By dynamic analysis, the 4 QTLs controlled SCT were detected at different stages. During cold treating, SCT was controlled by qSCT-1, qSCT-2 (both from *japonica*), and qSCT-4 (from *indica*). In the earlier stage of recovery it was controlled by qSCT-3, and in the late stage of recovery, it was by qSCT-1 and qSCT-2. This suggested that SCT was controlled by the different combinations of the 4 QTLs (fig. 3). This is in accordance with our preparatory experiment for this study (unpublished data).

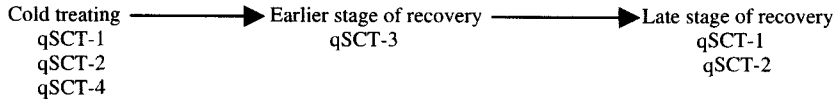

Fig. 3. Four QTLs effect model.

On the 6th and 7th d during cold treating, the died DH lines increased rapidly, indicating that the most serious injury occurred in 3-leaf stage. This is in accordance with Yan's study[5]. By genotype analysis we found that the individuals with 4 positive QTL alleles for SCT showed stronger cold tolerance than the parent JX17, while the individuals with no positive QTLs showed even weaker tolerance than ZYQ8. This indicates that the QTLs for SCT detected in this study can be used in rice molecular breeding for SCT. By integrating the QTLs for SCT, selecting *indica* or *japonica* type, and improving agronomic characters, it is possible to transfer the genes for cold tolerance from *japonica* to *indica* varieties.

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Effects of metal ions on recombinant calcineurin A subunit

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Abstract Effects of metal ions on activities and solution conformations of calcineurin A subunit have been examined. The ability of several metal ions to activate calcineurin A has been tested with Ni^{2+}>Mn^{2+}>Mg^{2+}/Ca^{2+}. The corresponding CD spectra and intrinsic fluorescent emission spectra show that calcineurin A exists in different metal ion-dependent conformation states. Effects of the different concentritions of Ni^{2+} on activities and solution conformations of calcineurin A have been tested too. Results indicate that effects of these metal ions to activate calcineurin are due to their conformational changes.

Keywords: calcineurin, metal ion, CD spectrum, intrinsic fluorescent emission spectrum.

Calcineurin (CN) is a heterodimer consisting of a catalytic subunit (CNA) and a regulatory subunit (CNB). It is an only protein phosphatase that is dependent on Ca^{2+} and calmodulin. Calcineurin, a multifunctional phosphatase[1—4], is of considerable interest because of its essential role in the brain function and the T cell activation pathway. In addition to regulation by Ca^{2+}, CN requires certain

divalent metal ions for activity. The binding of the two most potent activators Mn^{2+} and Ni^{2+} to calcineurin has been reported[5]. Once CN is activated by Mn^{2+} or Ni^{2+} it cannot be deactivated by removal of the free metal activators by dialysis or gel filtration. In a previous work[6] we have indicated that there are several binding sites of exogenous metal ions on CN. In order to address the relationship of CNA structure and function regulated by metal ions, in this study we have shown the effects of Mn^{2+}, Ni^{2+}, Mg^{2+}/Ca^{2+}, and different concentrations of Ni^{2+} on activities and solution conformations of recombinant CNA.

1 Methods

(i) Preparation of Calcineurin A subunit. Expression and isolation of CNA were basically performed by the procedure reported by Wei et al.[7]. The rat δCNA cDNAs encoding sequence was inserted into pET21a expression vector and expressed in *E. coli* strain HMS174 (DE3). The CNA subunit was isolated and purified to near-homogeneity by the procedure of ultracentifugation, Calmodulin-Sepharose 4B and so on. The protein fractions were collected and concentrated by centrifugation through Certriplus 30 membranes (Amicon) or to 50% glycerol for several hours or overnight. The purified materials were stored at -20℃. Protein determination was performed by the procedure described in ref. [8].

(ii) Assay of phosphatase activity[9]. Assay for the activity towards p-nitrophenyl phosphate as the substrate using Mn^{2+} as the activator was performed in 50 mmol/L Tris-HCl, pH 7.4, 1 mmol/L $MnCl_2$, 0.5 mmol/L DTT, 0.2 mg/mL bovine serum albumin, and 20 mmol/L pNPP. When Ni^{2+} was used as the activator, $MnCl_2$ was replaced with 1 mmol/L $NiCl_2$. When Ca^{2+} was used as the activator, $MnCl_2$ was replaced with 6 mmol/L $MgCl_2$ and 0.2 mmol/L $CaCl_2$. Reactions were performed in a volume of 0.2 mL at 30℃ for 20 min and terminated by the addition of 1.8 mL of 0.5 mmol/L sodium carbonate. The absorbance was read at 410 nm. Units of activity were defined as nanomoles of pNPP hydrolyzed per minute.

(iii) Determination of circular dichroism spectroscopy. The enzymes were prepared to a final concentration of 0.4 mg/mL. CD spectra were recorded with a Jasco spectropolarimeter J-700 using 1 mm spectral path length.

(iv) Determination of fluorescent emission spectra. The fluorescent emission spectra were recorded with a Hitachi F-4010 fluorescence spectrophotometer using 1 cm spectral path length. EX and EM bandpasses were 5 nm, respectively. EX wavelength was 280 nm. The enzymes were prepared to a final concentration of 0.08 mg/mL when the interaction of metal ions was tested. The time of incubation is the same as that of assaying enzyme activity.

2 Results and discussion

(i) Effects of different metal ions on CNA activities. CN is not only the Ca^{2+}/calmodulin dependent protein phosphatase, but also an enzyme regulated or activated by many metal ions[10]. It is not clear how those exogenous metal ions affect catalytic subunit and what their mechanism is. In this study, the activities of recombinant CNA subunit were examined and compared at presence of Ni^{2+}, Mn^{2+}, Mg^{2+}/Ca^{2+}, respectively.

The results indicated that these metal ions stimulated CN catalytic subunit itself too and CNA subunit exhibited different activities in the presence of different metal ions. CNA subunit exhibited no activity in the absence of metal ions. The order of CNA activities stimulated by metal ions was $Ni^{2+}>Mn^{2+}>Mg/Ca^{2+}$.

(ii) Effects of different metal ions on CNA solution conformation. High quality and quantity of CNA expression in *E. coli* laid the basis for studying the relationship between the activity and conformation of CN[7]. Under the same condition of CNA activity measurement, the CNA solution conformations were examined in the presence of different metal ions. The corresponding CD spectra of CNA have been examined. Fig. 1 shows that the positive peaks and negative grooves changed greatly after adding Ni^{2+}, Mn^{2+} or Mg^{2+}/Ca^{2+}. The patterns of negative grooves among different ion-dependent conformation states are shown. The difference of these metal ions in activating calcineurin is due to conformational changes.

(iii) Effects of different concentrations of Ni^{2+} on activities and solution conformation of CNA. Whether different concentrations of metal ion Ni^{2+}, which is the strongest ion to stimulate CNA, affected the activities and solution conformation of CNA was studied.

Fig. 2(a) shows that different conentrations of Ni^{2+} affected the activity of CNA. The activities of CNA increased gradually with Ni^{2+} concentration increasing, and they reached the highest at 0.5 mmol/L and 1 mmol/L. The corresponding intrinsic fluorescence emission spectra of CNA subunit at different concentrations of Ni^{2+} are shown in fig. 2(b). Fluorescence quenching increased gradually with increasing Ni^{2+} concentration. It is indicated that with the changing concentration of Ni^{2+}, the conformations of CNA changed correspondingly. These results further showed the relationship between metal ions and solution conformation of CNA subunit.

The above results suggested that the activation of CNA by metal ions is based on the changing conformation of CNA.

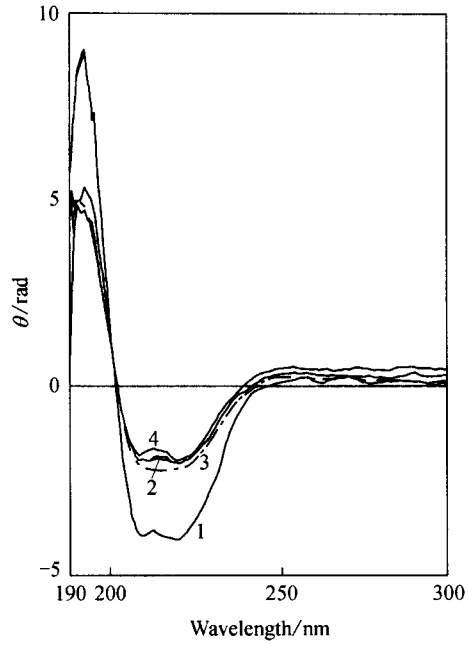

Fig. 1. CD spectra of calcineurin A subunit. 1, Calcineurin A in the presence of free divalent cation; 2, with Mg^{2+}/Ca^{2+} added; 3, with Mn^{2+} added; 4, with Ni^{2+} added.

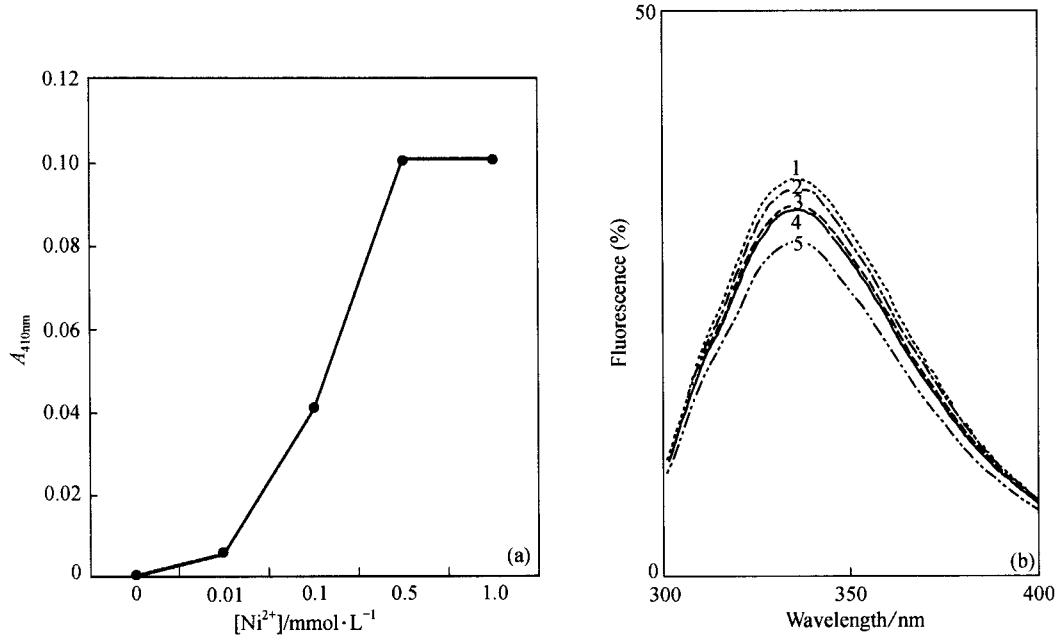

Fig. 2. Effects of different concentrations of Ni^{2+} on activities (a) and intrinsic fluorescence emission spectra (b) of CNA. 1, Calcineurin A with free Ni^{2+}; 2, with 0.01 mmol/L Ni^{2+}; 3, with 0.1 mmol/L Ni^{2+}; 4, with 0.5 mmol/L Ni^{2+}; 5, with 1 mmol/L Ni^{2+}.

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Establishment and application of both FLP and Cre site-specific recombination systems at the same position in the genome

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Abstract Both FRT-FRT and LoxP-LoxP sites that are the target sequences of site-specific recombinases have been constructed in a vector, called C4LFY, using the recombinant DNA technique. C4LFY also contains P elements, 2 exons and 1 intron of Drosophila *yellow* gene, *yellow* promoter and enhancers, and flanking DNA. Since C4LFY made use of two pairs of FRT and LoxP sites, this vector included two site-specific recombination systems. C4LFY was then integrated into Drosophila genome by P-element-mediated germ line transformation. In the presence of the FLP or Cre recombinase, either FLP/FRT or Cre/LoxP recombination reaction was successfully created at the same position in the genome. Using this system, the molecular basis of *yellow* gene expression and regulation during development have been investigated. Results indicate that the tissue-specific expression of *yellow* gene is directly regulated by transcriptional enhancers. In addition, the 5′ and 3′ genomic sequences flanking the *yellow* gene have been preliminarily studied and their potential role is discussed.

Keywords: **site-specific recombination, transgene, gene expression and regulation, *cis*-acting elements.**

Site-specific recombination is a recombinase-mediated DNA recombination reaction[1]. The FLP and Cre site-specific recombinases recognize and interact with their target sites, FRT and LoxP sites, respectively. FLP/FRT and Cre/LoxP recombination systems have been employed in a wide range of biological studies[2-4]. In some cases, however, these systems are still limited to studies of expression and regulation of eukaryotic genes. For example, some experiments demand that the analyzed multiple *cis*-acting elements of a gene be present in identical genomic position. Therefore, function of certain *cis*-acting elements may not be studied effectively because it has not been possible to analyze multiple elements at the same genomic position by single recombination system. Since FRT site consists of only three 13 bp symmetry elements and LoxP site only two 13 bp symmetry elements separated by an 8 bp spacer, there exists the possibility of constructing a vector that contains two pairs of FRT and LoxP sites. Thus, a vector named C4LFY was constructed and a system that included both FLP/FRT and Cre/LoxP recombination reactions at identical genomic position was successfully created by P-element-mediated germ line transformation and recombinant DNA techniques. Using this system, we have advanced the understanding of molecular basis of expression and regulation of *yellow* gene during development.