

# Role of cdk5 and parkin in parkinsons diseases biology essay

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James parkinsons disease is a prevailing neurodegenerative disease that was associated with Cdk5 and parkin activity. Recent research has shown the mechanism behind the Cdk5 and parkin interaction, but has yet to look at the map of this interaction in relation to Parkinson ' s disease and whether there is an component of PD causing behind this interaction.

A parkin concept with the Cdk5 phosphorylation site residues replaced to render these sites functionless was used in vitro and in vivo parametric quantities. In these experiments, low Dopastat degrees compared to wildtype were used as a marker for PD. Experiments on mice with these two parkin ( parkin concept and wildtype parkin ) phenotypes were performed to measure whether there were any behavioural traits similar to mice with PD.

Problem. Methods. How it would take us at that place. Literature

ReviewParkinson ' s Disease is a common neurodegenerative upset that has a lifetime incidence of about two per centum.

The clinical manifestations of it include resting shudder, muscular rigidity, bradykinesia, and postural inability. PD is characterized by the progressive devolution of dopaminergic nerve cells in the substantia nigra. A specific pathological characteristic of PD is the intracytoplasmic inclusion organic structure, known as the Lewy organic structure. The Lewy Body is found in many parts, including the substantia nigra, venue ceruleus, nucleus basalis, hypothalamus, intellectual cerebral mantle, cranial motor nervus karyon, and the cardinal and peripheral divisions of the autonomic nervous system<sup>1</sup>. One protein that was found to play a cardinal function in both familial and sporadic Parkinson ' s disease is parkin. Parkin<sup>2</sup> belongs to a household of

proteins with conserved ubiquitin-like sphere ( UBL ) at the N end point  
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connected to a C-terminal truly interesting new finger ( RING ) sphere by a linker part. The RING finger sphere gives parkin the ability to work as an E3 ubiquitin-ligase enzyme. By attaching ubiquitin to a lysine on the mark protein through an isopeptide bond, it targets specific protein substrates for debasement by the proteasome.

It was found that mutants in parkin are the most common cause of familial PD and besides do a part of autosomal recessive juvenile parkinsonism<sup>3</sup>. From the neuropathologic studies<sup>4</sup> of patients with parkin mutants, a selective loss of dopaminergic nerve cells of the substantia nigra and loss of noradrenergic nerve cells in the ventral tegmental nucleus was observed. In a part of these surveys, Lewy organic structures were found. Further research, showed that parkin ubiquitylates the  $\alpha$ -synuclein-interacting protein synphilin-1 and the p38 fractional molecular weight of aminoacyl-tRNA synthetase/JTV-15. This is of importance when speaking of Parkinson's disease because mutants of the  $\alpha$ -synuclein gene have been found to play a function in some familial forms of PD, and the accumulation of  $\alpha$ -synuclein protein has been found in the brains of patients with this pathology<sup>6</sup>. Specifically, parkin protects against the toxicity that may originate from the accumulation of  $\alpha$ -synuclein.

Experiments on Drosophila Parkin null mutations found that they display mitochondrial dysfunction<sup>7</sup>, a mark of PD, and this conveys that Parkin may play a function in the care of mitochondrial map. Parkinson's disease, like many other neurological disorders, consequences in loss of CNS nerve cells due to apoptotic or necrotic neural death. Not merely does the apoptotic

machinery have important maps during development, but it can besides be reactivated under pathological conditions in the grownup nervous system. One of the constituents of this apoptotic machinery is Cdk5. Despite being expressed in most tissues, Cdk5 activity is about entirely restricted to nerve cells. Experiments performed by Bibb et Al, and White and Cooper, that Cdk5 plays a function in modulating Dopastat signaling in dopaminergic. Furthermore, the research workers found that with chronic cocaine disposal there would be upregulation of Cdk5.

The PD marker mentioned earlier, Lewy organic structures, contain Cdk5, farther cogent evidence that a Cdk5 Parkinson ' s nexus does be ( Pierre ) . In an experiment performed by Avraham et Al. ( 2007 ) , the research workers found parkin was phosphorylated in vitro and in vivo by Cdk5. They deemed that this phosphorylation by Cdk5 regulated the catalytic activity of Parkin, and was responsible for parkin ' s ability to ubiquitylate its synphilin-1 and p38 substrates. Furthermore, the consequences they obtained suggested that the phosphorylation of parkin by Cdk5 decreased the E3 ubiquitin-ligase activity. After happening that parkin has four Cdk5 phosphorylation sites, all located in the connecting linker part, in vitro and in vivo phosphorylation experiments were carried out.

Previous research had indicated that parkin was phosphorylated at serine residues 101, 131, 136, 296, and 378, uniting that cognition with the determination from there research that serine 131 mutations had a important sum of lower phosphorylation than the other sites. In trying to happen the in vivo specificity of Ser-131 to Cdk5, the research workers

generated a parkin concept that had the serine residues at 101, 136, 296, and 278 mutated to alanine and merely the serine 131 was left unaltered. By utilizing different protein kinase inhibitors that preferentially inhibited different protein kinases, the experimenters found that merely the Cdk5 inhibitor, roscovitine, inhibited phosphorylation. This suggests that Cdk5 was chiefly responsible for the phosphorylation of parkin, and as a consequence decreased parkin's E3-ubiquitin ligase activity. The lessening in ligase activity may lead to the accretion of p38. The toxic protein p38, which accumulates in PD encephalon tissues and contributes to the decrease of dopaminergic nerve cells, is a constituent of the PD model that may be affected by the phosphorylation of parkin by Cdk5.

This research provided the mechanism behind the phosphorylation of parkin by Cdk5, but did not near the subject of its functional significance with regard to Parkinson's disease. I hope to research whether the phosphorylation of parkin by Cdk5 leads to Parkinson's disease or other types of neural harm. The difference in dopamine release between wildtype nerve cells and mutations that prevent phosphorylation will be used as a marker for Parkinson's disease. Through the conducting of necessary and sufficient trials, I hope to research the functionality behind this phosphorylation. Experimental Proposal Further elucidation of the Parkinson's disease theoretical account needs to happen for there to be effectual, targeted drug interventions that help in slaking the effects of, or bring about PD. One such possible manner is to see if Cdk5 inhibitors could be used to handle PD. Before such intervention could be administered, the

neurophysiological response of phosphorylation of parkin by Cdk5 must be observed.

Specific Aim: To find if the phosphorylation of parkin by Cdk5 leads to a lessening in Dopastat release and if it is either necessary or sufficient for such a phenotype. Besides, to detect the behavioural effects this Cdk5 phosphorylation of parkin has on a theoretical account mouse being.

Proposed Experiments  
Cell Culture ( in vitro )  
The in vitro facet of the experiment will utilize cells cultured from the striate body of a mutant mouse.

The cells are being cultured from the striate body because these cells are dopaminergic, and since the release of Dopastat is being studied as the marker for PD it makes sense to utilize cells from the striate body. The mutants will be made through site-directed mutagenesis. This the same process conducted in the Avraham survey. Full-length parkin concepts mutated at predicted Cdk5 sites or extra kinase sites will be generated by PCR utilizing primers that contained alanine codons alternatively of serine 1s. The mutants of Cdk5 sites were confirmed by double-strand sequencing.

These mutants will suppress the ability of Cdk5 to phosphorylate parkin, and Dopastat degrees could so be measured. This trial will mensurate the necessity of the phosphorylation of parkin by Cdk5. For the in vitro sufficiency trial, the wildtype parkin cistron should be added back into the mutated civilization cells and Dopastat degrees should be measured. During this measure of the process the cistrons responsible for the proteins that have been implicated in Parkinson ' s disease must all be knocked out, so

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that merely the staying proteins involved in the Parkinson ' s disease tract are parkin and Cdk5. Proteins such as PINK1, HtrA2, and p38 should all be knocked out, or it ' s look should be silenced.

The wildtype parkin cistron can be added back utilizing a bacterial unreal chromosome ( BAC ) along with Ampicillin to do certain the cistron was taken up. BAC would be used alternatively of a plasmid vector because the plasmid vector can merely incorporate inserts of about 1-10 kbp while parkin can incorporate inserts greater than 700 kbp. The parkin wildtype cistron is greater than 10 kbp and because of this the plasmid vector can non suitably reassign the cistron back in. The wildtype parkin cistron is inserted into a bacteria and coupled to the cistron bla, a cistron encoding for ampicillin opposition.

After this intervention, the bacteriums are so grown in a medium incorporating Principen. The cistrons that successfully take up the wild-type parkin besides possess an ampicillin opposition, thereby, lasting in this ampicillin medium. Striatum cell civilization with wild type parkin and wild type Cdk5 should be used as a control and mention for Dopastat degrees. The measuring of Dopastat in civilized cells can be measured by coulometry<sup>9</sup> coupled to a high public presentation liquid chromatography ( HPLC ) . This attack is better for cell civilizations than pieces because unlike pieces that lack cell organic structures or nervus terminuss, the cell cultures contain integral nerve cells. Model Organism ( in vivo )As in the earlier illustration, site-directed mutagenesis at the Cdk5 phosphorylation sites on parkin should present the parkin concept. Alternatively of the parkin concept, a Cdk5

smasher could hold been used. Like the other Cdks, Cdk5 plays an of import function development and to avoid possible confounds a Cdk5 smasher was decided against.

Alternatively of the wildtype parkin cistron, the parkin concept should be expressed in my mouse. This serves as an in vivo necessity trial. Wildtype parkin and wildtype Cdk5 should be used as a control and serve as a mention for Dopastat degrees. The degree of Dopastat can be observed utilizing Positron Emission Tomography ( PET ) . Two determine if these two proteins, parkin and Cdk5, interact with one another in both in vivo and in vitro a coimmunoprecipitation check has to be performed, severally.

The underlying mechanism behind the Co-IP check is that interaction between two proteins should ensue in their precipitation together. The check will be similar to that performed by Avraham et Al in that an anti-parkin antibody will be used. This means that Cdk5 and parkin should precipitate together and be captured on an agrose gel.

Gel cataphoresis and Western smudge analysis will be used to find if the protein associated with parkin is so Cdk5. Another control for both the in vivo and in vitro experiments is proving whether the parkin was really phosphorylated. This can be done through a radioactive label and autoradiography visual image. Animal Behavioral Studies To acquire a holistic apprehension of the effects Cdk5 has on parkin and its function in the development of Parkinson ' s disease, it may be good to execute behavioural surveies that test for PD.



Such experiments have been performed before, and they chiefly comprise of looking at behavioural symptoms that are associated with Parkinson ' s disease. Trials for akinesia ( stationariness ) will be performed by putting the different discrepancies of mice described above on a level surface, and latency is will be measured until the mouse moves all four of its limbs. Catalepsy, or the inability of the animate being to rectify an externally imposed position, can be quantified by setting the animate being ' s fore-limbs and hind-limbs on a little saloon that is a few centimetres above the land, and finding the clip it takes for the animate being to rectify its place ( this behaviour is usually observed in normal mice ) . There is besides a swim trial that can be performed on the animate beings to measure the degree of motor damage. In this experiment mice were put in a H<sub>2</sub>O basin and there swimming techniques were dichotomized for active vs. inactive natation.

Last, the pole trial introduced by Ogawa et Al ( 1985 ) to mensurate bradykinesia in mice will be used. The trial uses a 50cm high, gauze-taped pole that is 1cm in diameter with a little ball of cork at the top and topographic points mice with their caput upwards right below the top. The clip until the animate beings have turned by 180 grades, and the clip they have come to the land is measured. In such experiments, control animate beings have been show to turn around and fall in approximately 20 seconds.

12Expected Results and InterpretationsThe in vitro assay with the parkin concept should exhibit phosphorylation when examined by autoradiography, this consequence is predicted because there are no sites for Cdk5 phosphorylation and merely served as a control. Since there are Cdk5

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phosphorylation sites on the wildtype parkin, phosphorylation should be observed at these sites, specifically at the serine residue 131, a major site of Cdk5 phosphorylation. The coimmunoprecipitation check should uncover that merely in the instance of wildtype parkin did Cdk5 besides precipitate along with it. Since there is phosphorylation, and therefore no interaction, between the mutated parkin and Cdk5 the two proteins should not precipitate together.

The dopamine readings from the cell civilization that contains the parkin concept should hold more elevated degrees than the 1s with knockin wildtype parkin because the wildtype parkin has unblocked Cdk5 phosphorylation sites, phosphorylation that has been linked to programmed cell death and is a constituent of the apoptotic machinery. As a consequence, this experiment should give both necessary and sufficient consequences for the lift of Dopastat degrees in non-Cdk5 phosphorylated parkin. The direct nexus between PD and the phosphorylation of parkin can not be found in these experiments. Further research may take to happen this direct nexus, and it could be guided by the Brion et Al. ( 1995 ) experiment that found cortical and brainstem-type Lewy organic structures that were immunoreactive for Cdk5. This research could take to happen whether parkin was besides present in these cells, and exemplify a physiological nexus between the interaction of Cdk5 and parkin to Parkinson ' s pathology.

The in vivo experiment with mice possessing mutated parkin should demo greater Dopastat activity, this is the same consequence as those expected in

civilization. Without a cardinal constituent in apoptotic map ( Cdk5 activity ) , the dopaminergic nerve cells should go on prevailing and as a consequence maintain bring forthing Dopastat. The mice with wildtype parkin should demo less dopamine activity because this apoptotic mechanism is still conserved. Coimmunopercipitation and autoradiography for phosphorylation should exemplify the same consequences it did in vitro, this is because the interplay of the proteins and the parametric quantities are basically the same.

One facet that is different between the two experiments ( in vitro vs. in vivo ) is that there were both necessary and sufficiency trials performed in the in vitro parametric quantity, but merely necessary trials were performed in the in vivo instance. This was done because strike harding out all the cistrans that have been antecedently implicated in Parkinson ' s disease would hold had enormous developmental effects, and the mice likely would non hold survived to hold any significant trials run on them.

All four behavioural trials would non hold provided any nexus between Cdk5 phosphorylation of parkin and symptoms of Parkinson ' s disease, intending all the mice discrepancies would exhibit similar behaviour. There would be no important informations found from such experiments unless behavioural manifestations of the interaction between Cdk5 and parkin were found. This is expected because there are a huge figure of proteins and mechanisms such as chondriosomes disfunction and deregulating of the apoptotic rhythm that has been associated with PD. Possible Pitfalls and Solutions One of the chief booby traps of the experiment is that there is no mensurable manner to associate this interaction to the subject of concern, Parkinson ' s disease. A

more direct marker for the disease would be to detect the formation of Lewy organic structures.

The job with that attack is that Lewy organic structures may be formed through agencies unrelated to the dopaminergic nerve cells observed, whereas, the survivability of dopaminergic nerve cells and therefore the production of Dopastat can be straight observed with the striate body nerve cells being tested upon. Another chief booby trap of the experiment is that the Parkinson ' s disease theoretical account associates many different proteins and tracts with the causing of the disease. Examples of proteins implicated in this theoretical account are: PINK-1 that recruits parkin to mitochondria during mitophagy, and p38 that forms a complex with PINK-1 to trip HtrA2 activity to bring on the debasement of misfolded proteins. However, this experiment may farther clear up the function of Cdk5 and parkin in the loss of dopaminergic nerve cells, an of import facet of PD.