# Regulation of gene expression and degradation of tyrosine hydroxylase in Parkinson's disease.

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Midbrain dopaminergic neurons play a central role in a wide range of behaviors, from attention and motivation to motor control and reinforcement. Dopaminergic systems, originating in the Ventral Tegmental Area (VTA) and in the substantia nigra pars compacta (SNpc), and mainly projecting to the ventral and dorsal striatum (mesostriatal pathway) and to the prefrontal cortex (mesocortical pathway), play a major motivational role in behavioral actions [1-3]. Dysfunction of the dopaminergic system is involved in neurological disorders such as Parkinson's disease, Tourette's syndrome, and schizophrenia, as well as in pituitary tumors [4]. Although the etiology of dopamine-related disorders has been studied and molecular evidence for maintenance of a dopaminergic phenotype has been produced in the past quarter of century, they are not yet completely understood. The development of fundamental therapies for such diseases is expected. Here, I focus on Parkinson's disease and present regulation of the gene expression of Tyrosine Hydroxylase (TH), the rate-limiting enzyme of dopamine biosynthesis. I also refer to the quality control system for the clearance of TH, and offer perspectives on the fundamental therapies for Parkinson's disease.

TH [5] and Aromatic Aminoacid Decarboxylase (AADC) [6] are dopamine-biosynthesizing enzymes. TH is responsible for catalyzing the conversion of the amino acid L-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA), and subsequently L-DOPA is converted to dopamine by AADC. Tetrahydrobiopterin ($BH_4$), which is biosynthesized by GTP cyclohydrolase I (GTPCHI), is required for the enzymatic activity of TH. Unlike other enzymes, TH protein has its enzymatic activity strictly controlled at the rate-limiting step of dopamine biosynthesis [7, 8]. TH expression level and activity are directly involved in regulating intracellular dopamine levels. The activity of TH can be modulated by two mechanisms: medium- to long-term regulation of gene expression, for example by effect on enzyme stability, transcription, RNA stability, alternative RNA splicing and translation; and short-term regulation of enzyme activity, for example by feedback inhibition, allosteric regulation and phosphorylation. Regulation of TH expression is dependent on a cAMP-dependent responsive element (CRE) [8], AP-1 [9, 10], ATF-2 [11], and Nurr1 [12].

A degradation of the nigrostriatal dopaminergic system to reduce TH protein level is the key component of pathogenesis of Parkinson's disease. In contrast, TH activity from parkinsonian tissue was reportedly increased by 48% compared to controls [13], or activity per enzyme protein of TH in the parkinsonian nigrostriatal region elevated by 3-4-fold [14]. The increased activity of residual TH in parkinsonian brain suggests facilitation of TH phosphorylation as result in a compensatory increase in TH activity to compensate for the loss of dopamine caused by the disease [15]. Intriguingly, we recently reported that dopamine or biopterin deficiency potentiates phosphorylation in cultured cells [16]. It is noteworthy that we revealed that the potentiated TH phosphorylation is accompanied by degradation of TH protein by the ubiquitin proteasome system [16, 17]. This mechanism suggests the possibility that dysfunction of dopamine biosynthesis in dopamine-related disorders such as Parkinson's disease and dopa-responsive dystonia leads to the induction of TH phosphorylation, which is accompanied by a reduced total TH protein level induced by degradation via the ubiquitin proteasome system. Consistently, phosphorylation of the N-terminal portion of TH reportedly triggers proteasomal digestion of the enzyme [18]. Phosphorylated TH also easily forms aggregates in living cells. We previously reported that accumulated TH forms intracellular inclusion bodies under conditions of proteasomal inhibition [17]. This phenomenon supports a putative mechanism in which elevation of TH phosphorylation to prevent a lack of dopamine in Parkinson's disease leads to the formation of intracellular aggregates of TH protein. Importantly, we observed TH and phosphorylated TH-immunopositive Lewy bodies in the brains of patients with Parkinson's disease [19]. These data suggest that phosphorylated TH is vulnerable and that the dopamine-deficient state in Parkinson's disease and related disorders may accelerate the decrease in TH protein level through the degradation and/or aggregation of phosphorylated TH.

Fundamental therapies for Parkinson's disease are not yet well established. Almost half a century after it was first introduced [20], L-DOPA ($C_9H_{11}NO_4$) is still the most effective medication available for the palliative treatment of the motor symptoms in Parkinson's disease [21]. Recent progress of treatment technique for Parkinson's disease is remarkable; deep brain stimulation has been shown to be efficacious in the treatment of parkinsonian tremor [22], striatal injection of AADC-coding vectors stably restore the response to L-DOPA and contribute to a decrease of the effective L-DOPA dose [23], and stem cell transplantation therapies may effectively restore and replace cells in the damaged tissues [24]. Recently, we identified a novel regulatory mechanism of dopamine-biosynthesizing enzymes, that is functionally coupled to actin polymerization [25, 26]. This mechanism positively regulates TH, AADC and GTPCHI gene expression cooperatively, which may contribute to the recovery of dopaminergic function in nigrostriatal dopaminergic system. We also identified natural compounds that reverse memory dysfunction [27]. These novel compounds facilitated nigrostriatal dopamine biosynthesis [28, 29] promoting the motivation in behavioral actions. Interestingly, in addition to the pharmacotherapies and surgical operations described above, music therapy is receiving attention in the medical care for Parkinson's disease [30, 31]. It is noteworthy that music therapy

does improve motor, affective, and behavioral functions [30]. In fact, listening to music reportedly facilitates dopaminergic neurotransmission in a calcium/calmodulin-dependent manner [32, 33]. This phenomenon suggests the potential mechanism to facilitate CRE-mediated TH gene expression by the activation of calcium/calmodulin-dependent kinase II [34]. These reports mentioned above raise expectations for fundamental therapeutics and the benefits of music in neurodegenerative disorders.

Parkinson's disease and related disorders are currently difficult to cure. However, although fundamental therapies have not been established, development of novel therapeutics is remarkable. Understanding of the regulation of gene expression and degradation of dopamine-biosynthesizing enzymes can contribute to the development of chemical compounds and music therapy for dopamine-related disorders. Studied from the molecule to the organism level, it may provide an effective and sustainable therapeutic approach for treatment of Parkinson's disease and related disorders.

## References

1. Montague PR, Hyman SE, Cohen JD. Computational roles for dopamine in behavioural control. Nature 431 2004; (7010): 760-767.
2. Schultz W. Behavioral theories and the neurophysiology of reward. Annu Rev Psychol 2006; 57: 87-115.
3. Berridge KC. From prediction error to incentive salience: mesolimbic computation of reward motivation. Eur J Neurosci 2012; 35: 1124-1143.
4. Jackson DM, Westlind-Danielsson A. Dopamine receptors: molecular biology, biochemistry and behavioural aspects. Pharmacol Therapeut 1994; 64: 291-370.
5. Nagatsu T, Levitt M, Udenfriend S. Tyrosine Hydroxylase. The initial step in norepinephrine biosynthesis. J Biol Chem 1964; 239: 2910-2917.
6. Blaschko H. The activity of l(-)-dopa decarboxylase. J Physiol 1942; 101: 337-349.
7. Dunkley PR, Bobrovskaya L, Graham ME, von Nagy-Felsobuki EI, Dickson PW. Tyrosine hydroxylase phosphorylation: regulation and consequences. J Neurochem 2004; 91: 1025-1043.
8. Kumer SC, Vrana KE. Intricate regulation of tyrosine hydroxylase activity and gene expression. J Neurochem 1996; 67: 443-462.
9. Chae HD, Suh BC, Joh TH, Kim KT. AP1-mediated transcriptional enhancement of the rat tyrosine hydroxylase gene by muscarinic stimulation. J Neurochem 1996; 66: 1264-1272.
10. Guo Z, Du X, Iacovitti L. Regulation of tyrosine hydroxylase gene expression during transdifferentiation of striatal neurons: changes in transcription factors binding the AP-1 site. J Neurosci 1998; 18: 8163-8174.
11. Suzuki T, Yamakuni T, Hagiwara M, Ichinose H. Identification of ATF-2 as a transcriptional regulator for the tyrosine hydroxylase gene. J Biol Chem 2002; 277: 40768-40774.
12. Kadkhodaei B, Ito T, Joodmardi E, Mattsson B, Rouillard C, Carta M, Muramatsu S, Sumi-Ichinose C, Nomura T, Metzger D, Chambon P, Lindqvist E, Larsson NG, Olson L, Bjorklund A, Ichinose H, Perlmann T. Nurr1 is required for maintenance of maturing and adult midbrain dopamine neurons. J Neurosci 2009; 29: 15923-15932.
13. Rausch WD, Hirata Y, Nagatsu T, Riederer P, Jellinger K. Tyrosine hydroxylase activity in caudate nucleus from Parkinson's disease: effects of iron and phosphorylating agents. J Neurochem 1988; 50: 202-208.
14. Mogi M, Harada M, Kiuchi K, Kojima K, Kondo T, Narabayashi H, Rausch D, Riederer P, Jellinger K, Nagatsu T. Homospecific activity (activity per enzyme protein) of tyrosine hydroxylase increases in parkinsonian brain. J Neural Transm (Vienna) 1988; 72: 77-82.
15. Nagatsu T. Change of tyrosine hydroxylase in the parkinsonian brain and in the brain of MPTP-treated mice as revealed by homospecific activity. Neurochem Res 1990; 15: 425-429.
16. Kawahata I, Ohtaku S, Tomioka Y, Ichinose H, Yamakuni T. Dopamine or biopterin deficiency potentiates phosphorylation at Ser and ubiquitination of tyrosine hydroxylase to be degraded by the ubiquitin proteasome system. Biochem Biophys Res Commun 2015; 465: 53-58.
17. Kawahata I, Tokuoka H, Parvez H, Ichinose H. Accumulation of phosphorylated tyrosine hydroxylase into insoluble protein aggregates by inhibition of an ubiquitin-proteasome system in PC12D cells. J Neural Transm (Vienna) 2009a; 116: 1571-1578.
18. Nakashima A, Mori K, Kaneko YS, Hayashi N, Nagatsu T, Ota A. Phosphorylation of the N-terminal portion of tyrosine hydroxylase triggers proteasomal digestion of the enzyme. Biochem Biophys Res Commun 2011; 407: 343-347.
19. Kawahata I, Yagishita S, Hasegawa K, Nagatsu I, Nagatsu T, Ichinose H. Immunohistochemical analyses of the postmortem human brains from patients with Parkinson's disease with anti-tyrosine hydroxylase antibodies. Biogenic Amines 2009b; 23: 1-7.
20. Cotzias GC, Papavasiliou PS, Gellene R. Modification of Parkinsonism - chronic treatment with L-dopa. N Engl J Med 1969; 280: 337-345.
21. Fernandez HH. 2015 Update on Parkinson disease. Cleve Clin J Med 2015; 82: 563-568.
22. Miocinovic S, Somayajula S, Chitnis S, Vitek JL. History, applications, and mechanisms of deep brain stimulation. JAMA Neurol 2013; 70: 163-171.
23. Coune PG, Schneider BL, Aebischer P. Parkinson's disease: gene therapies. Cold Spring Harb Perspect Med 2012; 2: a009431.
24. Fu MH, Li CL, Lin HL, Chen PC, Calkins MJ, Chang YF, Cheng PH, Yang SH. Stem cell transplantation therapy in Parkinson's disease. Springerplus 2015; 4: 597.
25. Kawahata I, Morita J, Tabuchi A, Tsuda M, Ichinose H,

Izumi Y, Kume T, Akaike A, Yamakuni T. V-1 regulates tyrosine hydroxylase gene expression via SRF-mediated transcription coupled with actin polymerization. J Pharmacol Sci 2012; p: 61P.

26. Kawahata I, Yanxin L, Ohtaku S, Morita J, Kato S, Tabuchi A, Fukuchi M, Tsuda M, Ohashi K, Mizuno K. Novel actin-based regulatory mechanism of nigrostriatal dopaminergic function by V-1 *in vitro* and *in vivo*. J Pharmacol Sci 2014; p: 79P.
27. Kawahata I, Yoshida M, Sun W, Nakajima A, Lai Y, Osaka N, Matsuzaki K, Yokosuka A, Mimaki Y, Naganuma A, Tomioka Y, Yamakuni T. Potent activity of nobiletin-rich *Citrus reticulata* peel extract to facilitate cAMP/PKA/ERK/CREB signaling associated with learning and memory in cultured hippocampal neurons: identification of the substances responsible for the pharmacological action. J Neural Transm (Vienna) 2013; 120: 1397-1409.
28. Yamakuni T, Kawahata I, Yoshida M, Ando H. Composition for improving and/or treating central nervous system degenerative disease. 2013. Int Patent WO/2013/191236A1.
29. Oizumi Y, Yamakuni T, Kawahatal I, Yoshida M. Dried plant tissue and plant tissue extract for ameliorating central nervous system degenerative diseases accompanied by learning/memory disorders, movement disorders and the like, and pharmaceutical agent and food or beverage each comprising the dried plant tissue and the plant tissue extract. 2011. Int Patent WO/2011/105568A1.
30. Pacchetti C, Mancini F, Aglieri R, Fundaro C, Martignoni E, Nappi G. Active music therapy in Parkinson's disease: an integrative method for motor and emotional rehabilitation. Psychosom Med 2000; 62: 386-393.
31. Pacchetti C, Aglieri R, Mancini F, Martignoni E, Nappi G. Active music therapy and Parkinson's disease: methods. Funct Neurol 1998; 13: 57-67.
32. Sutoo D, Akiyama K. Music improves dopaminergic neurotransmission: demonstration based on the effect of music on blood pressure regulation. Brain Res 2004; 1016: 255-262.
33. Akiyama K, Sutoo D. Effect of different frequencies of music on blood pressure regulation in spontaneously hypertensive rats. Neurosci Lett 2011; 487: 58-60.
34. Matthews RP, Guthrie CR, Wailes LM, Zhao X, Means AR, McKnight GS. Calcium/calmodulin-dependent protein kinase types II and IV differentially regulate CREB-dependent gene expression. Molecul Cell Biol 1994; 14: 6107-6116.

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