Title
Conformation and dynamics of the kinase domain drive subcellular location and activation of LRRK2

Permalink
https://escholarship.org/uc/item/6p34v30d

Journal
Proceedings of the National Academy of Sciences of the United States of America, 118(23)

ISSN
0027-8424

Authors
Schmidt, Sven H
Weng, Jui-Hung
Aoto, Phillip C
et al.

Publication Date
2021-06-08

DOI
10.1073/pnas.2100844118

Peer reviewed
Conformation and dynamics of the kinase domain drive subcellular location and activation of LRRK2

Sven H. Schmidt,1,2,3,4,5,6,7 Ju-Hung Weng,1,7,8,9,10 Phillip C. Aoto,2,11 Daniela Boassa,6,12,13 Sebastian Mathe,14 Steve Silletti,15 Junru Hu,16,17 Maximilian Wallbott,18 Elizabeth A. Komives,12,19,20 Stefan Knapp,21,22,23,24 Friedrich W. Herberg,25,26,27,28,29,30,31 and Susan S. Taylor32,33,34,35

To explore how pathogenic mutations of the multidomain leucine-rich repeat kinase 2 (LRRK2) hijack its finely tuned activation process and drive Parkinson’s disease (PD), we used a multitiered approach. Most mutations mimic Rab-mediated activation by “unleashing” kinase activity, and many, like the kinase inhibitor MU-2, trap LRRK2 onto microtubules. Here we mimic activation by simply deleting the inhibitory N-terminal domains and then characterize conformational changes induced by MLi-2 and PD mutations. After confirming that LRRK2RCKW retains full kinase activity, we used hydrogen-deuterium exchange mass spectrometry to capture breathing dynamics in the presence and absence of MLi-2. Solvent-accessible regions throughout the entire protein are reduced by MLi-2 binding. With molecular dynamics simulations, we created a dynamic portrait of LRRK2RCKW and demonstrate the consequences of kinase domain mutations. Although all domains contribute to regulating kinase activity, the kinase domain, driven by the DYGΨ motif, is the allosteric hub that drives LRRK2 regulation.

Significance

To achieve a mechanistic understanding of LRRK2, a multidomain protein kinase, we must understand how the conformational landscape is changed by specific mutations that cause LRRK2 to become a driver of Parkinson’s disease (PD). To meet this challenge, we used a construct, LRRK2RCKW, that lacks the N-terminal inhibitory domains. Both catalytic domains as well as full activity are retained in LRRK2RCKW. To capture solvent-exposed/protected regions, we used hydrogen-deuterium exchange mass spectrometry and showed in detail how the conformation changed in the presence of a kinase inhibitor, MLi-2. Using molecular dynamics simulations, we explored the effects of MLi-2 as well as PD mutations on dynamics. Our multitiered analysis defines the kinase domain as a dynamic allosteric hub for LRRK2 activation.

Author contributions: S.H.S., J.-H.W., P.C.A., D.B., S.M., S.S., J.H., F.W.H., and S.S.T. designed research; S.H.S., J.-H.W., P.C.A., D.B., S.M., S.S., and J.H. performed research; S.H.S., J.-H.W., P.C.A., D.B., S.S., J.H., M.W., E.A.K., S.K., and F.W.H. analyzed data; and S.H.S., J.-H.W., P.C.A., and S.S.T. wrote the paper.

Reviewers: M.E., Dana-Farber/Harvard Cancer Institute; C.J.G., German Center for Neurodegenerative Diseases; and E.G., University of Padova. The authors declare no competing interest.

This open access article is distributed under Creative Commons Attribution License 4.0 (CC BY).

Published June 4, 2021.
moieties and is the smallest fragment that is capable of docking onto MTs (5). In contrast to full-length LRRK2 and the G2019S mutant, the corresponding LRRK2<sub>RCKW</sub> constructs formed filaments spontaneously in the absence of MLi-2, a high-affinity kinase inhibitor. To confirm that the catalytic machinery is conserved in LRRK2<sub>RCKW</sub>, we characterized the kinase activity of WT and mutant LRRK2<sub>RCKW</sub> proteins using LRRKtide and Rab8a as substrates. We focused, in particular, on the DYGI motif in the kinase domain which is a hotspot for PD mutations and a critical part of the switch mechanism that leads to LRRK2 activation (10).

Using hydrogen-deuterium exchange mass spectrometry (HDX-MS) to map the conformational state of LRRK2<sub>RCKW</sub>, we obtained a comprehensive profile of the solvent-accessible and protected regions confirming large multidomain protein is well-folded. We next mapped changes in the solvent exposure of the LRRK2<sub>RCKW</sub> domains following binding of MLi-2. A detailed analysis of these changes mapped onto a model of the active kinase domain provided a comprehensive allosteric portrait of the kinase domain as a hub for driving long-range conformational changes. At a final level, to create a dynamic portrait of LRRK2<sub>RCKW</sub>, we used Gaussian accelerated molecular dynamics (GaMD) simulations to observe in silico at an atomistic level how single-amino acid mutations in the kinase domain contribute to the intrinsic dynamic regulation of LRRK2.

Our multiscale approach allowed us to achieve a deeper appreciation of the intrinsic molecular features of LRRK2 and emphasized the crucial role of the DYGI motif in regulating LRRK2 structure and function. We hypothesized that the NTDs of LRRK2 (ARM, ANK, and LRR) play an inhibitory regulatory role acting as a “lid,” which is “unleashed” physiologically by activated Rab proteins but also by some of the PD mutations. Experimentally, the NTDs can be unleashed from the CTDs by kinase inhibitors or by simply deleting the NTDs, leaving the CTDs catalytically intact. Strikingly, our resulting LRRK2 model for activation and subcellular localization closely resembles the activation process of Raf kinases, which further supports our model and the central concept of kinases serving as the hub for driving conformational switching in multidomain signaling proteins.

**Results**

To characterize and dissect the functional properties of the catalytic domains of LRRK2, in particular the kinase domain, we used a multiscale approach that extends from testing real-time filament formation in live cells to assessing the consequences of MD simulations of PD mutations in the kinase domain. Of primary importance initially was to characterize the biochemical properties of LRRK2<sub>RCKW</sub> following deletion of the catalytically inert NTDs. To next confirm that LRRK2<sub>RCKW</sub> was a well-folded protein, we used HDX-MS. To capture the allosteric features of the kinase domain, we mapped by HDX-MS the conformational changes in LRRK2<sub>RCKW</sub> that result from adding MLi-2. Finally, we explored single-site mutants in silico using MD simulations.

**Capturing Filament Formation in Real Time.** We and others previously showed that treatment with a highly specific LRRK2 inhibitor (MLi-2) induces filament formation of WT LRRK2 and the G2019S mutant when these proteins are transiently expressed in mammalian cells (7, 10). To capture the dynamics of such redistribution, we performed time-lapse imaging of yellow fluorescent protein (YFP)-tagged G2019S and WT LRRK2 (G2019S: Movie S1, WT: Movie S3). As shown in Fig. 2 and Movies S1, S2, S3, and S4, under normal conditions G2019S LRRK2 is mostly diffuse in the cytosol; however, 15 to 30 min following MLi-2 treatment the protein begins to concentrate first in “satellite” structures diffuse throughout the cells. It then polymerizes to form intricate filaments by 2.0 to 2.5 h after treatment. Although WT LRRK2 follows a similar redistribution upon treatment with MLi-2, in general it takes longer, ∼30 min to 1 h, before the first structures are observed (Movies S1, S2, S3, and S4). In both cases this effect is readily reversible: After washout of MLi-2 for 2 h, the proteins gradually diffuse back into the cytosol (G2019S: Movie S2, WT: Movie S4). To verify that this protein rearrangement was truly dependent on the specific MLi-2 inhibitor, we performed time-lapse imaging using a type 2 inhibitor, rebastinib (Fig. 2A). Although rebastinib tightly binds to the LRRK2 kinase domain, as established from the stabilization of a kinase–WD40 construct in a thermal shift assay (SI Appendix, Fig. S1), it did not induce changes in the localization of G2019S proteins even after 8-h treatment, confirming the prediction of Deniston et al. (5).

**LRRK2<sub>RCKW</sub> Variants Spontaneously Form Filaments around Microtubules in an MLi-2–Independent Manner.** In our filament formation assay, Flag-tagged variants of the LRRK2<sub>RCKW</sub> construct were overexpressed and cells were analyzed after fixation via antibody staining or by confocal laser-scanning microscope. The majority of the transfected cells, regardless of the mutation, displayed constitutive filamentation (Fig. S1), it did not induce changes in the localization of G2019S proteins even after 8-h treatment, confirming the prediction of Deniston et al. (5).

Fig. 1. Schematic domain organization of LRRK2. Full-length protein (blue box) consists of the Armadillo domain (Arm), Ankyrin repeat (Ank), leucine-rich repeat, a GTPase domain called Ras of complex, C terminus of the Roc domain, kinase domain, and WD40 domain. The N-terminal domains contain the Arm, Ank, and LRR domains. The C-terminal domains, corresponding to the LRRK2<sub>RCKW</sub> construct (red box), contain the ROC, COR, kinase, and WD40 domains.

LRRK2<sub>RCKW</sub> full length

| Domain | Start | End |
|--------|-------|-----|
| Arm    | 156   | 510 |
| Ank    | 690   | 860 |
| LRR    | 985   | 1274|
| ROC    | 1335  | 1510|
| COR    | 1879  | 2138|
| Kinase | 2142  | 2498|
| WD40   | 2527  | 2527|

LRRK2<sub>RCKW</sub> (aa 1327–2527)
heterologous proteins as well as inhibition of the catalytic domains. We hypothesize that most of the PD mutations use different mechanisms to circumvent or “hijack” this normal process.

Of the mutants tested, only LRRK2_RCKW D2017A, a kinase-dead mutant, showed strongly reduced docking onto MTs, which is consistent with our earlier findings showing that the full-length D2017A mutant did not dock onto MTs even in the presence of MLi-2 (SI Appendix, Fig. S2). We confirmed here that MLi-2 did not have an additive effect on the percentage of cells showing LRRK2_RCKW filaments and did not induce binding of the D2017A mutant (SI Appendix, Fig. S2). We conclude that the high-affinity binding of MLi-2 to the kinase domain is sufficient to unleash the N-terminal protective lid that normally shields the catalytic domains and promotes localization in the cytosol. We also show that

**Fig. 2.** Localization of the LRRK2-G2019S mutant and LRRK2_RCKW variants. (A) Time-lapse imaging of HEK293T cells transiently expressing YFP-LRRK2-G2019S: Confocal images (YFP fluorescence signal, maximum-intensity projections) were acquired every 11 min. Representative images show the typical diffuse cellular localization of the proteins (t = 0 h) prior to treatment with 100 nM MLi-2; following MLi-2 addition, proteins relocalize to form cytoplasmic filamentous structures (yellow arrows; +MLi-2, t = 2.5 h). After washout of the inhibitor, the proteins gradually dissociate from the filaments into the cytosol (washout; t = 2 to 3 h). (B) Time-lapse imaging of HEK293T cells transiently expressing YFP-LRRK2-G2019S before (t = 0 h) and after treatment with 100 nM rebastinib. No changes in the localization of the proteins are observed. (Scale bar, 20 μm.) (C) Plasmids, encoding LRRK2_RCKW variants, were transfected into HEK293T cells for LRRK2_RCKW overexpression. Transfected cells were then analyzed for the spatial distribution of LRRK2_RCKW by immunostaining. All tested LRRK2_RCKW variants displayed a high likelihood (80 to 90%) of forming filaments inside the HEK293T cells except for LRRK2_RCKW D2017A (20 to 30%). Interestingly, in contrast to full-length LRRK2, the percentage of cells showing filament formation was independent of MLi-2 treatment or a specific LRRK2_RCKW mutation. The figures show the filaments of WT and G2019S. The figures of other LRRK2_RCKW variants are shown in SI Appendix, Fig. S2. Error bars represent standard deviations (SD) for the percentage of filament forming cells on six to ten representative images taken per transfection (n = 2).
simply removing the N-terminal lid is in most cases sufficient to promote docking onto MTs. The exception is the D2017A mutant, which cannot bind well under any conditions either because it lacks the ability to undergo a subsequent essential autophosphorylation step or, most likely, because it is locked into an open conformation similar to what we saw with rebastinib. We next asked whether LRRK2<sub>RCKW</sub> retained its full kinase catalytic activity even though the regulatory machinery embedded in the N-terminal domain is removed.

**Protein Kinase Activity Is Conserved in the LRRK2<sub>RCKW</sub> Proteins.** To assess kinase activity, we used both LRRKtide, a small synthetic peptide, and Rab8a as substrates for the LRRK2<sub>RCKW</sub> proteins. In addition to WT LRRK2<sub>RCKW</sub>, we measured the kinase activities of two ROC–COR domain mutations (R1441C and Y1699C) and four mutations in the kinase domain, more precisely in the DYG<sub>ψ</sub> motif (D2017A, Y2018F, G2019S, and I2020T). R1441 and Y1699 are located in the ROC and COR domains, respectively, and, based on homology models, are predicted to be part of the ROC-COR domain interface (5, 17, 18). Importantly, we found that WT LRRK2<sub>RCKW</sub> has kinase activity that is comparable to full-length LRRK2 (10) although in the absence of the N-terminal scaffolding domains the activity is no longer dependent on Rab activation. Using LRRKtide as a substrate, we found that the pathogenic mutation R1441C slightly increased the kinase activity while Y1699C had only a minor effect on LRRKtide phosphorylation (Fig. 3). In contrast, when we used a physiological substrate, Rab8a, Y1699C led to an enhanced pT72 phosphorylation in vitro, comparable to the phosphorylation by Y2018F, whereas R1441C behaved like WT (Fig. 3). The fact that kinase activity is dependent on substrate may account for some of the confusion in the literature about the activity of various LRRK2 mutants and suggests that some of the mutations may simply change substrate specificity. If these residues are indeed at a domain interface, as predicted, they could also introduce a conformational change that would result in the unleashing of the N-terminal scaffolding domains and/or promote dimerization which is associated with membrane localization and activation of LRRK2 (14, 19).

The strongest effects on kinase activity for LRRK2<sub>RCKW</sub> were observed for mutations embedded within the activation segment of the kinase domain, specifically in the DYG<sub>ψ</sub> motif, where ψ is typically conserved as a hydrophobic residue. The Tyr is a Phe in most other kinases, and Y2018 was predicted earlier, based on activation when the Tyr is replaced with Phe, to serve as a brake that keeps LRRK2 in an inactive state (10). We measured the effect of mutating each of these residues on kinase activity. The D2017A (DYG) mutant was not able to phosphorylate either LRRKtide or Rab8a (Fig. 3) which is consistent with other kinases, since this residue is part of the regulatory triad and is crucial for the correct coordination of the Mg<sup>2+</sup> ions and the -phosphate of adenosine triphosphate (ATP) in the kinase active-site cleft (20). Reintroducing the classical DFG<sub>ψ</sub> motif to LRRK2<sub>RCKW</sub> increases the kinase activity for LRRKtide by a factor of 3 to 4, whereas Rab8a phosphorylation was only enhanced by a factor of 1.7 (Fig. 3). LRRKtide phosphorylation by LRRK2<sub>RCKW</sub> G2019S was comparable to LRRK2<sub>RCKW</sub> Y2018F. When comparing Rab8a pT72 phosphorylation, G2019S showed two times higher Rab8a pT72 phosphorylation than Y2018F. The other tested pathogenic DYG<sub>ψ</sub> mutation, I2020T, displayed a reduced phosphorylation of LRRKtide as well as Rab8a (Fig. 3). This is also in accordance with our full-length LRRK2 data of I2020T, albeit full-length I2020T Rab8a phosphorylation was comparable to WT. The results for the I2020T mutation in full-length LRRK2 and LRRK2<sub>RCKW</sub> demonstrate that LRRK2 pathogenicity is not driven solely by increased kinase activity but also by changed substrate preferences such as serine/threonine specificity as well as changes in subcellular localization. We later show that the dynamic properties are also altered by these mutations.

**Mapping the Conformational Changes Induced by MLi-2 Using Hydrogen-Deuterium Exchange Mass Spectrometry.** To define the global conformational changes induced in LRRK2<sub>RCKW</sub> as a consequence of MLi-2 binding, we used HDX-MS, which allows us to determine the solvent-exposed regions of the protein over a time course of 5 min. Although this is a large protein (1,200 residues), we obtained excellent coverage (>96%), and the solvent-exposed regions are consistent with the predicted folding of all four domains (SI Appendix, Fig. S3). While we focus here primarily on the kinase domain, the graph summarizing the overall solvent accessibility of the entire protein shows not only that the four domains are well-folded but also identifies several regions that are highly solvent-exposed. Of particular note is the activation loop of the kinase domain as well as the segment that lies between the COR-B domain and the kinase domain and the segment that joins the GTPase domain to

![Fig. 3. LRRK2<sub>RCKW</sub> variants Y2018F and G2019S enhance LRRKtide and Rab8a phosphorylation. (A) An LRRKtide-based kinase assay for LRRK2<sub>RCKW</sub> variants revealed that it preserves full-length LRRK2 kinase activity. Additionally, the DYG<sub>ψ</sub> mutants tested here also resemble the results of their full-length counterparts. Interestingly, also the pathogenic mutations R1441C and Y1699C which are situated in the ROC-COR region of the LRRK2<sub>RCKW</sub> construct display a mild increase in kinase activity compared with LRRK2<sub>RCKW</sub> WT. Asterisks indicate the P value by one-way ANOVA test: 0.01 < * < 0.05; 0.001 < ** < 0.01; **** < 0.0001. Error bars represent SD for at least five independent measurements. (B) When testing Rab8a as a substrate for the LRRK2<sub>RCKW</sub> construct employing Western blotting against pT72 and the His tag of His-Rab8a, we revealed increased phosphorylation of Rab8a by LRRK2<sub>RCKW</sub> Y2018F, G2019S, and Y1699C. MLi-2 was shown to efficiently block phosphorylation of Rab8a which was also found for the kinase-dead mutant D2017A. Quantification was performed for three independent Western blots. For each quantification, the pT72 signals were referenced to the signal for the His tag of 6xHis-Rab8a and then normalized to the resulting WT signal. The dotted line therefore represents 100% of the WT signal. Error bars represent SD of the quantification of three independent Western blots.](https://doi.org/10.1073/pnas.2100844118)
the COR-A domain. The HDX-MS data suggest that these regions having high deuterium uptake are highly flexible or unfolded. Conversely, there are also regions on the surface of each domain that are highly protected from solvent, implying that these are domain–domain interfacial surfaces (SI Appendix, Fig. S3). It is important to appreciate that the HDX-MS profile is obtained independent of a solved structure and can thus serve as validation of a predicted model. Overall, LRRK2RCKW is a well-folded protein that is consistent with a complex topological model with interdomain interactions.

**Kinase domain.** Under apo conditions the N lobe of the kinase domain is more shielded from solvent than the C lobe (Fig. 4A). The αC-β4-loop, for example, is almost completely shielded from solvent. This is somewhat unusual in that the N lobe in the absence of nucleotide tends to be rather dynamic for many protein kinases. The ordered and stable structure of the N lobe of the kinase domain is predicted to be due to constraints imposed by the flanking domains. This is analogous to the way that cyclin binding orders the N lobe of CDK2 in contrast to the isolated kinase domain (21). Most kinase structures represent just an isolated kinase domain, so one cannot appreciate how other domains contribute to stabilization and, in turn, regulation of the N lobe. Our HDX-MS results also help to explain why it has not been possible so far to express the kinase domain independent of the rest of the LRRK2RCKW domains. For example, deletion of the ROC domain or deletion of even a few residues at the C terminus abolish the kinase activity (6).

In the apo protein the activation loop of the kinase domain in the C lobe has the highest deuterium uptake, suggesting it is highly disordered and exposed to solvent. In contrast to the activation loop, the helices of the C lobe for the most part are highly protected (Fig. 4B). The αE-helix, for example, is completely shielded from solvent, as is the middle of the αD-helix and the C terminus of the αH-helix. β7 is packed against a portion of the αC-β4-loop, and these two peptides that include the Mg-positioning loop and the last turn of the αC-helix are also completely shielded from solvent. These shielded regions nicely define the hydrophobic core of the kinase domain (Fig. 4B).

**Effect of inhibitor binding.** To gain insight into the allosteric impact of inhibitor binding, we next looked at the conformational changes in LRRK2RCKW following treatment with MLi-2. The overall changes, captured in the graph in Fig. 5A, show that there is subtle, albeit important, protection in regions that extend into the GTPase and COR-A–COR-B domains; however, the largest changes are concentrated in the kinase domain and in the linker that precedes the kinase domain. We focus here on the conformational changes that are localized to our kinase domain model. These changes lie not only in the N lobe and the active-site cleft but also in the C lobe in regions that lie far from the active-site cleft.

Among the protected regions, we saw that the binding of MLi-2 reduces the H-D exchange in the ATP-binding site, activation loop, αC-helix, and hinge region (Fig. 5B). These regions that would be predicted to contact the inhibitor (22) all show significantly reduced deuterium uptake. Peptides, for example, in the hinge region (amino acids 1948 to 1958), including the αD-helix, experienced a large increase in protection upon MLi-2 binding (50 vs. 20%). The peptide covering the catalytic loop (amino acids 2013 to 2022) including the YRD motif also experienced protection (30 to <10%), and the glycine-rich loop (amino acids 1884 to 1893) is also highly protected. Most importantly, we see that the peptide containing the DYGI motif (amino acids 1990 to 2002) is almost completely shielded as a consequence of MLi-2 binding: the deuterium exchange dropped from 70 to less than 10%, suggesting that this region, highly solvent-accessible in the absence of ligand, becomes almost completely protected by the coordination of the inhibitor. This is quite consistent with the prediction that the kinase domain assumes a compact and closed conformation in the presence of MLi-2. The C terminus of this peptide contains the beginning of the activation loop, which now also appears to be well-folded and shielded from solvent in contrast to the apo structure.

We looked more closely at the dynamic features of some of the critical peptides in the C lobe (Fig. 6). Of particular interest are the uptake spectra of the two peptides covering the activation loop: Both show an EX1 bimodal distribution, a feature that is indicative of two different conformations in solution (23). One of these peptides (amino acids 2028 to 2056) is shown in Figs. S5 and 5B. All other peptides, such as the DYGI peptide shown in Fig. 6A, show a single peak indicative of the more typical EX2 exchange kinetics. In addition, MLi-2 treatment also induced slow exchange in the DYGI loop even though it is highly protected. The peptide that covers the N terminus of the αC-helix (amino acids 1915 to 1921) shows significant slow exchange even in the absence of MLi-2 that most likely continues beyond 5 min (Fig. 6C). Although the exchange is quenched in the presence of MLi-2, the slow exchange still persists.

Although the protection of the ATP-binding site and the hinge region by MLi-2 is consistent with other inhibitor-bound homolog kinase structures (22, 24), the detailed information revealed by HDX-MS without structure is remarkable. In addition, our data also reflect the dynamic change that the binding of MLi-2 has not only on the kinase domain but also on LRRK2RCKW overall. Essentially any region in LRRK2RCKW that interfaces with the kinase domain will sense binding of nucleotide or an inhibitor. This also includes the NTDs, not included in our construct, which are predicted to lie over the kinase domain and inhibit kinase activity when LRRK2 is in its inactive state (5, 25). As demonstrated earlier, the NTDs would be displaced by the high-affinity binding of
GaMD Simulations Indicate That the LRRK2 Kinase Domain Mutations Y2018F, G2019S, and I2020T Attenuate Flexibility of the Activation Segment of the Kinase Core. GaMD simulations were performed on the activated kinase domain of LRRK2 (amino acids 1865 to 2135) to investigate changes in the conformational landscape that are caused by the D2017A, Y2018F, G2019S, and I2020T mutations. Enhanced sampling was used to broadly sample conformational space in order to build an accurate representative model of the LRRK2 kinase and the changes induced by mutation. During all 10 replicate accelerated simulations the WT kinase favors an open and inactive active-site cleft conformation as measured by the relative positions of the N and C lobes and the αC-helix (Fig. 7). The degree of stabilization of the closed conformation roughly correlates with the observed changes in MT association: D2017A < WT < G2019S < Y2018F < I2020T; it does not correlate with activity. The I2020T mutant is trapped in a mostly closed state without extensive open-to-close transitions and αC in-to-out motion compared with WT, Y2018F, and G2019S. This loss of breathing dynamics may partially explain the reduced kinase activity of I2020T, where substrate/product kinetics may be impacted. Likewise, Y2018F and G2019S both populate a wide range of open and also closed active conformations likely contributing to their increased kinase activity. The ability of all of the activating mutants to populate a closed conformation may play a role in their altered MT association compared with WT, where Y2018F and I2020T spontaneously form filaments and G2019S forms filaments faster than WT upon treatment by the type I inhibitor MLi-2.

The hydroxyl moiety of Y2018 in the DYGI motif forms persistent hydrogen bonds between the backbone of both I1933 in the αC-β4-loop and I2015 (Fig. 7B). This interaction stabilizes the tyrosine side chain in an orientation that restricts the αC-helix from assembling the active site due to steric clash with L1924.
This simulation agrees very well with a recent cryoelectron microscopy structure of an inactive conformation of LRRK2 (5). These authors identify the same hydrogen bond between Y2018 and the shell residue I1933. Our simulations provide strong independent evidence that Y2018 in WT LRRK2 is a key stabilizer of the inactive kinase conformation and may also act as a sensor of the αC-β4-loop conformation, a conserved hotspot for kinase allosteric modulation (26). Absence of the OH hydrogen bonds in the Y2018F αC-peptide (amino acids 1915 to 1921), the deuterium increases without reaching a plateau over 5 min for both states.

Discussion

The detailed signaling cascades that control LRRK2 are still being elucidated, and the molecular mechanisms that control its intrinsic regulation are also not well-characterized. Here we investigated a four-domain construct of LRRK2 consisting of the ROC, COR, kinase, and WD40 domains, which is the shortest functional construct to date that retains kinase as well as GTPase activity and is also the smallest construct that can dock onto MTs (5). In the current work, we elucidate different aspects of the intrinsic regulation of LRRK2 using a multilayered approach focusing on the importance of the kinase domain. We first concentrated on the spatial and temporal distribution of full-length LRRK2 in cells as a function of the high-affinity kinase inhibitor MLi-2, which provided us with a real-time assay for reversible filament formation in live cells. The effects of removing the N-terminal targeting domains on cellular distribution were then explored with our LRRK2RCKW variants, which led us to predict that NTDs shield and inhibit the catalytic domains when LRRK2 is in its inactive resting state. Biochemical characterization of LRRK2RCKW variants demonstrated that substrate-specific kinase activity comparable to full-length LRRK2 was retained by LRRK2RCKW; the catalytic machinery for mediating phosphoryl transfer remained intact. We next used HDX-MS analysis of LRRK2RCKW to provide a portrait of the conformational states of LRRK2RCKW in the presence and absence of MLi-2. Mapping the solvent-accessible regions in a model of the LRRK2 kinase domain not only provides an allosteric portrait of the “breathing” kinase domain but also suggests multidomain crosstalk in LRRK2RCKW. Finally, we performed GaMD calculations on the LRRK2 kinase domain to elucidate at a molecular level the differences in breathing dynamics between WT LRRK2 and the pathogenic kinase domain mutations Y2018F, G2019S, and I2020T, explicitly establishing the role of the DYGI motif as a dynamic regulator of the switch mechanism. With this multiscale approach, we were able to clearly demonstrate that the kinase activity and the spatial distribution of LRRK2 are regulated by a complex interplay of all the embedded protein domains. The highly dynamic kinase domain, nevertheless, appears to be the driver that coordinates the overall domain cross-talk and serves as a central regulatory hub for the intrinsic regulation of LRRK2.

Filament Formation Is Dependent on Unleashing the Catalytic Domains and on the Conformation of the Kinase Domain. Although multiple functions are associated with the many domains of LRRK2, these
domains can be structurally and functionally divided into the catalytically inert NTDs and the catalytic CTDs, and distinct functions are embedded in each. Further complexity is introduced by heterologous proteins such as Rab GTPases and 14-3-3 proteins, which contribute to the activation and subcellular localization of LRRK2. LRRK2 also exists in multiple oligomeric states where the most active state is thought to be a dimer in contrast to the less active monomer (19). The stability of the monomers and dimers can be further facilitated by heterologous proteins, in particular the 14-3-3 proteins. Physiologically, LRRK2 is thought to be activated by Rab GTPases, such as Rab29, which dock onto the NTDs and target LRRK2 to the trans-Golgi network (14). Other Rabs may also activate LRRK2 and target it to different organelles (13) while autophosphorylation on residues such as S1292 likely is a subsequent step in the activation process (5, 28). Many of the PD mutations hijack these finely tuned regulatory mechanisms. Using cryoelectron tomography (cryo-ET), Watanabe and coworkers were able to capture the precise way in which this LRRK2 mutant (I2020T) can polymerize and dock onto MTs. They show specifically how this LRRK2 mutant forms periodically repeating dimers, which then polymerize in a helical array onto MTs. The cellular phenotypes associated with G2019S, I2020T, and R1441C/Y1699C and other PD-associated mutations include perturbation of MT-related processes such as vesicular trafficking, autophagy, cilia formation, and nuclear/mitochondria morphology, so it is very likely that LRRK2 dysfunction physiologically interferes globally with dynamic cross-talk with MTs (9, 29–31).

With live-cell imaging in the absence and presence of a kinase inhibitor, MLi-2, we were able to capture at low resolution in real time the relocalization of cytoplasmic WT and G2019S LRRK2 to decorated MTs, a process that is fully reversible when the inhibitor is removed. MLi-2 binding to WT and G2019S thus captures the pathogenic phenotype constitutively observed for I2020T, R1441C, or Y1699C. In contrast to MLi-2 and LRRK2-IN-1, which are both type I kinase inhibitors, we show that in the presence of a type II kinase inhibitor, rebastinib, G2019S LRRK2 remains cytosolic, confirming the predictions of Deniston and coworkers that docking into MTs can be blocked by type II inhibitors.

**Fig. 7.** Mutations in the DYGI loop alter kinase dynamics. (A) Kinase conformational free-energy landscape, represented by “open–close”: the distance from the top (K1906/β3-sheet) to the bottom (D1994/YRD motif) of the active site; and “αC in–out”: the distance between K1906 and E1920/αC-helix. The white line shows the closed-active kinase conformation. WT samples the active state infrequently, whereas the mutants more readily access the closed-active conformation. However, D2017A is destabilized to a more open conformation. PMF, potential of mean force. (B) In WT, Y2018 is locked in an inactive orientation by hydrogen bonds with I2015 and I1933. Y2018F packs with L1924 and releases the DYGI loop from an inactive state helping to assemble the active site. Y2018F breaks the interaction leading to increased side-chain dynamics, measured by the distance between the 2018ζ-carbon and the backbone of I1933. (C) I2020T makes a hydrogen bond with the backbone of Y1992, coupling the DYGI and catalytic loops, which decreased backbone dynamics. The mutation brings the DYGI and YRD motifs together, measured as the distance from the 2020 Cβ and the backbone of Y1992. (D) G2019S bridges the DYGI loop to the αC-helix and β3-sheet, through E1920 and K1906. This stabilizes the DYGI loop, shown by rmsd, and promotes the closed kinase conformation.
The Switch Mechanism for Activation of LRRK2 Is Embedded in the DYGI Motif of the Kinase Domain. With HDX-MS, we confirm that a shift in conformation is induced by the binding of MLi-2 to LRRK2RCKW and, significantly, we find that stabilizing the active kinase conformation with MLi-2 drove changes in conformation and domain organization throughout LRRK2RCKW. The global decrease in backbone deuterium exchange measured across all four domains particularly in the linker between the COR-B and kinase domains and in flexible regions throughout LRRK2RCKW (Fig. 5A) is suggestive of changes in domain–domain packing as well as changes in global conformational dynamics. We propose that changes in the CTD organization and dynamics, driven by stabilization of the active kinase conformation, are likely coupled with association and dissociation of the NTDs. This explains why mutations such as R1441C and Y1699C, that lie far from the kinase active site but at a domain interface, are capable of unleashing the NTDs. Using the MLi-2–bound LRRK2RCKW as a proxy for the frozen closed and active-like conformation of the kinase domain, we explored each of the activating DYGI mutants using MD simulations and asked how each mutation perturbs the conformational ensemble of the kinase domain.

Our first hint that the DYGI motif impacts the kinase domain with consequences for LRRK2 global conformation and regulation came from our previous work with the DYGI mutations Y2018F and I2020T, where we were able to correlate changes in the internal organization of the kinase domain, specifically assembly of the R spine, with in situ MT association (10). Our data here show that deletion of the NTDs induces a similar MT association phenotype independent of mutation. Although full-length G2019S, the most prevalent PD mutation, does not form significant filaments spontaneously, we show that its kinetics of MT association are

to MTs is extremely sensitive to the conformational state of the kinase domain (5). Although the MLi-2 complex is catalytically inactive, MLi-2, which is a competitive inhibitor of ATP, nevertheless locks the kinase into an active-like conformation (24). In contrast, rebastinib is likely to stabilize a DYGI/DFG-out/open and inactive conformation of the kinase domain. In the absence of the NTDs, both WT and G2019S LRRK2RCKW spontaneously form filaments independent of MLi-2. Our HDX-MS data confirm that deletion of the NTDs in LRRK2RCKW does not unfold the remaining CTDs as solvent exchange shows that the protein is properly folded with the domains well-packed against each other. Collectively, our results support a model where the catalytically inert NTDs function as a lid that shields the active sites of the CTDs. The lid can be unleashed physiologically by activating Rab GTPases or by mutations that make LRRK2 a risk factor for PD. The Switch Mechanism for Activation of LRRK2 Is Embedded in the DYGI Motif of the Kinase Domain. With HDX-MS, we confirm that a shift in conformation is induced by the binding of MLi-2 to LRRK2RCKW and, significantly, we find that stabilizing the active kinase conformation with MLi-2 drove changes in conformation and domain organization throughout LRRK2RCKW. The global decrease in backbone deuterium exchange measured across all four domains particularly in the linker between the COR-B and kinase domains and in flexible regions throughout LRRK2RCKW (Fig. 5A) is suggestive of changes in domain–domain packing as well as changes in global conformational dynamics. We propose that changes in the CTD organization and dynamics, driven by stabilization of the active kinase conformation, are likely coupled with association and dissociation of the NTDs. This explains why mutations such as R1441C and Y1699C, that lie far from the kinase active site but at a domain interface, are capable of unleashing the NTDs. Using the MLi-2–bound LRRK2RCKW as a proxy for the frozen closed and active-like conformation of the kinase domain, we explored each of the activating DYGI mutants using MD simulations and asked how each mutation perturbs the conformational ensemble of the kinase domain.

Our first hint that the DYGI motif impacts the kinase domain with consequences for LRRK2 global conformation and regulation came from our previous work with the DYGI mutations Y2018F and I2020T, where we were able to correlate changes in the internal organization of the kinase domain, specifically assembly of the R spine, with in situ MT association (10). Our data here show that deletion of the NTDs induces a similar MT association phenotype independent of mutation. Although full-length G2019S, the most prevalent PD mutation, does not form significant filaments spontaneously, we show that its kinetics of MT association are
increased relative to WT following treatment with MLi-2 (Movies S1, S2, S3, and S4), suggesting that this mutation may share a common deregulating mechanism through changes in the kinase domain conformation. Indeed, our GaMD calculations reveal that in WT LRRK2 the DYGI motif exists in a strained conformation and that release of the strain leads to stabilization of the DYGI dynamics and promotion of the active kinase conformation by all three mutations Y2018F, G2019S, and I2020T. However, each of the mutants releases the strained DYGI motif and stabilizes the closed active conformation by different atomistic mechanisms. Significantly, the WT kinase in these simulations still fluctuates between both inactive and active states while favoring the inactive conformation. This means input from external factors, such as the NTDs or heterologous proteins, is required to fully modulate the conformational equilibrium to inhibit kinase activity. Activating DYGI mutations subvert the built-in regulation by distorting the inherent conformational equilibrium to a degree that breaks these layers of control. On the other hand, the kinase-dead D2017A, which has significantly reduced localization to MTs even in the absence of the NTDs, cannot be stabilized in an active-like kinase conformation because its active-site cleft is further dynamically destabilized. Together with our findings for rebastinib and MLi-2, this further emphasizes that filament formation is not solely dependent on the inhibitory lid function of the NTD but also on kinase domain integrity and conformational state.

Our findings highlight that the dynamic activation of LRRK2 is accurately defined in terms of a shift in conformational ensemble and associated dynamics. This is illustrated in the MD simulations by changes in bulk conformations due to DYGψ mutations and also by changes in timescales of dynamics highlighted by live-cell imaging and by the induction of bimodal HDX kinetics after binding MLi-2. HDX shows that the activation loop peptide has two distinct conformational populations. One represents a minor population of a highly solvent-exposed species similar to what was seen in the apo state and an additional highly protected species, which gradually becomes more solvent-exposed (Fig. 6). In MD simulations the DYGψ-activating mutants mirror this behavior and shift the equilibrium toward an ordered and less solvent-accessible activation loop (SI Appendix, Fig. S5). MLi-2 binding appears to lead to a large decrease in the kinetics of the exchange between states, effectively trapping a major population of a closed and active-like state of the kinase domain. Extending this concept of regulation by tuning of conformational dynamics to the cellular level, our work implies that changes in the balance of LRRK2 conformational equilibrium will lead to proportional changes in its cellular distribution and activity, that is, the population of LRRK2 in the cytosol vs. associated with MTs will mirror the conformational distribution of expanded and closed LRRK2.

LRRK2 and Braf Share the Same Kinase Activation Mechanism. By comparing the resulting finely tuned multilayered regulation mechanism of LRRK2 with other related homologs of the kinase tree, we recognized that our model for LRRK2 regulation closely resembles the activation process of another multidomain kinase: Braf (32, 33). LRRK2, like Braf, is activated by the interaction of its N-terminal noncatalytic domains with a small GTPase: Rab vs. Ras. In both cases, autoinhibitory sites/domains (AIs) in the NTDs become displaced when the activated GTPase binds, and this unleashes the kinase domain (34, 35). The kinase domains, no longer locked in their inactive conformations, are free to toggle between their inactive and active states. Only in the active conformation are the catalytic domains able to dimerize. In Braf this pushes the kinase domain into the active conformation and induces cis-autophosphorylation of the activation loop. Dimerization of the catalytic domains of LRRK2, as seen in the cryo-ET structure (25), also requires an active conformation of the kinase domain, although in the case of LRRK2 the dimer interface most likely does not directly involve the kinase domain. In both cases this last step stabilizes the kinase domain in a conformation where the R spines are assembled, rendering them ready to bind and phosphorylate their substrates. When this process is disrupted by a mutation such as Y2018F or I2020T in the kinase domain of LRRK2, kinase activation becomes independent of Rab binding, as these mutations shift the equilibrium to a more active kinase conformation which also promotes displacement of the NTDs (Fig. 9A). This recapitulates a theme observed in Braf, wherein the most activating cancer-driving mutation, V600F, renders the kinase active without the need for heterologous activation by Ras (36).

Other mutations that contribute to stabilization of the assembled R spine also lead to constitutive activation that is independent of Ras (36, 37). Kinase inhibition was also shown to facilitate downstream signaling as the inhibition of Braf stabilized the active kinase conformation which is sufficient to promote cis-autophosphorylation through heterodimerization (38). This closely resembles the situation we observed for LRRK2 where MLi-2 stabilizes the active kinase conformation and thereby induces filament formation (Fig. 9A). The recently solved LRRK2 filament structure on MTs shows that each LRRK2 monomer interacts with two adjacent monomers through COR and the WD40 domain while the N terminus interacts with the
upper and lower turns of the LRRK2 filament (25). Furthermore, the number of LRRK2 dimers needed for one turn correlates well with the number of MT protofilaments. Therefore, we believe that this is a pathogenic but specific interaction with MTs resulting from impaired regulation of LRRK2 by stabilizing an active conformation of the kinase domain of LRRK2 either by mutations or by binding of ML1-2.

Another feature common to LRRK2 and BRaf is that the deletion of the N terminus of either protein generates a constitutively active kinase (Fig. 9B) (39–41). Finally, the kinase-dead mutations in both proteins are unable to form productive dimers (42, 43).

Since many aspects of the kinase domain regulation of LRRK2 and BRaf appear to be quite similar, it can be concluded that activation mechanisms, in general, where the kinase domain switching between active and inactive conformations serves as a central hub are likely to be conserved throughout much of the kinome. In addition, from the recent structures of full-length BRaf in complex with its substrate MEK and 14-3-3 proteins, we can see how these auxiliary proteins can stabilize either an inactive or an active conformation (44). This will certainly also be true for LRRK2. Clearly, we can learn much about LRRK2 activation by looking at close homologs that have been studied for a longer time, and hopefully this will facilitate the discovery of new therapeutic strategies for attacking LRRK2 as a driver of PD.

Conclusion

Analysis of multidomain kinases suggests that the conformation of the kinase domain might, as a general principle, regulate much more than just the activity of the kinase. Our results with LRRK2 demonstrate that the active kinase conformation not only switches the kinase into an “on” state but also unleashes inhibitory domains, can promote dimerization, and can facilitate translocation to anchored substrates. In the case of LRRK2 this is a complex and tightly regulated process where more domains and other proteins than just the kinase domain are involved; however, the paradigm is similar for BRaf and probably for most kinases such as Src and PKC where the kinase domain is also embedded in multidomain proteins. In each case, the conformation of the kinase domain seems to play a crucial role in these intrinsic regulatory processes. We also further confirm here how a switch mechanism for activation, embedded in the DFGw motif of the kinase core, allows the kinase to toggle between inactive and active conformations which are then communicated to all parts of the protein. We also demonstrate here that the noncatalytic NTDs play an important regulatory role by shielding the catalytic CTDs in the absence of physiological activators. Interacting proteins like 14-3-3 or Rab proteins are likely to further fine-tune this regulation either positively or negatively by stabilizing certain conformations of LRRK2. This precisely controlled signaling process can also be hijacked by a variety of disease-metabolic mutations such as those that lead to PD.

Materials and Methods

Detailed materials and methods are included in SI Appendix, Materials and Methods. LRRK23CXXK proteins were expressed and purified from Sf9 or HEK293T cells. All S9 proteins that were used for HDX-MS were monodisperse and monomeric based on size-exclusion chromatography (SI Appendix, Fig. S6). Phosphorylation of Rab8a was measured by Western blotting using a pT272-specific antibody (MF-P20; abbac; ab231706) and anti-His antibody (GE Healthcare; mouse). The kinase activity of the LRRK23CXXK variants was measured by a microfluidic mobility-shift kinase assay using LRRKtide (RLGDKYKLTRIRQQ-amine; GeneCust) as substrate. The AMBER16 package was used for GaMD simulations.

Data Availability. All study data are included in the article and/or supporting information.

ACKNOWLEDGMENTS. We acknowledge the excellent technical assistance of Brian Benitez, Hanh and Imraud Hammerl-Witzel and the assistance of Alexandre Kornew in the preparation of models. This work was supported by Michael J. Fox Foundation Grant 11425 (https://www.michaeljfox.org/) (to D.B., S.S.T., and F.W.H.), the Brainman Family Foundation (D.B.), NIH Grant NIH ROI GM086197 (to D.B.), and Ruth L. Kirshstein National Research Service Award NIH National Cancer Institute T32 CA009523 (to P.C.A.). S.M. and S.K. are grateful for support from the Deutsche Forschungsgemeinschaft (DFG) (HE 1818/11) and Structural Genomics Consortium (SGC), a registered charity that receives funds from AbbVie, Bayer Pharma AG, Boehringer Ingelheim, Canada Foundation for Innovation, Ethelain Institute for Innovation, Genome Canada, Innovative Medicines Initiative (875510), Janssen, Merck KGaA Darmstadt Germany, Merck Sharp and Dohme (MSD), Novartis Pharma AG, Ontario Ministry of Economic Development and Innovation, Pfizer, Sao Paulo Research Foundation, Takeda, and Wellcome. The National Center for Microscopy and Imaging Research was supported by Grant P41 GM103412. The Synapt G2Si HDX mass spectrometer was obtained from shared instrumentation NIH Grant S10 OD016234 (to E.A.K. and S.S.). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

1. B. A. Benitez et al., Resequencing analysis of five Mendelian genes and the top genes from genome-wide association studies in Parkinson’s disease. Mol. Neurodegener. 11, 28 (2016).
2. G. Guaitoli et al., Structural model of the dimeric Parkinson’s protein LRRK2 reveals a compact architecture involving distant interdomain contacts. Proc. Natl. Acad. Sci. U.S.A. 113, E4357–E4366 (2016).
3. J. E. Tomkins et al., Comparative protein interaction network analysis identifies shared and distinct functions for the human RCOG proteins. Proteomics 18, e1700444 (2018).
4. R. Migheli et al., LRRK2 affects vesicle trafficking, neurotransmitter extracellular level and membrane receptor localization. PLoS One 8, e77198 (2013).
5. C. K. Deniston et al., Structure of LRK2 in Parkinson’s disease and model for microtubule interaction. Nature 598, 344–349 (2020).
6. L. R. Kett et al., LRRK2 Parkinson disease mutations enhance its microtubule association. Hum. Mol. Genet. 21, 890–899 (2012).
7. M. Blanca Ramirez et al., GTP binding regulates cellular localization of Parkinson’s disease-associated LRRK2. Hum. Mol. Genet. 26, 2747–2767 (2017).
8. X. Deng et al., Characterization of a selective inhibitor of the Parkinson’s disease kinase LRRK2. Nat. Chem. Biol. 7, 203–205 (2011).
9. X. Chen et al., Parkinson’s disease-related leucine-rich repeat kinase 2 modulates nuclear morphology and genomic stability in striatal projection neurons during aging. Mol. Neurodegener. 15, 12 (2020).
10. S. H. Schmidt et al., The dynamic switch mechanism that leads to activation of LRRK2 is embedded in the DFGw motif in the kinase domain. Proc. Natl. Acad. Sci. U.S.A. 116, 14979–14988 (2019).
11. J. T. Manschuetz et al., Binding of the human 14-3-3 isoforms to distinct sites in the leucine-rich repeat kinase 2. Front. Neurosci. 14, 302 (2020).
12. N. J. Lavalley, S. R. Stone, H. Ding, A. B. West, T. A. Yacoubian, 14-3-3 proteins regulate mutant LRRK2 kinase activity and neurite shortening. Hum. Mol. Genet. 25, 109–122 (2016).
13. E. McGrath, D. Waschbisch, B. M. Baker, A. R. Khan, LRRK2 binds to the Rab32 subfamily in a GTP-dependent manner via its armadillo domain. Small GTPases 12, 133–146 (2021).
14. E. Purlyte et al., Rab29 activation of the Parkinson’s disease-associated LRRK2 kinase. EMBO J. 37, 1–18 (2018).
15. N. Dizamko et al., Inhibition of LRRK2 kinase activity leads to dephosphorylation of Ser910/Ser935, disruption of 14-3-3 binding and altered cytoplasmic localization. Biochem. J. 430, 405–413 (2010).
16. R. J. Nichols et al., 14-3-3 binding to LRRK2 is disrupted by multiple Parkinson’s disease-associated mutations and regulates cytoplasmic localization. Biochem. J. 430, 393–404 (2010).
17. E. Deyaert et al., Structure and nucleotide-induced conformational dynamics of the Chlorobium tepidum Roco protein. Biochem. J. 476, S1–S6 (2019).
18. E. Deyaert et al., A homologue of the Parkinson’s disease-associated protein LRRK2 undergoes a monomer-dimer transition during GTP turnover. Nat. Commun. 8, 1008 (2017).
19. Z. Berger, K. A. Smith, M. J. Lavoie, Membrane localization of LRRK2 is associated with increased formation of the highly active LRRK2 dimer and changes in its phosphorylation. Biochemistry 49, 5511–5523 (2010).
20. S. S. Taylor, H. S. Meharena, A. P. Kornev, Evolution of a dynamic molecular switch. IUBMB Life 71, 672–684 (2019).
21. P. D. Jeffrey et al., Mechanism of CDK activation revealed by the structure of a cyclin-CDK2 complex. Nature 376, 313–320 (1995).
22. J. D. Scott et al., Discovery of a 3-(4-pyrimidinyl) indazole (ML-2), an orally available and selective leucine-rich repeat kinase 2 (LRRK2) inhibitor that reduces brain kinase activity. J. Med. Chem. 60, 2983–2992 (2017).
23. J. M. Roberts et al., Dynamics of the Tec-family tyrosine kinase SH3 domains. Protein Sci. 25, 852–864 (2016).
24. D. S. Williamson et al., Design of leucine-rich repeat kinase 2 (LRRK2) inhibitors using a crystallographic surrogate derived from checkpoint kinase 1 (CHK1). J. Med. Chem. 60, 8945–8962 (2017).
25. R. Watanabe et al., The in situ structure of Parkinson’s disease-linked LRRK2. Cell 182, 1508–1518.e16 (2020).
26. W. Yeung, Z. Ruan, N. Kannan, Emerging roles of the αC-β4 loop in protein kinase structure, function, evolution, and disease. IUBMB Life 72, 1189–1202 (2020).
27. P. C. Aoto, R. L. Stanfield, I. A. Wilson, H. J. Dyson, P. E. Wright, A dynamic switch in inactive p38 leads to an excited state on the pathway to an active kinase. Biochemistry 58, 5160–5172 (2019).
28. Z. Sheng et al., Ser1292 autophosphorylation is an indicator of LRRK2 kinase activity and contributes to the cellular effects of PD mutations. Sci. Transl. Med. 4, 164ra161 (2012).
29. A. R. Esteves, S. M. Cardoso, LRRK2 at the crossroad between autophagy and microtubule trafficking: Insights into Parkinson’s disease. Neuroscientist 23, 16–26 (2017).
30. H. S. Dhekne et al., A pathway for Parkinson’s disease LRRK2 kinase to block primary cilia and Sonic hedgehog signaling in the brain. eLife 7, e40202 (2018).
31. A. Marku et al., The LRRK2 N-terminal domain influences vesicle trafficking: Impact of the E193K variant. Sci. Rep. 10, 3799 (2020).
32. A. S. Shaw, A. P. Kornev, J. Hu, L. G. Ahuja, S. S. Taylor, Kinases and pseudokinases: Lessons from RAF. Mol. Cell. Biol. 34, 1538–1546 (2014).
33. E. Park et al., Architecture of autoinhibited and active BRAF-MEK1-14-3-3 complexes. Nature 575, 545–550 (2019).
34. M. Jaleel et al., LRRK2 phosphorylates moesin at threonine-558: Characterization of how Parkinson’s disease mutants affect kinase activity. Biochem. J. 405, 307–317 (2007).
35. E. Greggio et al., The Parkinson disease-associated leucine-rich repeat kinase 2 (LRRK2) is a dimer that undergoes intramolecular autophosphorylation. J. Biol. Chem. 283, 16906–16914 (2008).
36. J. Hu et al., Kinase regulation by hydrophobic spine assembly in cancer. Mol. Cell. Biol. 35, 264–276 (2015).
37. J. Hu et al., Allosteric activation of functionally asymmetric RAF kinase dimers. Cell 154, 1036–1046 (2013).
38. G. Hatzivassiliou et al., RAF inhibitors prime wild-type RAF to activate the MAPK pathway and enhance growth. Nature 464, 431–435 (2010).
39. H. Cin et al., Oncogenic FAM131B-BRAF fusion resulting from 7q34 deletion comprises an alternative mechanism of MAPK pathway activation in pilocytic astrocytoma. Acta Neuropathol. 121, 763–774 (2011).
40. H. Chong, K. L. Guan, Regulation of Raf through phosphorylation and N terminus-C terminus interaction. J. Biol. Chem. 278, 36269–36276 (2003).
41. M. Zang, C. Hayne, Z. Luo, Interaction between active Pak1 and Raf-1 is necessary for phosphorylation and activation of Raf-1. J. Biol. Chem. 277, 4395–4405 (2002).
42. T. Ikenoue et al., Functional analysis of mutations within the kinase activation segment of B-Raf in human colorectal tumors. Cancer Res. 63, 8132–8137 (2003).
43. P. T. Wan et al.; Cancer Genome Project, Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. Cell 116, 855–867 (2004).
44. N. P. D. Liau et al., Negative regulation of RAF kinase activity by ATP is overcome by 14-3-3-induced dimerization. Nat. Struct. Mol. Biol. 27, 134–141 (2020).
45. M. V. Shapovalov, R. L. Dunbrack Jr, A smoothed backbone-dependent rotamer library for proteins derived from adaptive kernel density estimates and regressions. Structure 19, 844–858 (2011).