numerous components involved in the ubiquitinylation pathway. All these components are lumped together in the parameter σ which represents the rate constant for the formation of an active proteasome complex and the associated activity of the ubiquitin targeting system. Thus, changes in the value of σ can also reflect targeting of other parts of the UPS system besides the proteasome by αSN aggregates.

The maintenance of cell homeostasis is centered around the proteasome response to protofilaments (pfa), filaments (F) and finally aggregation of these into LBs. While all three stages of aggregation may put a load on the system, we show that the interplay between fast degradation of protofilaments and a slower degradation of larger filaments in itself opens for an abnormal oscillation in proteasome availability. When the proteasome is occupied by the degradation of αSN fibrils, there is nothing to prevent the accumulation of oligomers with consequent deleterious effects for the cell function and viability.

Results

Our model is based on a feedback loop between the development of fibrils and proteasome response; see figure 1. Denoting F, C and P as the concentration of fibrils, proteasome-fibril complex ($C = [P : F]$) and free proteasome, respectively, the changes in their concentration levels over time t are described by

$$\frac{\mathrm{d}F}{\mathrm{d}t} = \frac{m}{1+P} - \gamma \cdot F \cdot P, \tag{1}$$

$$\frac{\mathrm{d}C}{\mathrm{d}t} = \gamma \cdot F \cdot P - \nu \cdot C, \tag{2}$$

$$\frac{\mathrm{d}P}{\mathrm{d}t} = \sigma - P - \gamma \cdot F \cdot P + \nu \cdot C. \tag{3}$$

The role of the parameters m, γ, ν and σ is indicated in figure 1, where m is the constant influx of protofilaments, γ is the reaction constant associated with the formation of the proteasome–fibril complex, $1/\nu$ is the degradation time for fibrils in the complex (a process during which proteasomes are recycled), and σ as described above is the production rate of proteasome proteins. Time is measured in units of proteasome lifetime (the second term in equation (3)), which is estimated to be around 8–15 days in the cell [61, 62]. The concentration is measured in terms of the concentration level at which the proteasome starts to inhibit fibril production (the first term in equation (1)).

The model constitutes the first attempt to encapsulate the interplay between the ongoing fibril formation and the counteracting proteasome, as outlined in the introduction section and in figure 1. It only encompasses the minimal set of essential reactions in such a feedback system, and thus leaves out parallel actions in particular lysosome activity or feedback of proteasome upon its own production rate. We accordingly analyze the model with emphasis on its robustness against variations in parameters as well as on the feedback concerning the adjustment of proteasome concentration to counteract the stress imposed by aggregating proteins.

The important outputs of the dynamics of equations (1)–(3) are the accumulation levels of the LBs, $\propto \int F \,\mathrm{d}t$ and oligomers $\propto \int (1/P)\,\mathrm{d}t$ (see the methods section for more details). The fraction of filaments that forms LB is unknown. However, since its contribution can be simulated by a smaller value of m, it does not need to be considered in detail. The prefactor associated with oligomer production depends on processes embedded in the αSN network (indicated by the gray area in figure (1)). For example, an A30P mutation in the αSN gene could affect the formation rate of dangerous oligomer seeds $\alpha\alpha\alpha$ [63], and consequently influence the oligomer accumulation. In any case, since this study is mainly focused on how fibrils occupy the proteasome needed for the removal of $\alpha\alpha\alpha$, we omit a detailed modeling of the αSN network here and conclude simply that oligomer accumulation is substantial when the proteasome concentration is low.

Apart from *in vitro* estimates of fibril nucleation time (minimum 12–24 h but often longer; see the introduction section), very little is known about the four key parameters γ, ν, σ and m entering equations (1)–(3). Therefore, in order

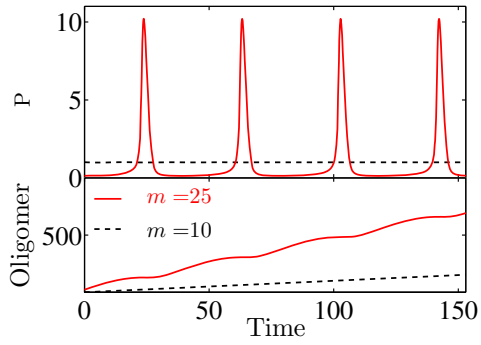

Figure 2. (Upper panel) Dynamics of free proteasome for $m = 10$ (black) and $m = 25$ (red), respectively, as predicted by equations (1)–(3). (Lower panel) Oligomer accumulation, here equals to $\int (1/P)\,dt$, for the two m values. Remaining parameters were set to $\nu = \gamma = \sigma = 1$.

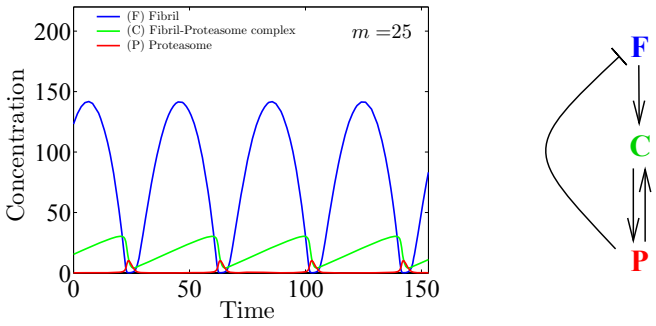

Figure 3. Dynamics of available proteasome (P), fibrils (F) and the complex (C) for $m = 25$ and $\nu = \gamma = \sigma = 1$ (i.e. same as in figure 2). The overall feedback is negative, as $C \to P$ mediate a repression of F, which, in turn, participate in production of C. In addition, $P + F \to C$ mediate a switch where increased F sequester P, unless there is enough P to repress and remove F. As a result, P and F are mutually exclusive with spikes in P associated with the depletion of F, whereas the slow build up of large F is associated with low P.

to trace the different dynamical behaviors of the model, we performed a numerical scan in which the parameters were varied over a wide range. Simulation of equations (1)–(3) was done numerically using MATLAB (ODE15s) or, equivalently, the fifth-order Runge–Kutta method. Overall, two different classes of feedback dynamics, both illustrated in figure 2, were found:

- *Healthy (dashed black lines).* The system is in steady state where the concentration of proteasome is kept at a constant level $P = \sigma$, allowing the proteasome to degrade fibrils and oligomers. Under steady-state conditions, P remains decoupled from F and may, therefore, work independently of the rate of fibril formation (m).
- *Sick (red lines).* The system performs spiky/pulsatile oscillations during which the concentration of P is very low for long periods of time between bursts of high levels. This opens for increased oligomer formation which may trigger PD.

The oscillatory behavior of F, C and P is depicted in figure 3, and is driven by a negative feedback loop where C indirectly removes F which, in turn, produce C (see supplement figure S3 at stacks.iop.org/PhysBio/6/036005). The negative feedback loop is associated with a time delay [48] during which C builds up until it reaches a level where it indirectly can remove F by producing enough P. The slow build up of C is rate limited by the production of P. The spiky oscillations [64] of P seen in figure 2 is associated with the mutual exclusion between free P and F by the process $F + P \to C$.

In the supplementary material (stacks.iop.org/PhysBio/6/036005), we derive conditions for the collapse of stable homeostasis and associated onset of oscillations. For example, if the complex formation is fast ($\gamma/\nu \gg 1$ in normalized units), and proteasome production is reasonable ($\sigma > 1$), the system oscillates when

$$m > m_T = (1 + \nu)(1 + \sigma)^2, \tag{4}$$

with a period that decreases with ν as seen in supplementary material figure S1 at stacks.iop.org/PhysBio/6/036005. In addition, the model predicts, interestingly, that the period may easily exceed proteasome decay time by a large factor (figures 4(*a*) and (*d*)). During most of this period, P is very low and the cell therefore is unable to prevent accumulation of oligomers. Both the long period and the possibility for a partial recovery during high P spikes may play a role for the long time it normally takes to develop PD.

An interesting feature of the feedback motif in figure 1 is that oscillations can be driven by increasing fibril formation m regardless of the values of the remaining parameters in the model. In other words, we can associate with m a threshold value, m_T, above which one is guaranteed to have oscillations. This contrasts with other oscillatory motifs, where one has to tune the parameters collectively in order to access the oscillatory regime, or assume a high level of cooperativity (high Hill coefficients) [65]. Our working hypothesis is therefore that the development and outbreak of PD is related to a change in the key parameters in the model in such a way that m_T becomes smaller than the protofilaments production rate m, triggering the disease.

The breakdown of stable homeostasis occurs abruptly when m exceeds m_T, leading to the development of spiky oscillations in proteasome concentration which, in turn, facilitates huge accumulation of both LBs and oligomers. Figure 4 shows the range of m and σ in which we predict the accumulation of LBs and oligomers, respectively. Outside this region, the concentration of $P = \sigma$ is independent of m, and the accumulation of oligomers is kept below some non-damaging level which is independent of eventual fluctuations in protofilament production. Note, however, that LBs are accumulated even in the steady-state region (at a rate proportional to m) which is consistent with the observation of LBs in patients not suffering from PD [14]. Thus, as long as the system is in steady-state conditions, there can be a substantial accumulation of LBs without any substantial oligomer production.

The model outlined in equations (1)–(3) describes a simplified set of core reactions involved in the proteasome–fibrillation 'battle'. In addition to this minimal model, we analyzed a number of cases in which additional processes were

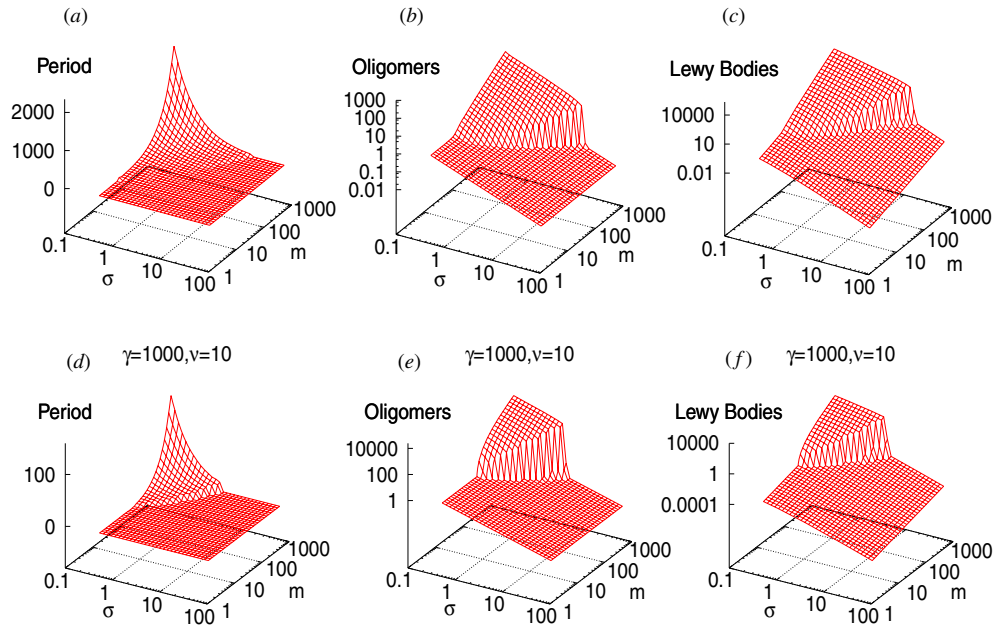

Figure 4. Panels (*a*), (*b*) and (*c*) show, respectively, the oscillation period, average accumulation of oligomers ($\propto \int (1/P)\,\mathrm{d}t$) and LBs ($\propto \int F\,\mathrm{d}t$), as a function of m and σ (for fixed $\nu = \gamma = 1$). Note the sharp onset in the formation of oligomers and LBs. The onset is approximately given by equation (4). Panels (*d*) and (*f*) illustrate the behavior of the model for higher rates for proteasome–fibril reactions, $\gamma = 1000$ and $\nu = 10$.

(This figure is in colour only in the electronic version)

added on. One example is given in the methods section where the oligomers also have the possibility of forming seeds for LB formation. In such a scenario, oligomers can act as on-pathway fibril precursors rather than being a strict off-pathway species. In short, this extended model has an additional source term for filament production ($\sim 1/(\epsilon + P)$, where ϵ is a small constant) added to the right-hand side of the rate equation governing the concentration of F (equation (1)). As the functional form of such an addition is similar to the standard production term, $m/(1 + P)$, its presence tends to destabilize homeostasis. This means that if oligomer production becomes high, and a sizeable amount of oligomers add to fibril formation, this could result in the onset of oscillation that may induce PD. This is an important observation which indicates that the role of the cytotoxic oligomer with regard to the fibrillation pathway (as yet unresolved) does not qualitatively affect the outcome. That is because this additional pathway for forming filaments, in fact, adds to the instability of the system.

We should also emphasize that the effect of mitochondrial dysfunction is implicit in our model. The mitochondrial dysfunction caused by, e.g., the disruption of the proton gradient by cytotoxic oligomers or other environmental insults will increase oxidative stress and thus enhance the production of oligomer precursors $\alpha\alpha\alpha$. In the model, this corresponds to a rescaling of the $1/P$ parameter associated with oligomer production. Unless oligomers can be converted to a sizeable amount of filaments (favoring oscillations, see above), this will not have an effect on the oscillations.

Another variation of the model that was considered was the possibility that the proteasome effectively autoregulates its own production. This kind of negative feedback is known from numerous shock-response systems [46, 47], and can be modeled by replacing equation (3) by

$$\frac{\mathrm{d}P}{\mathrm{d}t} = \frac{\sigma^*}{1 + P/K} - P - \gamma \cdot F \cdot P + \nu \cdot C, \qquad (5)$$

where, for example, $\sigma^* = 10\sigma$ and $K = \sigma/9$ correspond to our standard model supplemented with the proteasome production that can be up-regulated by a factor 10 from the normal steady state at $P = \sigma$. This model also generates 'spiky' oscillations for a wide range of K values, with oligomer accumulation for m values that are sufficiently large; see supplementary material at stacks.iop.org/PhysBio/6/036005. The negative feedback of the proteasome in equation (5) enables the cell to maintain an unchanged level of free P even if it is exposed to large additional stresses such as a rise in temperature or a change in pH. In the supplementary material at stacks.iop.org/PhysBio/6/036005, we show that a proteasome–fibrillation system with self-regulating P has similar stability against oscillations as the corresponding system described by equations (1)–(3). However, the self-regulation, indeed, opens for more production of P, and therefore both the oscillation period and the oligomer production rate are reduced by an amount proportional to the strength of self-regulation.

Interestingly, we find overall that the oscillatory feature of the system seems to be robust to most alterations of the core model we investigated. The only variation with a qualitative change is when proteasomes are degraded together with the complexes: when a degrading complex destroys the involved proteasome with a probability that exceeds σ/m, then the proteasome collapses completely without any oscillations. When the degradation of proteasomes in the complexes is less than this threshold, oscillations prevail with a period that

increases as the threshold is approached. If we include the self-regulation of proteasome, as in equation (5), the system will sustain oscillations for substantially larger loss of proteasome during fibril degradation. In any case, when we pass the threshold set by m_T, the proteasome collapses and oligomers start to accumulate.

As a final variation, we have considered the possibility of a relatively large turnover of filaments to LB (a rate which is neglected in the standard model above). As the filament to LB formation is increased to dominate the rate of complex C formation, the stress on the proteasome is weakened and the threshold m_T for oscillations/break down of proteasome is increased. A similar stabilizing effect would occur if some of the filaments are degraded in parallel by lysosomes, because the remaining filaments then would provide a smaller drain on the proteasome. Nevertheless, in both cases it is important to note that the phenomenon of proteasomal oscillations will still prevail under a broad range of physiological conditions.

Conclusions and outlook

In this paper, we have modeled the onset and progression of the Parkinson disease as the interplay between the production of fibrils and the response of the cell in attempting to remove these filaments. Despite the simplifications of the model, there is a clear and testable outcome: the model predicts that the interplay of fast and slow degradation of filaments along the aggregation pathway easily induces huge oscillations in the availability of the proteasome in the cell.

The onset of oscillation is associated with a proteasome which fails to maintain filaments production at a low steady level. Periods of ultra-low free proteasome concentrations make the system hyper-sensitive to the accumulation of toxic oligomers. We, therefore, suggest that the onset of oscillations is associated with the development of PD. Our model easily predicts an oscillation period that is substantially longer than the characteristic proteasome half life, estimated to be 8–15 days [61, 62]. If the accumulation of dangerous oligomers could involve several oscillations (e.g., due to the large number of mitochondria in the cell), the model would suggest why the progression of the disease could take decades despite typical nucleation times of filaments on the order of a day.

Under all conditions, our model predicts that oscillations start when the ratio of proteasome to protofilament production is reduced. *In vivo*, such a change could be related to

(1) Increased formation rate of protofilaments and oligomers by duplication mutation of α-synuclein gene. In the model, this corresponds to increasing protofilament rate m, and, in addition, increasing the basal oligomer formation rate proportionally. As demonstrated in figure 4, such a mutation increases the propensity to enter the regime of pathological oscillations.

(2) Increased formation rate of protofilaments (m) due to mutations such as A53T. This essentially works as the gene duplication described just above. In contrast, the A30P mutation increases the rate of oligomer formation [20] but slows down the filament formation, and we would therefore predict that its ability to form Parkinson relies on a significantly increased rate of oligomers formation that is sufficiently large to partly survive the action of an intact proteasome.

(3) Direct reduction in proteasome production as cells age (reduction in σ). It is generally recognized that protein turnover slows down in aging cells, leading to the accumulation of oxidized cells [66–68]. This is probably caused by the decreased production or post-translational modification of proteasome subunits [69, 70].

(4) Proteasomes could be titrated out by LBs as they tend to co-localize with them, which in effect should be modeled by introducing a increased degradation rate of P. This is not included in our equations (1)–(3), but its effect would be simulated by a decreased P degradation, e.g. replacing $-P \rightarrow -P/\tau$ where $\tau < 1$. This would decrease the concentration of P with the increasing amount of LB. Our model predicts that the presence/absence of oscillations, as well as the length of their period, is fairly independent of τ (see supplementary material figure S2 at stacks.iop.org/PhysBio/6/036005).

(5) Proteasomes could be partly occupied by other misfolded proteins in the cell, implying that only a fraction of F is available for fibril degradation. Such an 'external' stress indeed perturbs proteasome availability transiently. After the transient, the proteasome concentration adjusts to the new stress level, and both proteasome oscillations and oligomer accumulation behave as if there was no external stress (assuming that the degradation of misfolded proteins is relatively fast, see supplementary material at stacks.iop.org/PhysBio/6/036005).

(6) The predicted spikes in the proteasome concentration caused by their release from the C complexes might also have cellular consequences. When proteasome or other related components of the protein repair machinery reaches concentrations which can be order of magnitudes larger than their normal steady-state levels, other potentially essential proteins may be destroyed, and the cell may die during the spikes in P. If only some cells die during each spike, this way of interpreting our model may suggest an entirely new way of understanding the disease. At the same time, this would reconcile the slow development of the disease with the short timescale associated with production and decay of the involved protein complexes.

The insecticide neurotoxicant MPTP can induce PD without leading to the visible accumulation of LBs [71–73]. This merits a few comments with regard to our model. The most likely way for MPTP to induce PD would be to increase the flux on the path leading to oligomers, e.g. by increasing the concentration of reactive oxygen species through mitochondrial dysfunction. If oligomers can act as substantial seeds for protofilaments these could indeed induce oscillations. If oligomers do not act as seeds for filaments, then MPTP should not influence filament production and proteasome availability. Under these circumstances, the disease should develop without any oscillations, simply because the proto-oligomer formation rate in itself becomes so large that even a small fraction that survives the proteasome

may be lethal. Note also that even if MPTP, in addition, increases the load on the proteasome by increasing misfolded proteins in general, then this should not lower the threshold for the onset of PD (see point 4 above).

Oxidative stress is also expected to increase the amount of misfolded proteins and consequently further increases the load on the UPS system. Even in the absence of proteasome self-autoregulation such an increased load will only have a transient effect on the concentration of free P. Our model only allows oxidative stress to induce oscillations, if it effectively increases the fibril formation rate. This could be done directly (through m) or through conversion of oligomers to fibrils, as discussed above.

The fact that a simple gene duplication of αSN can induce early onset of Parkinson [74] indicates that the wild-type system is pruned to operate fairly close to disease onset. Accordingly, the model suggests a number of follow-up experiments. First of all, an investigation on the possibility of inducing proteasomal oscillation in single cells by over-expressing αSN. Second, it would be useful to measure as many of model's parameters as possible in a living cell or appropriate cellular models of PD, and thereby determine how close a typical *in vivo* system is to initiate oscillations. Proteasome activity can itself lead to cyclical variations in the concentration of other proteins [75], and oscillations in proteasomal activity are linked to different stages in the cell division cycle [76]. In addition, some proteasome fluctuations in fibroblast cells have been reported as a consequence of aging [77]. Nevertheless, to the best of our knowledge proteasome oscillations have as yet not been studied in healthy non-dividing neuronal cells.

The sensitivity of the UPS system to many other aggregating proteins [31] suggests that these oscillations may be a general feature of aggregation-driven neurodegenerative diseases. In fact, the pathology associated with the onset of oscillations as described by equations (1)–(3) is so robust that it may be relevant to degenerative diseases such as Alzheimer's, Huntingtons disease, ALS or even multiple sclerosis. Our mechanism for oscillations only requires the competition between a fast and a slow inhibition of a proteasome or other chaperone system along a path of ongoing aggregate formation. Perhaps, some of these degenerative diseases are associated with oscillations generated by a failing proteasome or a particular overused chaperone as this becomes occupied with repair processes (point 5 above).

Whereas the clinical symptoms for PD increases steadily over time, multiple sclerosis is known to progress in attacks. Such speculations suggest yet another way that spiky oscillations in P could be lethal (point 5). That is, instead of the depletion in free P, it could be the short duration of a hugely over-active proteasome that kills the cells by eliminating some essential but unstable protein. For example, up-regulation of proteasome activity in cells infected with Heres Simplex virus leads to the degradation of host RNA polymerase, thus effectively preventing the cell from mounting an interferon-based defense [78].

Acknowledgments

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Appendix

The model outlined in equations (1)–(3) is a minimal version of a more complex set of equations which describe both the details of the protofilament degradation, and the paths leading to the formation of non-fibrillous oligomers through direct nucleation processes or internal modifications of the αSN itself. The more complete set of equations for the proteosome repression of fibril formation, to which the minimal model is a special case, is given by

$$\frac{ds}{dt} = m - \gamma_s \cdot s \cdot P - \omega \cdot s, \tag{A.1}$$

$$\frac{d(sP)}{dt} = \gamma_s \cdot s \cdot P - \nu_s \cdot (sP), \tag{A.2}$$

$$\frac{dF}{dt} = \omega \cdot s - \gamma \cdot F \cdot P, \tag{A.3}$$

$$\frac{dC}{dt} = \gamma \cdot F \cdot P - \nu \cdot C, \tag{A.4}$$

$$\frac{dP}{dt} = \Sigma - \frac{P}{\tau} - \gamma \cdot F \cdot P + \nu \cdot C - \gamma_s \cdot s \cdot P + \nu_s \cdot (sP), \tag{A.5}$$

where s and (sP) denote the concentration of pre-filament and sP-complex, respectively.

Under certain conditions, equations (A.1)–(A.3) may be compressed into a single rate law for F (equation (1)). First, if it is assumed that the (sP)-complex is short lived, i.e. when γ_s and ν_s are large, equation (A.2) gives $(sP) = (\gamma_s/\nu_s)s \cdot P$. Second, if the pre-filaments s adjust themselves rapidly to the conditions specified by P, one obtains from equation (A.1) $s = m/(\gamma_s P + \omega)$. Inserting these relations into equation (A.3) leads to $dF/dt = m/(1 + (\gamma_s/\omega)P) - \gamma \cdot F \cdot P$. Moreover, if the concentrations are measured in units of ω/γ_s, and time in units of τ, equations (1)–(3) are recovered after proper rescaling of the remaining rate equations (A.4) and (A.5), where $\sigma = (\tau\gamma_s/\omega)\Sigma$.

In addition, the αSN may have the possibility of forming oligomers directly. The paths from the unfolded monomeric αSN to the oligomer include a traditional nucleation path involving several identical monomers $(\alpha\alpha\alpha)$ (figure 1). This is an inefficient process which under optimal *in vitro* conditions at best can generate few extra percent of oligomer content [13]. In addition, αSN may be modified chemically by oxidative stress and high concentration of dopamine [20], which lowers the threshold for oligomer formation and increases the rate of its formation. Our treatment of oligomer formation is based on the creation of a critical nucleus $(\alpha\alpha\alpha)$ which can either nucleate an oligomer or decay by the proteasome or something else:

$$\alpha SN \underset{\leftarrow}{\rightarrow} (\alpha\alpha\alpha)$$
$$(\alpha\alpha\alpha) + P \rightarrow P \quad \text{(A.6)}$$
$$(\alpha\alpha\alpha) \rightarrow \emptyset$$

$$(\alpha\alpha\alpha) \rightarrow \text{olig} \quad \text{(A.7)}$$

leading to

$$\frac{\mathrm{d}(\alpha\alpha\alpha)}{\mathrm{d}t} = k_{\rightarrow}[\alpha SN] - k_{\leftarrow}[(\alpha\alpha\alpha)] - \upsilon P \cdot [(\alpha\alpha\alpha)], \quad \text{(A.8)}$$

where $k_{\leftarrow}$ includes all non-proteasomal decay processes of $\alpha\alpha\alpha$ plus the rate at which $\alpha\alpha\alpha$ form oligomers. In the steady state, the proto-oligomer concentration is

$$[(\alpha\alpha\alpha)] = \frac{k_{\rightarrow}[\alpha SN]}{k_{\leftarrow} + \upsilon P}, \quad \text{(A.9)}$$

giving an oligomer formation rate

$$\frac{\mathrm{d}[\text{olig}]}{\mathrm{d}t} = \frac{S}{\epsilon + P}, \quad \text{(A.10)}$$

where $\varepsilon = k_{\leftarrow}/\upsilon$ is small when the proteasome contribution is dominating the decay of proto-oligomers. The constant S parameterizes the total source terms for oligomers, $S \propto k_{\rightarrow}[\alpha SN]/\upsilon$. To avoid discussing the upper limit of oligomer formation above which apoptosis may occur (effect of $k_{\leftarrow}$) we estimate oligomer accumulation [olig] as proportional to $1/P$, throughout the paper.

In the model, we assumed that oligomers represent a small population that it in itself does not perturb the proteasome substantially. However, we have also analyzed the effect of a substantial conversion of oligomers to filaments, adding the process,

$$\text{olig} \rightarrow F, \quad \text{(A.11)}$$

to the processes in equation (A.6). To allow the accumulation of oligomers we allow a sub-part of oligomers to aggregate in a form that cannot form fibrils, and write the dynamics in terms of the remaining free oligomers [olig]. The corresponding model is still given by equations (1)–(3), where equation (1) is replaced by the additional source from the free oligomers:

$$\frac{\mathrm{d}[\text{olig}]}{\mathrm{d}t} = \frac{S}{\varepsilon + P} - d_1 \cdot [\text{olig}] \quad \text{(A.12)}$$

$$\frac{\mathrm{d}F}{\mathrm{d}t} = \frac{m}{1 + P} + d_2 \cdot [\text{olig}] - \gamma \cdot F \cdot P, \quad \text{(A.13)}$$

where $d_1 \geqslant d_2$ and $d_1 - d_2$ quantify oligomers that accumulate in places where they cannot form fibrils. As the functional forms of the production terms for oligomers and filaments are similar, the olig $\rightarrow$ F conversion tends to destabilize the homeostasis even further. Formally this comes about because S adds directly to m, and oscillations may start when the sum of these two sources exceeds the threshold m_T (see equation (4)).

Glossary

Proteasome. The main function of the proteasome is to degrade unneeded or damaged proteins.

Lewy bodies. Large aggregates of α-synuclein that may accumulate in certain nerve cells in the brain.

Parkinson disease. A disease associated with the aggregation of α-synuclein into Lewy bodies.

Negative feedback. Coupled regulatory processes that tend to maintain homeostasis.

Spiky oscillations. Oscillations which are characterized by long periods with nearly vanishing signal and short periods with high activity.

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