*Chinese Science Bulletin* 2005 Vol. 50 No. 17 1887—1891

# Involvement of the crebellum in sequential finger movement learning: Evidence from functional magnetic resonance imaging

ZHU Yihong[1], DI Haibo[1,2], YUAN Yi[3], REN Jin'ge[3], YU Wei[4], ZHANG Zhaoqi[4], GAO Jiahong[5], WENG Xuchu[3] & CHEN Yizhang[1]

1. Medical School, Zhejiang University, Hangzhou 310006, China;
2. Medical School, Hangzhou Normal University, Hangzhou 310018, China;
3. Laboratory for Higher Brain Function, Institute of Psychology, Chinese Academy of Sciences, Beijing 100101, China;
4. Department of Radiology, Beijing Anzhen Hospital of Capital University, Beijing 100029, China;
5. Research Imaging Center, University of Texas Health Science Center, San Antonio, Texas, USA

Correspondence should be addressed to Weng Xuchu (email: wengxc@psych.ac.cn)

Abstract **Whether the cerebellum is involved in voluntary motor learning or motor performance is the subject of a new debate. Using functional magnetic resonance imaging (fMRI), we examined cerebellar activation in eight volunteers before and after an extended period of training. Activation volume on both sides of cerebellum after learning was significantly reduced compared to that before learning even under the same motor frequency. Remarkably, while motor frequency for the training sequence was significantly higher than the control sequence after 41 d of learning, activation in the cerebellum for both sequences, with respect to activation loci and volumes, was very similar. These results suggest that the cerebellum was involved in motor learning but not motor performance. Changes of cerebellar activation from training thus appear to be associated with learning but not with improvement on task performance.**

**Keywords: cerebellum, learning, performance, sequential movement, fMRI.**

**DOI: 10.1360/982005-520**

The recognition of cognitive functions of the cerebellum is a great breakthrough in the field of neuroscience[1−4]. As one of the important cognitive functions, learning ability of the cerebellum has been intensively investigated over the years. Many fundamental issues, however, still remain poorly understood. For instance, although several studies demonstrated significant changes of cerebellar activation after training, it is not clear whether these changes resulted from learning process or from the improvement of motor performance after learning[5,6]. A number of neurobiological studies showed that animals with lesions in the cerebellum were unable to establish eye-blink conditioning while the eye-blink reflexive remained intact[7,8]. A recent neuroimaging study found that activity in some regions in the cerebellum changed with maze learning, and these practice-related activations occurred in the same hemisphere regardless of the hand used, suggesting that these regions must code information at an abstract level, which can be considered as a kind of ability that does not depend on the task itself[9]. In contrast, a more recent study demonstrated that during a serial reaction time task with a concurrent distractor the reaction time for the fixed and random serial condition was almost identical, and the cerebellum was not activated in either serial. However, upon removal of the distractor, the reaction time for the fixed serial decreased and the cerebellum was activated. Consequently, the cerebellum was thought to be engaged primarily in the modification of performance but not the learning of the motor skill[10]. Such a discrepancy together with the involvement of the cerebellum in voluntary movement makes it difficult to separate the effects of motor learning from motor performance. Whether learning-induced changes of cerebellar activation comes from the change of motor performance or motor learning becomes a new debate[5,6,11].

Furthermore, it is not very clear whether learning leads to an increase or a decrease in cerebellar activation. While some studies showed that the volume of cerebellar activation decreased with the improvement of motor task[12−14], others observed decrease of activation after learning[15,16]. Since motor frequency has significant effect on the activation volume of the cerebellum[17,18], the conflicting results may be at least partly due to poor control of the motor frequency before and after learning (motor frequency often increases after learning).

In the present study, we used functional MRI and a sequential finger movement task to investigate changes of cerebellar activity during learning and examined the relationship between motor performance and motor learning. In order to exclude the effect of motor frequency on cerebellar activation, we controlled the frequency of the motor task before and after learning. This allowed us to more reliably separate motor performance and motor learning. Because short-term learning task is often affected by non-learning factors such as attention[19], long-time learning of 41 d was adopted to obtain more reliable results.

## 1 Materials and methods

### 1.1 Subjects

Eight healthy university students (4 males), aged from 19 to 22 years old, served as subjects. All were right-handed as determined by a Chinese version of a standardized inventory[20]. None had any history of psychiatric or neurological problems.

### 1.2 Motor task

The experimental design is shown in Fig. 1 **A** and **B**

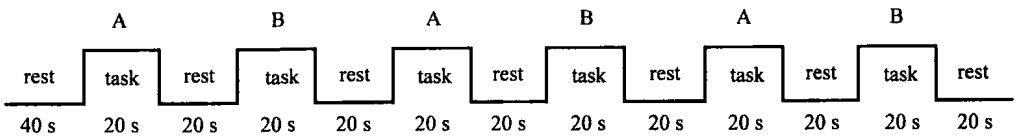


Fig. 1. Experimental design. A and B represent two different motor sequences. In half of the subjects A served as the learning sequence and B as the control sequence. Sequence allocation was opposite in the remaining subjects.

represent two different motor sequences. For counterbalance, in half of the subjects sequence **A** served as the learning sequence and **B** as the control sequence; in the remaining subjects sequence **B** served as the learning sequence and sequence **A** as the control sequence. During scanning, the subjects were asked to press the buttons using their right fingers (except the thumb) with sequence **A** or **B**. The motor frequency, which was always kept at 1 Hz, was paced by a visual cue shown on the screen. A block design was employed. Each task block lasted 20 s followed by a 20 s rest block. There were six task blocks in each run.

During the learning period of 41 d, the subjects practiced the learning sequence for 10 min in the frequency of 1 Hz every day, without any practice for the control sequence. The speed of motor performance was measured every day in the first 6 d of learning, and every 5 d in the remaining learning period. The subjects were asked to press the buttons as quickly as possible. The number of presses was recorded in a period of 25 s and the motor frequency was calculated (with wrong presses excluded).

After 41 d of training, the second scan was conducted. All imaging parameters were the same as the first one.

### 1.3 MRI equipments and scanning parameters

A 1.5T Siemens Sonata magnetic resonance imaging system equipped with a standard head coil was used.

(i) Two-dimensional anatomical images. A T1-weighted Fast Spin Echo (FSE) sequence was used (axial, TR/TE = 447/15 ms, slice thickness = 5 mm, skip = 2 mm, slices number = 20, FOV = 240×240 $mm^2$, matrix = 256×256).

(ii) Functional images. A T2*-weighted gradient-echo echo planar imaging (GRE-EPI) sequence was used (axial, TR/TE = 2000/60 ms, Flip Angle = 90°, slice thickness = 5 mm, skip = 2 mm, slices number = 20, FOV = 220×220 $mm^2$, matrix = 64×64). For each slice, 140 images were acquired with a total scan time of 280 sec in a single run.

(iii) Three-dimensional whole-brain anatomical images. 128 contiguous T1-weitghted sagital images, covering the whole brain volume, were collected with a fast low angle shot (FLASH ) sequence (TR/TE = 30/1.17 ms, Flip Angle = 35°, thickness = 1.3 mm, skip = 0.26, FOV = 325×325 $mm^2$, matrix = 192×256).

### 1.4 Data analysis.

AFNI software[21] was used to analyze and display the image. First, functional images were preprocessed, including motion correction, spatial normalization according to Talairach and Tournoux standard coordination[22], resliced in 3 mm, and spatial smoothing with an isotropic Gaussian kernel of full width at half-maximum (FWHM) 4 mm.

A multiple linear regression analysis was used to calculate the fitness between two regressors, one for sequence A and another for sequence B, and the observed data. *F* value of each voxel was obtained and transformed into standard *Z* value. Only those voxels, whose *Z* values were larger than 3.5 ($P<4.7\times10^{-4}$), were considered to be activated. *Z* values were displayed by pseudo-colors.

Activation volumes before and after learning in left, right and the whole cerebellum for control and learning sequences were calculated. The differences between control and learning sequence, before and after practice were tested with a paired *t* test.

## 2 Results

### 2.1 Behavioral data

All the behavioral data were obtained outside the MRI scanner. Before learning, the averaged motor frequency was 1.91±0.34 Hz for the control sequence and 1.90±0.34 Hz for the learning sequence. After 41 d training, the frequency was 2.58±0.26 Hz for the control sequence and 5.35±0.70 Hz for the learning sequence (Fig. 2). The motor frequencies before and after training were significantly different for the learning sequence ($p<0.01$) and the frequency for learning sequence after training was faster than that for the control sequence ($p<0.01$).

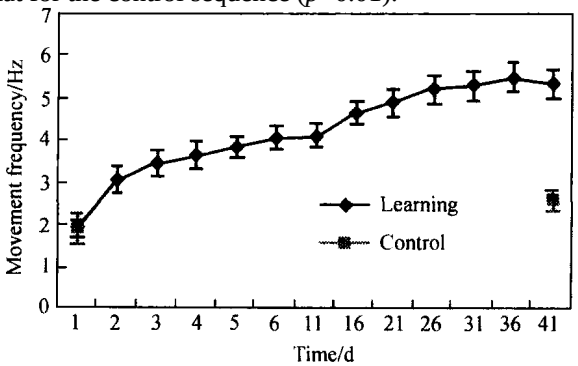


Fig. 2. Change of motor frequency during learning. With the process of learning, motor frequency for learning sequence increased.

### 2.2 Activation in the cerebellum

Before training, activations related to the task were found on both sides of the cerebellum and there were

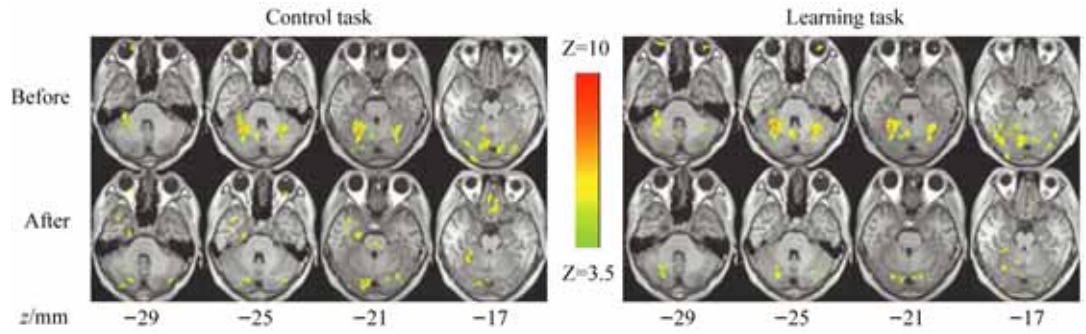


Fig. 3. Activation maps of the cerebellum before and after learning. The voxels whose $Z$ value was larger than 3.5 ($P<4.7\times10^{-4}$) were considered being activated. The left is the activation map for the control sequence and the right for the learning sequence. The upper panel is the activation map before learning and lower panel after learning. $z$ is the distance (mm) from the slice shown to the zero panel in the standard coordinates defined by Talairach and Tournoux atlas.

more activations in the ipsilateral cerebellum. After training, the activations were reduced both for the control and the learning sequence. Fig. 3 is the activation maps of one subject, which shows that activations were observed mainly in the right cerebellum either before or after training and was reduced after training. There were no significant difference between the control sequence and the learning sequence either before or after training.

### 2.3 Comparison of activation volume

To further verify the differences, the activation volumes (the number of activated voxels, the volume of each voxel is 3×3×3 $mm^3$) of the left, right, and whole cerebellum for each condition were calculated. For the learning sequence, the activation volume after practice was significantly less than that before practice. The change in the left cerebellum ($p$=0.02) was more significant than that in the right cerebellum ($p$=0.04). This was also true for the control sequence. The change in the left cerebellum ($p$=0.01) was greater than that in the right cerebellum ($p$=0.03) (see Fig. 4).

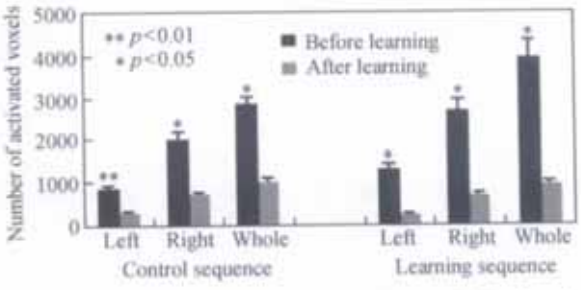


Fig. 4. Comparison of activation volume before and after learning. Either for the learning sequence or the control sequence, the activation volumes in the left, right and whole cerebellum before learning were larger than those after learning.

## 3 Discussion

Consistent with other studies and our previous results, the present study shows that both sides of cerebellum were involved in voluntary movements with dominant activation in the right cerebellum (ipsilateral to the hand used)[23−25]. Our study further shows that after practice, the activation of both sides of the cerebellum was significantly reduced. This result is quite consistent with some previous studies but not all. This discrepancy can be explained as follows. (i) Activations in the cerebellum depend on the duration and intensity of learning. Transient practice often leads to increase of activation volume in some areas of the cerebellum[26], while activation volume for skilled or over-learned sequence were always less than that for newly learned sequence[14,27,28]. (ii) Studies with implicit training paradigm often show decreased activation while those with explicit training paradigm show increased activation[14,27−29]. The present study adopted explicit training paradigm and the result is consistent with this pattern.

It should be noted that most of the previous studies failed to control motor performance in having the same motor frequency before and after learning[12,13]. Although within the range of relatively low frequency, change of the motor frequency has little or even no effect on activation of the cerebellum, the activation does significantly increase with the increase of motor frequency when the frequency is higher than 3 Hz[17,18]. Our behavioural data indicated that after 41 d training the frequency was well above 3 Hz. It is thus critical to control the motor frequency.

The present study clearly showed that even when the frequency was controlled, activation of the cerebellum was still significantly reduced after long-term learning. This finding strongly suggests that the change of activation volume of the cerebellum is related to learning rather than the change of movement frequency after learning. This conclusion is not only consistent with the studies of eye-blink conditioning in laboratory animals[7] and a number of neuroimaging studies in human subjects[9,12,14], but also in accord with the neuropsychological study of patients with cerebellum trauma. For example, Pascual-Leone et al. found that although without being aware

of a fixed sequence, the reaction time for this sequence in a serial reaction time task was reduced with repetition of the task in normal subjects but not in patients with lesions in the cerebellum. The cerebellum thus is believed to play an essential role in learning[30].

A dissociation of behavioral data and cerebellar activation in the present study provided further evidence in support of the involvement of the cerebellum in learning. Behavioral data indicated that the performance of the learning sequence after intensive training was much better than that of the control sequence, whereas imaging data demonstrated that regions and volume of activations in the cerebellum for the two sequences were almost identical. That activation of the cerebellum did not depend on the type of sequence indicates that the change of cerebellar activation is related to the improvement of ability in an abstract level rather than changes of motor performance[9,11]. Similarly, an imaging study with a maze tracing task demonstrated changes of activation in some brain regions after a mere of 10 min training changed and training-induced activations occurred in the same hemisphere regardless of the hand used. According to the authors, these results suggested that the regions that are directly related to maze learning must code information at an abstract level that is distinct from the motor performance of the task itself[9]. In resonance with van Mier et al. study that used hand trained and hand not trained to separate motor performance and motor learning, our study demonstrated the involvement of the cerebellum in motor learning by separating performance and learning with two different motor sequences and by control of the motor frequency.

Heun et al.[31] also showed that encoding and retrieval of sequential finger tapping task can elicit activation in the cerebellum. Although this study did not report change of activation volume in the cerebellum, it was suggested that the activation in the cerebellum was not task-specific. Heun et al. further compared activations of the cerebellum during retrieval between well- and poor-learners after training and no significant difference was found. This finding also suggests that activation of the cerebellum is not related to familiarity of the motor task. This and our present study on convergence support the conclusion that the change of cerebellum activation is related to learning rather than the change of motor performance itself.

Let it be noted that not all studies were consistent with the above conclusion. For example, Seidler et al.[10] recently found that when the participants performed a concurrent distractor task, the reaction time for a fixed sequence in a serial reaction time task did decrease along with learning and the cerebellum was not activated. However, after removal of the distractor, the reaction time decreased and the cerebellum became activated. The authors suggested that the cerebellum might not contribute to learning of the motor skill itself but was engaged primarily in the modification of performance. In contrary, some neuroscientists provided alternative explanations for these results[11]. (i) The distractor itself might change activation pattern of the cerebellum; (ii) learning task in Seidler et al. was a very simple repetition task, which might not be directly associated with particular muscles or movement types; (iii) Seidler et al. used implicit learning. In order to overcome these weaknesses, we adopted 41 d of intensive training, which not only avoided introducing the distractor, but also allowed the subjects to explicitly make practice everyday.

In summary, in the present study we recorded reduced cerebellar activation after 41 d of learning even motor frequency was controlled, and the activations for the control and learning sequence did not differ significantly. These results together indicate that significant changes in cerebellar activation after intensive learning can be observed even the influence of change in motor performance on cerebellar activation is excluded, and the activation appear not to be related to the intensity of training. We thus conclude that the cerebellum is involved in sequential movement learning.

**Acknowledgements** This work was supported by the National Natural science Foundation of China (Grant Nos. 30425008 & 30128005), and a foundation from Health Bureau of Zhejiang Province (Grant No. 2002B019).

## References

1. Gao, J. H., Parsons, L. M., Bower, J. M. et al., Cerebellum implicated in sensory acquisition and discrimination rather than motor control, Science, 1996, 272(5261): 545—547.
2. Xiang, H., Lin, C., Ma, X. et al., Involvement of the cerebellum in semantic discrimination: An fMRI study, Hum. Brain Mapp., 2003, 18(3): 208—214.
3. Hernandez-Muela, S., Mulas, F., Mattos, L., The contribution of the cerebellum to cognitive processes, Rev. Neurol., 2005, 40( Suppl 1): S57—64.
4. Schmahmann, J. D., Sherman, J. C., Cerebellar cognitive affective syndrome, Int. Rev. Neurobiol., 1997, 41: 433—440.
5. Freeman, J. H. Jr., Nicholson, D. A., Developmental changes in the neural mechanisms of eyeblink conditioning, Behav. Cogn. Neurosci. Rev., 2004, 3(1): 3—13.
6. Bracha, V., Role of the cerebellum in eyeblink conditioning, Prog. Brain. Res., 2004, 143: 331—339.
7. Freeman, J. H. Jr., Nicholson, D. A., Developmental changes in eye-blink conditioning and neuronal activity in the cerebellar interpositus nucleus, J. Neurosci., 2000, 20: 813—819.
8. Thompson. R., Neural mechanisms of classical conditioning in mammals, Phil. Trans. R. Soc. (Sect. B), 1990, 329: 161—170.
9. van Mier, H. I., Tempel, L. W., Perlmutter, J. S. et al., Changes in brain activity during motor learning measured with PET: Effects of hand of performance and practice, J. Neurophysiol., 1998, 80(4): 2177—2199.
10. Seidler, R. D., Purushotham, A., Kim, S G. et al., Cerebellum activation associated with performance change but not motor learning, Science, 2002, 296(5575): 2043—2046.
11. Hazeltine, E., Ivry, R. B., Can we teach the cerebellum new tricks? Science, 2002, 296(5575): 1979—1980.

12. Seitz, R. J., Roland, P. E., Learning of sequential finger movements in man: A combined kinematic and positron emission tomography (PET) study, Eur. J. Neurosci., 1992, 4(2): 154—165.
13. Toni, I., Krams, M., Turner, R. et al., The time course of changes during motor sequence learning: A whole-brain fMRI study, Neuroimage, 1998, 8(1): 50—61.
14. Friston, K. J., Frith, C. D., Passingham, R. E. et al., Motor practice and neurophysiological adaptation in the cerebellum: A positron tomography study, Proc. R. Soc. Lond. B Biol. Sci., 1992, 248(1323): 223—228.
15. Seitz, R. J., Roland, E., Bohm, C. et al., Motor learning in man: A positron emission tomographic study, Neuroreport, 1990, 1(1): 57—60.
16. Grafton, S. T., Woods, R. P., Tyszka, M., Functional imaging of procedural motor learning: Relating cerebral blood flow with individual subject performance, Hum. Brain Mapp., 1994, 1: 221—234.
17. Riecker, A., Wildgruber, D., Mathiak, K. et al., Parametric analysis of rate-dependent hemodynamic response functions of cortical and subcortical brain structures during auditorily cued finger tapping: A fMRI study, Neuroimage, 2003, 18(3): 31—739.
18. Jancke, L., Specht, K., Mirzazade, S. et al., The effect of finger-movement speed of the dominant and the subdominant hand on cerebellar activation: A functional magnetic resonance imaging study, Neuroimage, 1999, 9(5): 497—507.
19. Schulze, K., Luders, E., Jancke, L., Intermanual transfer in a simple motor task, Cortex, 2002, 38(5): 805—815.
20. Li, X. T., The distribution of left and right handedness in Chinese people, Acta Psychol. Sin., 1983, 3: 268—275.
21. Cox, R. W., AFNI: Software for analysis and visualization of functional magnetic resonance neuroimages, Comput. Biomed. Res., 1996, 29: 162—173.
22. Talairach, J., Tournoux, P., A Coplanar Stereotactic Atlas of the Human Brain, Stuttgart: Thieme Verlag, 1998.
23. Luft, A. R., Skalej, M., Stefanou, A. et al., Comparing motion- and imagery-related activation in the human cerebellum: A functional MRI study, Hum. Brain Mapp., 1998, 6(2): 105—113.
24. Nitschke, M. F., Hahn, C., Melchert, U. H. et al., Activation of the cerebellum by sensory finger stimulation and by finger opposition movements. A functional magnetic resonance imaging study, J. Neuroimaging, 1998, 8(3): 127—131.
25. Cui, S. Z., Li, E. Z., Zang, Y. F. et al., Both sides of human cerebellum involved in preparation and execution of sequential movements, Neuroreport, 2000, 11(17): 3849—3853.
26. van Mier, H. I., Petersen, S. E., Role of the cerebellum in motor cognition, Ann. NY Acad. Sci., 2002, 978: 334—353.
27. Jenkins, I. H., Brooks, D. J., Nixon, P. D. et al., Motor sequence learning: A study with positron emission tomography, J. Neurosci., 1994, 14(6): 3775—3790.
28. Jueptner, M., Frith, C. D., Brooks, D. J. et al., Anatomy of motor learning. II. Subcortical structures and learning by trial and error, J. Neurophysiol., 1997, 77(3): 1325—1337.
29. Doyon, J., Owen, A. M., Petrides, M. et al., Functional anatomy of visuomotor skill learning in human subjects examined with positron emission tomography, Eur. J. Neurosci., 1996, 8(4): 637—648.
30. Pascual-Leone, A., Grafman, J., Clark, K. et al., Procedural learning in Parkinson's disease and cerebellar degeneration, Ann. Neurol., 1993, 34(4): 594—602.
31. Heun, R., Freymann, N., Oliver, D. et al., Differences of cerebral activation between superior and inferior learners during motor sequence encoding and retrieval, Psychiatry Res., 2004, 132: 19—32.

(Received April 7, 2005; accepted July 6, 2005)

*Chinese Science Bulletin* 2005 Vol. 50 No. 17 1891—1895

# The induction of *Sinorhizobium meliloti* $C_4$-dicarboxylate transport system (Dct) is regulated by oxygen concentration

WEN Jin[1], NAN Beiyan[1], Fergal O'Gara[2] & WANG Yiping[1]

1. National Laboratory of Protein Engineering and Plant Genetic Engineering, College of Life Sciences, Peking University, Beijing 100871, China;
2. Microbiology Department, University College Cork, National University Ireland, Cork, Ireland

Correspondence should be addressed to Wang Yiping (email: wangyp@pku.edu.cn)

Abstract **The *Sinorhizobium meliloti* $C_4$-dicarboxylate transport (Dct) system is essential for symbiotic nitrogen fixation. The *dctA* gene, encoding the $C_4$-dicarboxylate permease, is expressed in both free living and symbiotic cells. But in free living cells expression of *dctD* and *dctB* is absolutely required for the expression of *dctA*. In this study, in order to investigate the effect of oxygen concentration on the induction of Dct system, *E. coli* DH5α strain which carries the plasmid-encoded *dctABD* operon was used in tube assays. It was found that the specific induction of Dct system occurred only at a certain depth under the surface of M63-0.6% agar media, suggesting that Dct system could respond to oxygen concentration during succinate-induced expression. Furthermore, when measured at different oxygen concentrations, the highest expression level was observed at oxygen concentration of 2%. Thus, we predict that in addition to dicarboxylates, the induction of Dct system may also regulated by oxygen concentration.**

**Keywords: *Sinorhizobium meliloti*, Dct system, oxygen concentration.**

**DOI: 10.1369/982004-174**

A mature nodule contains plant cells filled with nitrogen-fixing *rhizobia*, termed bacteroids. $C_4$-dicarboxylates are supplied to the bacteroids by leguminous plants as the major energy source fueling the symbiotic nitrogen fixation process. For this reason, the regulation mechanism of the $C_4$-dicarboxylate transport (Dct) system is of significance for studying symbiotic nitrogen fixation.

In *Sinorhizobium meliloti*, transport of $C_4$-dicarboxylates such as L-malate, fumarate, and succinate occurs via Dct system[1−4]. In free-living cultures, Dct system enables bacteria to use $C_4$-dicarboxylates as sole carbon source. The Dct system consists of three genes: *dctA* encodes the $C_4$-dicarboxylate permease, and *dctB* and *dctD* transcribe divergently from *dctA*. In the presence of $C_4$-dicarboxylate, DctB and DctD could sense this signal and activate the