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Investigation of the roles of dysbindin-1 and SATB2 in the progression of Parkinson's disease

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Abstract. – OBJECTIVE: Parkinson's disease is a neurodegenerative disease that typically results in the loss of dopaminergic neurons, especially in an area of the brain known as the substantia nigra. Here, we investigated the roles of two important neuronal development proteins, dysbindin-1 and SATB2, at different stages of Parkinson's disease.

MATERIALS AND METHODS: Using various concentrations of a neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), we established the mouse models at initial and advanced stages of the Parkinson's disease. The pole and rotarod tests were used to assess behavioral response and motor function, respectively. Histology was used to assess the disease pathology. Immunohistochemistry and Western blotting were used to analyze dysbindin-1 and SATB2 expression levels.

RESULTS: Compared with controls, the mice in the initial and advanced stages of Parkinson's disease required 2.3-fold and 3.8-fold longer to reach the floor in the pole test. Similarly, in the rotarod test, mice in the initial (168 ± 3.73 s) and advanced stages (91 ± 5.62 s) of Parkinson's disease were less able to maintain motor stability, compared with control mice (214 ± 4.18 s). The expression levels of dysbindin-1 and SATB2 in substantia nigra tissue from control mice were limited but were substantially increased (2.4-fold and 3.6-fold, respectively) in mice in the initial stage of the Parkinson's disease. However, in the mice in the advanced stage of Parkinson's disease, dysbindin-1 expression was 1.7-fold lower, and the SATB2 expression was 1.8-fold higher, than that in the control mice.

CONCLUSIONS: The increased expression levels of dysbindin-1 and SATB2 in the initial stage of Parkinson's disease may be due to their protective roles. However, the reduced expression levels in the advanced stage of Parkinson's disease may contribute to irreversible neuronal degeneration.

Key Words:
Substantia nigra, Dysbindin-1, SATB2, Parkinson's disease, MPTP.

Introduction

Parkinson's disease is a type of neurodegenerative disease that more commonly affects older adults, characterized by various abnormalities such as cognitive dysfunction, tremor, slow motion, sensory disorders, autonomic dysfunction, and sleep disorders[1,2]. Although advanced treatments are available, there has been no improvement in life expectancy[3]; moreover, reports[4] have shown that the incidence of Parkinson's disease is increasing. A variety of factors (e.g., age, industrialization, and socioeconomic, sociopolitical, and lifestyle changes) contribute to the development of non-communicable diseases, such as Parkinson's disease[5].

The pathogenesis of Parkinson's disease is not fully understood, but multiple processes play important roles in the onset of the disease; these include a buildup of misfolded proteins in cells, mitochondrial dysfunction, stress in the endoplasmic reticulum membrane, cellular calcium overload, autophagy, and apoptosis[6]. There is still no cure for Parkinson's disease; available treatments only slow or halt the progression of the disease[7]. In addition, the establishment of appropriate treatment is a complex process, due to the heterogeneous nature of the disease[7]. Therefore, new methods for early detection of Parkinson's disease are needed for its management.

Dystrobrevin-binding protein 1 (dysbindin-1) plays an important modulatory role in cognitive functioning; it controls both behavior and neurophysiological activity[8,9]. Dysbindin-1 forms and aggregates in patients with mental disorders, such as schizophrenia[10]. Special AT-rich sequence-binding protein 2 (SATB2) binds to DNA and regulates transcription by recruiting chromatin-modifying proteins[11]. Earlier studies[12] showed that mutations in SATB2 gene loci affect brain function, resulting in mental retardation

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and learning disability. Thus, it is important to investigate the roles of SATB2 and dysbindin-1 in the development of Parkinson's disease.

Materials and Methods

Mouse Model of Parkinson's Disease

Two-month-old male C57BL/6 mice (n = 18) were used to develop the experimental models of Parkinson's disease. The mice were maintained under controlled experimental conditions at 23 ± 1°C with alternating 12-h light and dark cycle. Animal care and experimental protocols for the establishment of Parkinson's disease models were approved by our Institutional Animal Care Committee, in accordance with the strict guidelines of the National Institutes of Health. The mice were divided into three groups, and each group (n = 6) was maintained separately. The mice in the first group served as controls; these mice were intraperitoneally injected with physiological saline, once a day for 15 days. To establish the models of the initial and advanced stages of Parkinson's disease, a standard neurotoxic chemical, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), was used as described previously[13]. The mice in the second group were intraperitoneally injected with MPTP (20 mg/kg), once a day for 10 days; while for the remaining 5 days, these mice were injected once a day with physiological saline. The mice in the third group were intraperitoneally injected with MPTP (35 mg/kg), once a day for 15 days. None of the mice received injections for an additional 5 days (i.e., days 16–20); at the end of day 20, the mice were sacrificed for further analysis. Before sacrifice, the mice were monitored at regular intervals for changes in the behavior and physical activity.

Pole Test

The pole test is a simple test used to evaluate the degree of bradykinesia following MPTP treatment[14]. Before performing the pole test, the mice were acclimated to the experimental environment for 3 days. A 60-cm high wooden pole was placed on a base stand, and the apparatus was then placed inside the home cage. The surface of the wooden pole was rough to prevent slipping. The mice were then placed at the top of the wooden pole. The time taken to reach the floor and the number of the turning movements were recorded. Each mouse performed three tests, with 10 min of rest between the tests.

Rotarod Test

The motor function in the mice was analyzed using the rotarod tests, as previously described[15]. Before the experiment was performed on day 20, all mice were trained at 8, 12, and 15 rpm on days 17-19. The length of time that each mouse could remain on the rolling rod was calculated based on the average of three experiments, with 10 min of rest between the tests.

Histology and Immunohistochemistry

After performing the above tests, on day 20, the mice were deeply anesthetized with an intraperitoneal injection of 1% pentobarbital sodium (10 mg/kg). To prevent tissue damage, the mice were perfused with 4% paraformaldehyde, and the brain tissue was carefully dissected from each mouse. The tissue was sliced into small pieces and then, fixed by incubation in 4% paraformaldehyde for 48 h. The tissue pieces were washed in the water and then, subjected to gradual dehydration, tissue clearing, wax infiltration, and histology analyses. For immunohistochemistry, the sections were incubated with anti-SATB2 antibody (ab212176; Abcam, Cambridge, UK) or anti-dysbindin-1 antibody (ab133652; Abcam, Cambridge, UK) for 6 h at 4ºC. After the sections have been washed with Tris-Buffered Saline with Tween-20 (TBST), they were incubated for 2 h at room temperature with a horseradish peroxidase (HRP)-conjugated secondary antibody diluted in TBST solution. After a final wash, the sections were incubated with the 3,3′-diaminobenzidine solution for 15 min to develop the staining signals; they were then stained with hematoxylin and visualized under a microscope.

Western Blotting

The mouse brain tissues were carefully dissected; the substantia nigra region was sliced and washed with ice-cold Phosphate-Buffered Saline. The substantia nigra tissue was transferred to an Eppendorf tube and subjected to cell lysis using a total protein extraction kit (Beyotime, Nanjing, China), in accordance with the manufacturer's instructions. Following the protein extraction, the total protein concentration was determined using the Lowry method. The samples were separated by 12% SDS-PAGE and then, transferred to polyvinylidene difluoride (PVDF) membranes. After transfer, the PVDF membranes were probed with anti-SATB2 antibody (ab212176; Abcam, Cambridge, UK) or anti-dysbindin-1 antibody (ab133652; Abcam, Cambridge, UK), and the

protein expression levels were detected with an HRP-conjugated secondary antibody and HRP detection kit. All protocols related to Western blotting were performed as previously described[16].

Statistical Analysis

All experiments were repeated independently three times to obtain data for statistical analysis. The data were analyzed using SPSS software (version 22.0; IBM Corp., Armonk, NY, USA). The differences between the groups were evaluated using analysis of variance, followed by Tukey's post-hoc test for multiple comparisons. The differences were considered statistically significant when $p < 0.05$. The results are shown as means ± standard deviation.

Results

Characterization of the Mouse Models of Early and Advanced Stages of MPTP-Induced Parkinson's Disease

To induce the initial stage of Parkinson's disease, C57BL/6 strains of mice were intraperitoneally injected with MPTP (20 mg/kg), continuously for 10 days, followed by saline injection for 5 days. Similarly, to induce the advanced stage of Parkinson's disease, the mice were intraperitoneally injected with MPTP for 15 days as described in the Materials and Methods section. Subsequently, the pole test was conducted in the control mice, as well as in mice in the initial and advanced stages of Parkinson's disease. The results clearly showed that MPTP treatment resulted in the development of the initial and advanced stages of Parkinson's disease, indicated by the onset of the gradual motor function instability in the pole test. Figure 1 shows the length of time that the control mice needed to reach the floor from the pole end (13.38 ± 1.21 s). Notably, the mice in the initial stage of Parkinson's disease needed 2.3-fold more time (30.61 ± 1.41 s) to reach the floor, while the mice in the advanced stage of Parkinson's disease needed 3.8-fold more time (50.44 ± 1.38 s) than the control mice. To further evaluate the motor activity of each group of mice, the rotarod test was performed. As shown in Figure 2, the control mice remained on the rotarod for a longer duration (214 ± 4.18 s) than mice in the initial (168 ± 3.73 s) and advanced stages (91 ± 5.62 s) of Parkinson's disease.

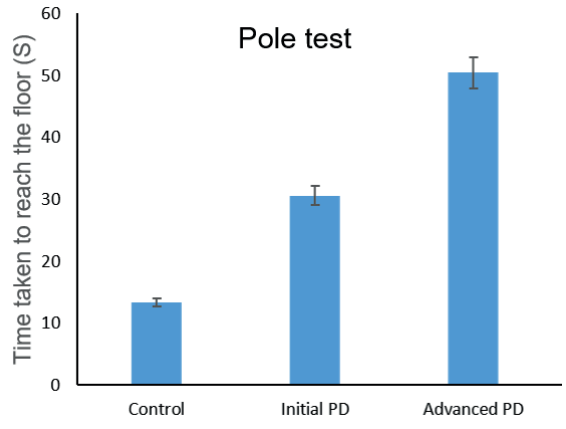

Figure 1. Pole test for assessment of bradykinesia. Control mice, as well as mice in the initial and advanced stages of Parkinson's disease, were subjected to pole tests. The speed of each mouse to reach the floor, from the top of the pole, was calculated in seconds. The data are shown as means ± standard deviation. $p < 0.05$ was considered statistically significant. PK – Parkinson's disease

Histopathological Changes in the Substantia Nigra of Mouse Brain Following MPTP Injection

The following assessment of the behavioral changes, the mouse brains were subjected to histological analysis to elucidate pathological changes following MPTP treatment (Figures 3A-C). In the control mice, the substantia nigra appeared as a well-defined structure with a diffuse, uniform neuronal arrangement (Figure 3A). Conversely, in the initial stage of Parkinson's disease, the neuronal degeneration was observed, together with the formation of Lewy bodies (Figure 3B). In the advanced stage of Parkinson's disease, greater cell loss was observed, as well as greater accumulation of Lewy body structures (Figure 3C).

Expression Levels of Dysbindin-1 and SATB2 in Different Stages of Parkinson's Disease

Immunohistochemistry was performed to detect the expression patterns of dysbindin-1 and SATB2 proteins in substantia nigra tissue at different stages of Parkinson's disease (Figure 4). Dysbindin-1 showed moderate expression in the substantia nigra tissue of the control mice (Figure 4A). Surprisingly, we observed an increased expression of dysbindin-1 in the substantia nigra tissue of mice in the initial stage of Parkinson's disease (Figure 4B), but minimal expression therein in mice in the advanced stage (Figure 4C). SATB2 showed limited expression in the substantia nigra tissue of the control mice (Figure 4D)

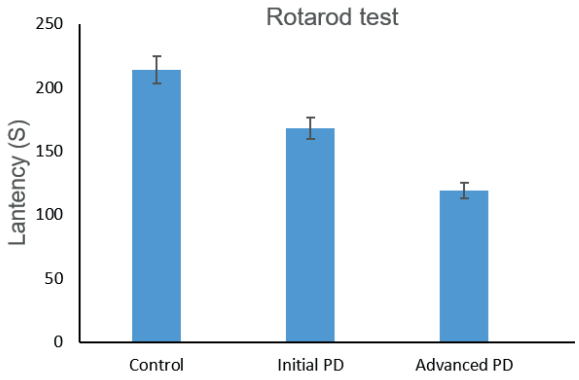

Figure 2. Rotarod test for assessment of motor activity. The length of time that each mouse remained on the rotarod was used as the input value. Graphs are plotted with time in seconds (y-axis) for each mouse group (x-axis). Values are shown as means ± standard deviation.

but showed more prominent expression therein in mice in the initial stage (Figure 4E). Similar to dysbindin-1 the expression of SATB2 was also reduced to minimal levels in the substantia nigra tissue of mice in the advanced stage of Parkinson's disease (Figure 4F).

Furthermore, we used Western blotting to analyze the expression levels of dysbindin-1 and SATB2 in the two different stages of Parkinson's disease (Figure 5). The results showed that dysbindin-1 expression in the substantia nigra tissue of the control mice was high. In addition, dysbindin-1 expression in the substantia nigra tissue of mice in the initial stage of Parkinson's disease was 2.4-fold greater than that in the control mice, which implied that it has a protective effect. However, we observed that dysbindin-1 expression in the substantia nigra tissue of mice in the advanced stage of Parkinson's disease was 1.7-fold lower than that in the control mice. Similar changes were observed with respect to SATB2, whose expression was increased by 3.6-fold in the substantia nigra tissue of mice in the initial stage of Parkinson's disease, compared with that in the control mice. Notably, the expression of SATB2 was reduced in the substantia nigra tissue of mice in the advanced stage of Parkinson's disease, compared with that in mice in the initial stage of Parkinson's disease. We noted that it was only 1.8-fold greater than that in the control mice (Figure 6).

Discussion

In Parkinson's disease, the substantia nigra is unable to produce dopamine, a neurotransmitter vital for motor activity. In this study, the motor activity was evaluated in a mouse model of Parkinson's disease using a pole test; the results confirmed that the motor activities of mice in the initial and advanced stages of Parkinson's disease mice were 2.3-fold and 3.8-fold lower than those of the control mice, respectively. Similarly, the results of the rotarod test confirmed that coordinated body movement was affected. Thus, the toxic effects of MPTP at different doses induced the initial and advanced stages of Parkinson's disease, effectively. The control mice remained on the rotarod for 214 ± 4.18 s, which was the baseline for fatigue[17] without loss of motor function. The present anal-

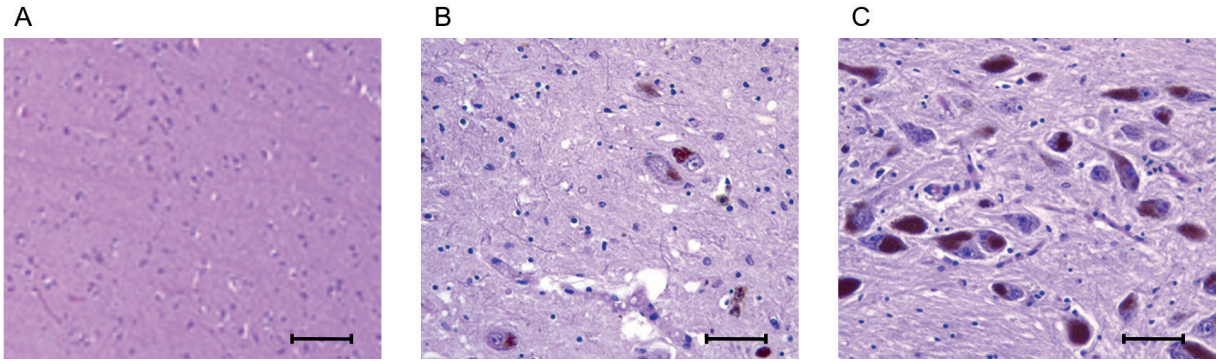

Figure 3. Histopathological changes in the substantia nigra following 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment. ***A,*** Hematoxylin and eosin (H&E)-stained tissue from a control mouse shows diffuse neuronal arrangement. ***B,*** H&E-stained tissue from a mouse in the initial stage of Parkinson's disease shows neuronal loss and the formation of Lewy bodies. ***C,*** H&E-stained tissue from a mouse in the advanced stage of Parkinson's disease shows additional neuronal loss and greater accumulation of Lewy bodies. Scale bar = 50 µm.

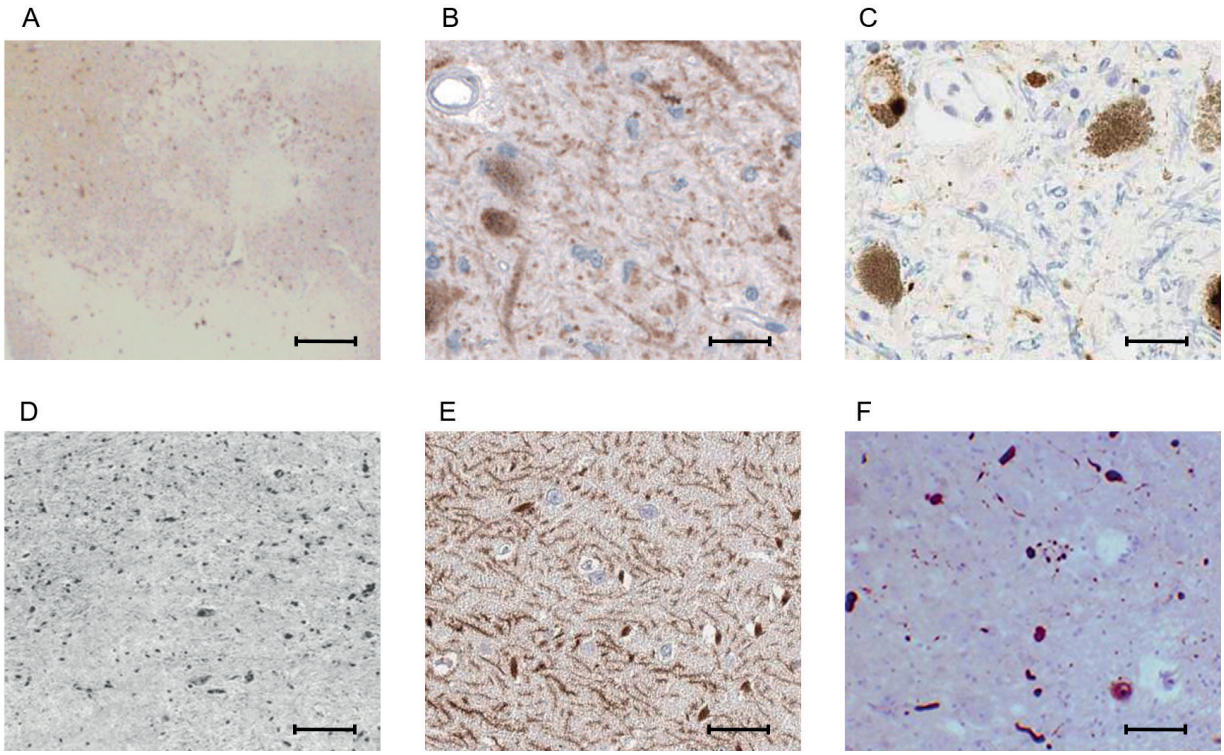

Figure 4. Immunohistochemical analysis of dysbindin-1 and SATB2 expression in the substantia nigra, with hematoxylin counterstain. ***A,*** The tissue from a control mouse shows substantial expression of dysbindin-1. ***B,*** The tissue from a mouse in the initial stage of Parkinson's disease shows overexpression of dysbindin-1. ***C,*** Tissue from a mouse in the advanced stage of Parkinson's disease shows limited expression of dysbindin-1. ***D,*** Tissue from a control mouse shows very limited expression of SATB2. ***E,*** Tissue from a mouse in the initial stage of Parkinson's disease shows prominent overexpression of SATB2. ***F,*** Tissue from a mouse in the advanced stage of Parkinson's disease shows limited expression of SATB2. Scale bar = 50 μm.

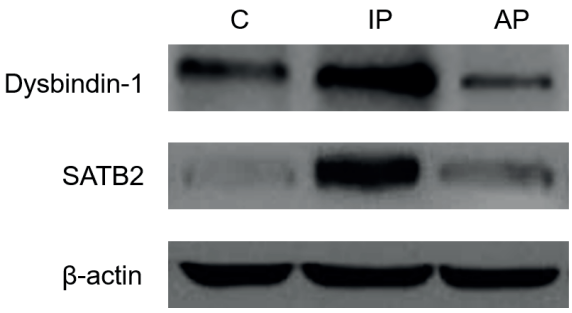

Figure 5. Western blotting analysis of dysbindin-1 and SATB2 expression in the substantia nigra. Lane 1 shows the expression of dysbindin-1 and SATB2 in tissue from a control mouse. Lane 2 shows the expression of dysbindin-1 and SATB2 in tissue from a mouse in the initial stage of Parkinson's disease. Lane 3 shows the expression of dysbindin-1 and SATB2 in tissue from a mouse in the advanced stage of Parkinson's disease. β-actin was used as a loading control.

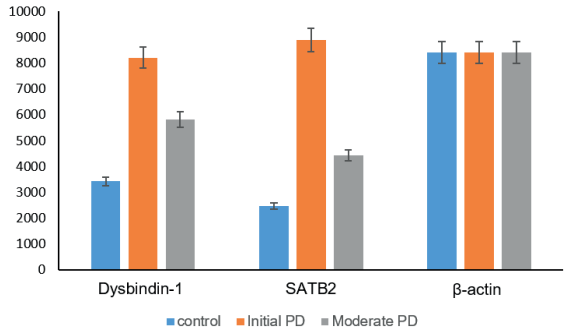

Figure 6. Quantification of dysbindin-1 and SATB2 expression in the substantia nigra of mice in the initial and advanced stages of Parkinson's disease. The expression levels were quantified based on the band intensity and are represented in the bar graph. The experiments were performed three times and the average values were used in the analysis. The data are shown as means ± standard deviation. $p < 0.05$ was considered statistically significant.

ysis was performed with C57BL/6 mice, which exhibit better motor performance than C57BL/10 and 129S2/Sv mice[18,19], and are therefore expected to exhibit a greater consistency in the test results.

Histological studies confirmed that neuronal integrity was unstable with disease progression, as indicated by the formation of additional Lewy bodies in the substantia nigra tissue of the brain in MPTP-treated mice. Although Lewy body formation does not directly correlate with neuronal cell loss, it reflects the clinical progression of dementia[20]. Dysbindin-1 is involved in the release of regulatory neurotransmitters, and in synaptic plasticity; thus, the mutations in this gene can result in schizophrenia[21]. Dysbindin-1 is widely expressed throughout the brain, but particularly in the basal ganglia, substantia nigra, and both temporal cortices[22]. Our results indicated that the increased expression of dysbindin-1 in the initial stage of Parkinson's disease may enhance neuronal plasticity and slow the disease progression. In the advanced stage of Parkinson's disease, the downregulation of dysbindin-1 may lead to irreversible disease progression.

SATB2 is a key transcriptional factor that modifies chromatin structure, thereby regulating other genes[23,24]. It also contributes to neuronal development and cognition, facilitating improved learning and memory[25]. In the present work, the enriched expression of SATB2 in the initial stage of Parkinson's disease may have been protective against disease progression. However, in the advanced stage of Parkinson's disease, expression of SATB2 was downregulated, and the protective effect was lost. The SATB2 expression in substantia nigra tissue in the control mice was limited, as previously reported[26], but abundant signals were observed in mice in both the initial and advanced stages of Parkinson's disease. This increased expression may be related to neuronal repair and efforts to prevent degeneration. In addition to our findings, recent investigations[27,28] regarding Parkinson's disease have identified miRNA-based molecular markers (e.g., miR-331-5p, miR-505, and miR-599), which may be useful in the detection and monitoring of Parkinson's disease. Importantly, further research is needed to fully elucidate the mechanisms of disease progression.

Conclusions

In summary dysbindin-1 and SATB2 were overexpressed in mice in the initial stage of Parkinson's disease, which suggests that they exhibit protective effects. Importantly, in mice in the advanced stage of Parkinson's disease, dysbindin-1 and SATB2 expression were limited, which may contribute to the disease progression.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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