

## 研究报告

# Expression, Purification and Biological Activity of a Cell-permeable Cre Recombinase\*

GUO Zhong-Min<sup>1)</sup> \*\*, XU Kang<sup>2)</sup> \*\*, YUE Ying<sup>1)</sup>, HUANG Bing<sup>1)</sup>, TANG Huan<sup>3)</sup>,  
Ma Yun<sup>1)</sup>, HONG Xun<sup>1)</sup>, CHEN Xi-Gu<sup>1)</sup>, XIAO Dong<sup>1)</sup> \*\*\*

<sup>1)</sup> Center of Experimental Animals, Sun Yat-sen (Zhongshan) University, Guangzhou 510080, China;

<sup>2)</sup> The First Affiliated Hospital of Sun Yat-sen University, Sun Yat-sen University, Guangzhou 510080, China;

<sup>3)</sup> The Third Military Medical University, Chongqing 400010, China)

**Abstract** The Cre/*lox* P site-specific recombinase system, which has two components: Cre recombinase and two 34-bp *lox* P sites that Cre recognizes, has been widely used in conditional gene knockout/activation to study the structure and functions of gene. In the present study, a cell-permeable fusion protein (His6-NLS-Cre-MTS) containing a 12-amino acid membrane translocation sequence (MTS), a nuclear localization signal (NLS) and an N-terminal His6 affinity tag, was expressed in BL21 strains (e. g., DE3) transformed with pDJHisCre by induction of IPTG. The fusion protein was purified with His-Bond Ni-NTA resin. Its functionality was confirmed in a cell-free recombination assay with a plasmid (e. g., pApoE-SCS-EGFP) containing *lox* P-flanked gene(s), and in an intracellular recombination system using *lox* P-flanked STOP cassette-modified BEL-7402 cells, by assaying the expression of enhanced green fluorescent protein (EGFP). This cell-permeable Cre recombinase provides a rapid alternative means of manipulating mammalian gene structure and function *in vitro* and *in vivo*. Its advantages and potential uses are discussed.

**Key words** Cre/*lox* P system, cell-permeable Cre recombinase, expression, purification, biological activity, cell-free and intracellular recombination systems

The Cre/*lox* P site-specific recombinase system, which has two components: Cre recombinase and two 34-bp *lox* P sites that Cre recognizes, has been frequently and widely used in conditional gene knockout/activation to study the structure and functions of gene(s) [1~4].

It is important to find ways of controlling Cre-mediated recombination to create more flexibility for conditional mutagenesis in mice. Temporal control has been achieved by using either inducible gene promoters or Cre fusion proteins with mutant ligand-binding domains of the progesterone or estrogen receptors which are activated when they bind RU486 or tamoxifen, respectively [1~4]. Although its use in the creation of conditional gene knockout or activation is now routine, progress has been limited by the limited number of transgenic mouse lines that express recombinase in the appropriate tissues/cell types and/or at the specific developmental stage (e. g., see <http://www.mshri.on.ca/nagy>). In addition, virus-mediated Cre gene transfer has been used to introduce Cre into specific cells or tissues [1~4]. Delivering enzymatically active and cell-permeable Cre directly into cells provides an alternative approach to manipulating gene function *in vitro* and *in vivo* [5~8]. Many potential applications of this newly designed Cre are probably equivalent to those that can be achieved via viral delivery, but generating fusion proteins (or buying commercial products) is likely to be much easier than manipulating

recombinant viruses. It is now routine in gene targeting strategies to flox selectable markers for elimination after homologous recombination. Moreover, many manipulations now require Cre-mediated steps to create sophisticated gene alterations. It might be possible to generate mouse embryo fibroblast feeder layers that secrete a cell-permeable Cre, i. e., His6-NLS-Cre-MTS, to delete floxed selectable markers or generate Cre-mediated gene alterations in embryonic stem cells (ES cells) by co-culture, bypassing electroporation and cell cloning steps. Alternatively, the cell-permeable Cre could also be easily added to cultures of developing pre- and post-implantation mouse embryos with floxed alleles to study gene functions in different tissues, for example the visceral endoderm. Another potential application of this protein would be to treat isolated tissues or organs containing floxed genes and use them for transplantation. Whatever the application, this marriage of two technologies promises to be very productive for genetic modification of the growing list of mice with conditional alleles (<http://www.mshri.on.ca/nagy>).

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\*\* Equal contribution. \*\*\* Corresponding author.

Tel: 86-20-87330450, Fax: 86-20-87331230

E-mail: Xiao\_d@hotmail.com

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Against this background, the present study was undertaken to express and purify His6-NLS-Cre-MTS, then to assay its biological activity *in vitro*. This lays a solid foundation for our future projects, which include the conditional activation of hepatitis B virus (HBV) surface antigen and hepatitis C virus (HCV) core protein expression in transgenic mice mediated by the Cre/*lox* P expression switching system, and the construction of a neurite outgrowth inhibitor — Nogo-A temporal gene targeting in mice mediated by the Cre/*lox* P system.

## 1 Materials and methods

### 1.1 Major chemicals and solvents

Lipofectamine™ 2000, RPMI 1640 medium, 0.25% trypsin solution, HEPES, dimethyl sulfoxide (DMSO), culture flasks and plates were supplied by GibcoBRL (Life Technologies). Other chemicals and materials employed throughout this work were obtained from commercial sources and were of the highest quality available, and were used without further purification.

### 1.2 Cell line

The human hepatoma cell line BEL-7402 was from Cell Bank, Center of Experimental Animals, Zhongshan University. The *lox* P-flanked STOP cassette-modified BEL-7402 cells were derived by stably transfecting BEL-7402 cells with pApoE-SCS-EGFP constructed in our laboratory (data not shown). pApoE-SCS-EGFP contains STOP cassette sequence (SCS) flanked by *lox* P sites from pBS302<sup>[9]</sup> positioned downstream of the liver-specific human apolipoprotein E promoter (apoE promoter)<sup>[10]</sup> and upstream of the enhanced green fluorescent protein (EGFP, Clontech). The validity of the *lox* P site sequences in the vector of pApoE-SCS-EGFP has been confirmed in extracellular and intracellular systems. Our previous research has clearly demonstrated that in the absence of Cre, EGFP expression is completely prevented by the intervening transcriptional STOP sequence<sup>[9]</sup>. In the presence of Cre, DNA recombination removes STOP sequence and EGFP expression is activated in the *lox* P-modified BEL-7402 cells.

### 1.3 Preparation of recombinant adenovirus carrying Cre gene

Ad5 CMV Cre virus DNA, in which Cre expression cassette containing human CMV promoter and metallothionein polyadenylation signal was from pBS185<sup>[11]</sup>, was obtained from Professor Beverly L. Davidson, Gene Transfer Vector Core, University of Iowa, USA<sup>[12]</sup>. Ad5CMVCre virus, which expresses Cre, was generated as described previously<sup>[12,13]</sup>. The full length viral DNA was transfected into HEK293 cells. A concentrated and purified virus stock was then prepared, and titers were determined as described

previously<sup>[14]</sup>. Lysates of infected cells were evaluated for Cre activity *in vitro* as described<sup>[15]</sup> (data not shown). Site-specific recombination was confirmed by PCR and sequencing (data not shown).

### 1.4 Expression, purification, and preparation of His6-NLS-Cre-MTS protein

The plasmid pDJHisCre encoding a cell-permeable Cre protein (His6-NLS-Cre-MTS) was generously provided by Ruley<sup>[5]</sup>. Expression and purification of His6-NLS-Cre-MTS protein were performed by standard techniques<sup>[16]</sup> following the manufacturer's instructions (Qiagen). To determine which strain over-expresses this soluble His-tagged Cre, various strains of *E. coli* (DH5 $\alpha$ , JM109, XL-1, BL21 (DE3), HB101, and SSC) from Novagen or Stratgene were transformed with an expression vector (e. g., pDJHisCre) of Cre fusion protein (His6-NLS-Cre-MTS) and induced to express the protein with IPTG (0.8 mmol/L, final concentration) for 2 h at 37°C. The findings from pilot experiments indicated that of the six bacterial strains tested, only BL21 strains, i. e. DE3, expressed high levels of this fusion protein (~56 ku), as demonstrated by SDS-PAGE of the cell lysate supernatants (data not shown). Subsequently, induction of affinity-tagged Cre fusion protein in DE3 transformed with pDJHisCre was performed on a large scale, followed by the isolation and purification of His6-NLS-Cre-MTS on a large scale, as directed by the supplier of the affinity matrix (His-Bond Ni-NTA, Qiagen), from *E. coli* BL21 cells grown to an  $A_{600}$  of 0.8 ~ 1.0 and induced for 2 h with 0.8 mmol/L IPTG (Sigma). After affinity purification, all the fusion proteins were concentrated by ultrafiltration with a 30 ku cut-off membrane at 4°C and dialyzed for 4 h against cell culture medium (RPMI-1640) containing 1% S/P and 3% glycerol at 4°C. The fusion protein solution was filtered with a syringe filter unit (0.45 ~ 5  $\mu$ m) for cultured cells. Finally the fusion protein was quantitated, aliquoted and stored at -80°C. The solution was thawed on a slow rotor at room temperature (RT) immediately before use.

### 1.5 Western blot analysis for fusion Cre protein

Western blots were carried out to detect His6-NLS-Cre-MTS fusion protein (~56 ku) in the BL21 cell lysate supernatant after transformation with pDJHisCre. Standard techniques were used<sup>[16]</sup> following the manufacturer's instructions on the highly sensitive ECL Kit (Amersham). Antibody reaction was performed with monoclonal rabbit anti-Cre antibody (Novagen; 1:200). Horseradish peroxidase-conjugated goat anti-rabbit IgG (Amersham; 1:2500).

### 1.6 Assay of Cre activity using a cell-free recombination system

A cell-free *in vitro* recombination assay using a plasmid (e. g., pApoE-SCS-EGFP) containing two

*lox* P sites in the same direction was employed to test the biochemical activity of the fusion Cre protein. The plasmid pApoE-SCS-EGFP was digested *in vitro* with the purified cell-permeable Cre protein; commercially available Cre (Novagen) was used as control. This was followed by plasmid transformation, digestion with restriction enzymes (Figure 4 and Table 1) and PCR (Figure 4) to confirm Cre-mediated recombination events. Cre digestion was carried out on pApoE-SCS-EGFP following the protocol<sup>[11]</sup> and the manufacturer's instructions (Novagen).

### 1.7 Detecting the biochemical activity of fusion Cre with an intracellular recombination system

The *lox* P-flanked STOP cassette-modified BEL-7402 cells (*lox* P-modified BEL-7402; see above) afforded an intracellular recombination assay for post-purified fusion Cre enzyme activity, following the protocol illustrated in Figure 1. Cell culture was performed using the standard techniques described by Spector *et al.*<sup>[17]</sup>. When the cells reached 50% ~ 80% confluence they were incubated with serum-free RPMI-1640 medium alone, or with serum-free RPMI-1640 medium containing His6-NLS-Cre-MTS (final concentration 10  $\mu$ mol/L), and infected with Ad5CMVCre virus. The cells were exposed to His6-NLS-Cre-MTS (final concentration 10  $\mu$ mol/L) for 2 h, washed extensively, and cultured with complete medium for another 24 ~ 72 h, providing time for EGFP expression. In this experiment, fetal bovine serum (FBS) would have inhibited the transduction of His6-NLS-Cre-MTS. After 24 ~ 96 h, Cre-mediated recombination events in the *lox* P-modified BEL-7402 cells were monitored by assaying for +/– EGFP expression using fluorescence microscopy (excitation 450 ~ 490 nm).

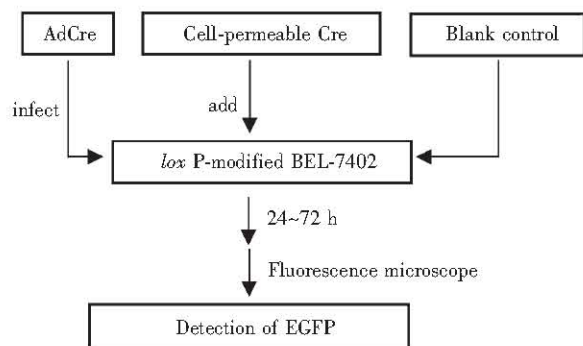


Fig. 1 Protocol for *in vitro* analysis of cell-permeable Cre-mediated DNA recombination

### 1.8 PCR analysis of Cre-mediated DNA recombination

The primers P1 (5'-GGAATTTTCTATGGAGG-CCG-3') and P2 (5'-GAACTCAGGGTCAGCTTG-3')

were used to confirm the post-Cre-mediated DNA recombination product. The above-mentioned PCR reactions involved 30 cycles of denaturation (45 s at 94°C), primer annealing (45 s at 62°C) and primer extension (2 min at 72°C). PCR was performed on plasmid DNA or genomic DNA (prepared by standard protocols<sup>[16]</sup>) (Figure 4) from *lox* P-modified BEL-7402 treated with or without His6-NLS-Cre-MTS or Ad5CMVCre virus (Figure 5).

## 2 Results and discussion

### 2.1 Experimental strategy for conditional gene knockout/activation in our future projects

The Cre/*lox* P system has been most widely used for conditional gene modification *in vivo*, either gene knockout or activation/inactivation<sup>[1,2]</sup>. The general strategies for conditional gene activation/inactivation have been fully demonstrated by Nagy<sup>[1]</sup>, Sauer<sup>[2]</sup> and Figure 2. Cre plays a critical part in such strategies by

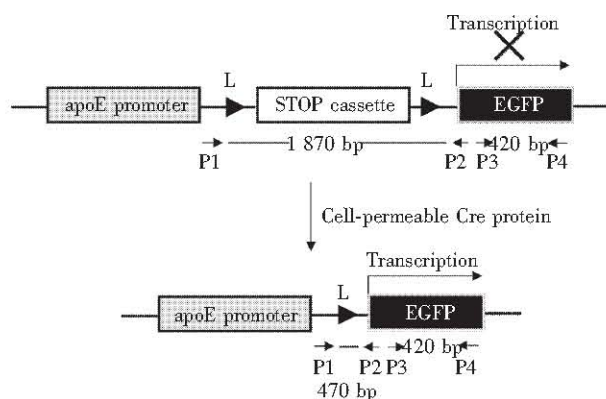


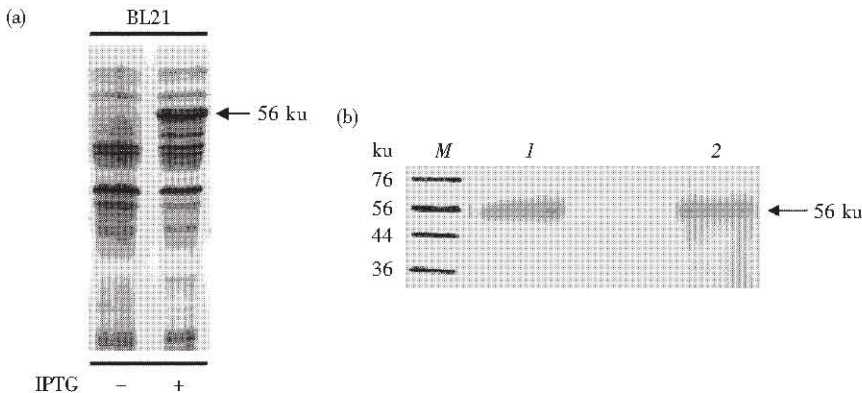
Fig. 2 Strategy for activation of the dormant EGFP gene *in vitro* and *in vivo* by the cell-permeable Cre protein

In the absence of Cre, EGFP expression is prevented by the intervening transcriptional STOP cassette sequence (SCS) flanked by two *lox* P sites in the same orientation (Lakso *et al.*, 1992). In the presence of Cre, DNA recombination removes the STOP sequence, and the Cre automatically joins the apoE promoter and EGFP cDNA, followed by activation of EGFP expression. The primers (P1 and P2) were used for PCR analysis of recombination events. Amplification with primers EGFP-P3 (5'-ACCTACGGCGTGCAGTGC TTC-3') and EGFP-P4 (5'-ACTGGTGCTCAG GTAGTGGTTG-3') resulted in a 420 bp amplified product of the EGFP coding sequence of the transgene. Primers P3/P4 were used as internal controls for PCR efficiency. Abbreviation: L, *lox* P site. The construct map is not drawn to the scale.

deleting *lox* P-flanked ("floxed") gene(s) to induce knockouts/activation. The systems currently used to deliver Cre *in vivo* mainly include transgenic mice, in which the Cre gene is expressed in a temporal or/and spatial pattern<sup>[1~4]</sup>; fusion Cre protein<sup>[5~8]</sup>; and retrovirus or adenovirus delivery systems<sup>[1]</sup>. These latter two approaches are more tedious than simple mating, but they are advantageous in not introducing a Cre transgene into the system investigated. Each of



these ways of introducing Cre into the intracellular environment has its own pros and cons. Depending on the actual strategy and the questions asked, one approach will be preferred. As mentioned in Jo *et al.*<sup>[5]</sup>, fusion Cre has definite advantages, so we plan to express and purify cell-permeable His6-NLS-Cre-MTS that are expected to mediate conditional expression of HBV surface antigen and HCV core protein in transgenic mice, and conditional gene knockout of Nogo-A.



**Fig. 3 The expression and purification of the cell-permeable Cre recombinase**

(a) Induction of affinity-tagged Cre fusion protein in BL21(DE3) strains. BL21(DE3) strains were transformed with an expression vector of a Cre fusion protein (His6-NLS-Cre-MTS) and induced to express the protein with 0.7 mmol/L IPTG for 2 h at 37°C. Samples of uninduced (first lane), and induced (second lane) material were fractionated by SDS-PAGE and the gel was stained with Coomassie blue. The arrow indicates the molecular mass of His6-NLS-Cre-MTS (~56 ku). (b) Western blot analysis of the cell-permeable Cre recombinase. SDS-PAGE and Western blot analysis were performed as described in **Materials and methods**. Western blot analysis was used to test the lysate supernatants from BL21 cells transformed with pDJHisCre (2) and post-purified His6-NLS-Cre-MTS (I). The molecular mass of His6-NLS-Cre-MTS band was ascertained as ~56 ku from the marker protein band (lane M).

To prepare a stock solution of cell-permeable Cre, the solution containing His6-NLS-Cre-MTS was concentrated, dialyzed, quantitated and aliquoted. Eventually, the protein was stored at -80°C until use.

2.3 Assays of Cre enzymatic activity using cell-free and intracellular recombination systems

2.3.1 A cell-free recombination assay

An extracellular recombination system using a plasmid (e. g. pApoE-SCS-EGFP) containing *lox* P-flanked (“floxed”) SCS sequence was used to examine the activity of His6-NLS-Cre-MTS. The recombination event mediated by Cre (Novagen) between two *lox* P sites in the pApoE-SCS-EGFP vector led to excision of the “floxed” SCS (1.481 kb) resulting in plasmid

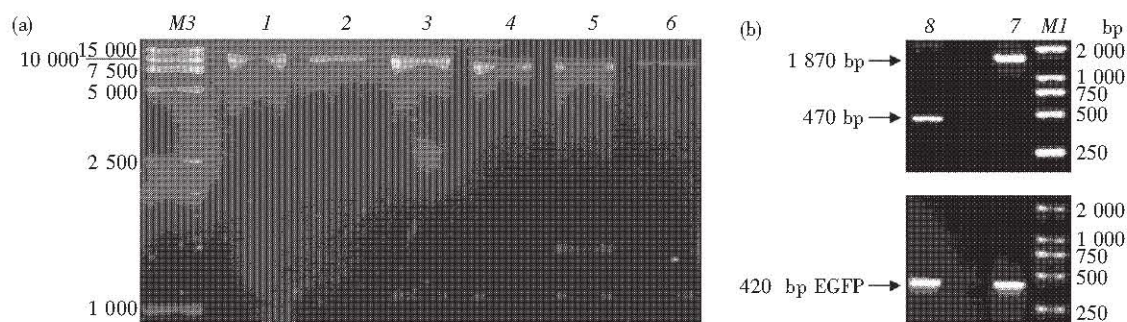
2.2 Expression and preparation of fusion cell-permeable Cre

Of the six bacterial strains tested, only BL21 strains, i. e. DE3, overexpressed this fusion Cre (~56 ku) (Figure 3a). Soluble His-tagged fusion proteins were affinity purified from DE3 cell lysate supernatants with His-Bond Ni-NTA resin (Qiagen), and His6B-NLS-Cre-MTS was identified by SDS-PAGE (data not shown) and Western blotting (Figure 3b).

pApoE-*lox* P-EGFP (Table 1), as confirmed by enzyme digestion analysis (Table 1). DNA recombination mediated by Cre (Novagen) was also detected by PCR (data not shown). PCRs were performed on pApoE-SCS-EGFP and pApoE-*lox* P-EGFP plasmids as templates, with primers P1/P2 to amplify the non-recombined (1 870 bp) and recombined transgene (470 bp) fragments (data not shown), respectively. pApoE-SCS-EGFP was digested with His6-NLS-Cre-MTS prepared in this study, followed by plasmid DNA transformation and analysis of enzyme digestion products (Figure 4a) and PCR (Figure 4b). The recombination results were the same as those mediated by Cre (Novagen).

**Table 1 The identity of each plasmid verified by digestion with the indicated enzyme(s)**

Plasmids	Size	Diagnostic restriction enzyme (s)		
		<i>Kpn</i> I	<i>Hind</i> III	<i>Kpn</i> I and <i>Hind</i> III
pApoE-SCS-EGFP	10.477 kb	10.477 kb	9.481 kb, 0.996 kb	8 kb, 1.481 kb, 0.996 kb
pApoE- <i>lox</i> P-EGFP	8.996 kb	8.996 kb	8 kb, 0.996 kb	8 kb, 0.996 kb



**Fig. 4 The cell-permeable Cre-mediated *in vitro* recombination**

A cell-free *in vitro* recombination assay was employed to examine the biochemical activity of the expressed and purified His6-NLS-Cre-MT followed by the analysis of enzyme digestion (a) and PCR (b) (see **Materials and methods** for details). After recombination, the resulting plasmid (RP; here referred to as pApoE-*lox* P-EGFP) and pApoE-SCS-EGFP (control) DNAs were used as the substrate for enzyme digestion (a) and template for PCR (b). In (b), the arrows indicated the positions of predicted PCR products, 470 bp and 1870 bp fragments amplified from plasmid DNA were diagnostic for the recombined DNA and non-recombined DNA, respectively, 420 bp PCR product was a result of EGFP amplification. See Figure 2 for further details. M3: DL2 000 + DL15 000 Marker (TaKaRa); M1: DL2 000 Marker (TaKaRa); 1: pApoE-SCS-EGFP digested by *Kpn* I; 2: RP digested by *Kpn* I; 3: pApoE-SCS-EGFP digested by *Hind* III; 4: RP digested by *Hind* III; 5: pApoE-SCS-EGFP digested by *Kpn* I and *Hind* III; 6: RP digested by *Kpn* I and *Hind* III; 7: pApoE-SCS-EGFP used as PCR template; 8: RP used as PCR template.

The fact that His6-NLS-Cre-MTS efficiently excised STOP cassette between two *lox* P sites of pApoE-SCS-EGFP demonstrates its enzymic activity.

### 2.3.2 Site-specific recombination in the cultured cells

The strategy for activating the dormant EGFP gene by Cre is shown in Figure 2. The *lox* P-modified BEL-7402 (see above) served as an intracellular recombination assay system for analyzing the biochemical activity of the post-purified fusion Cre.

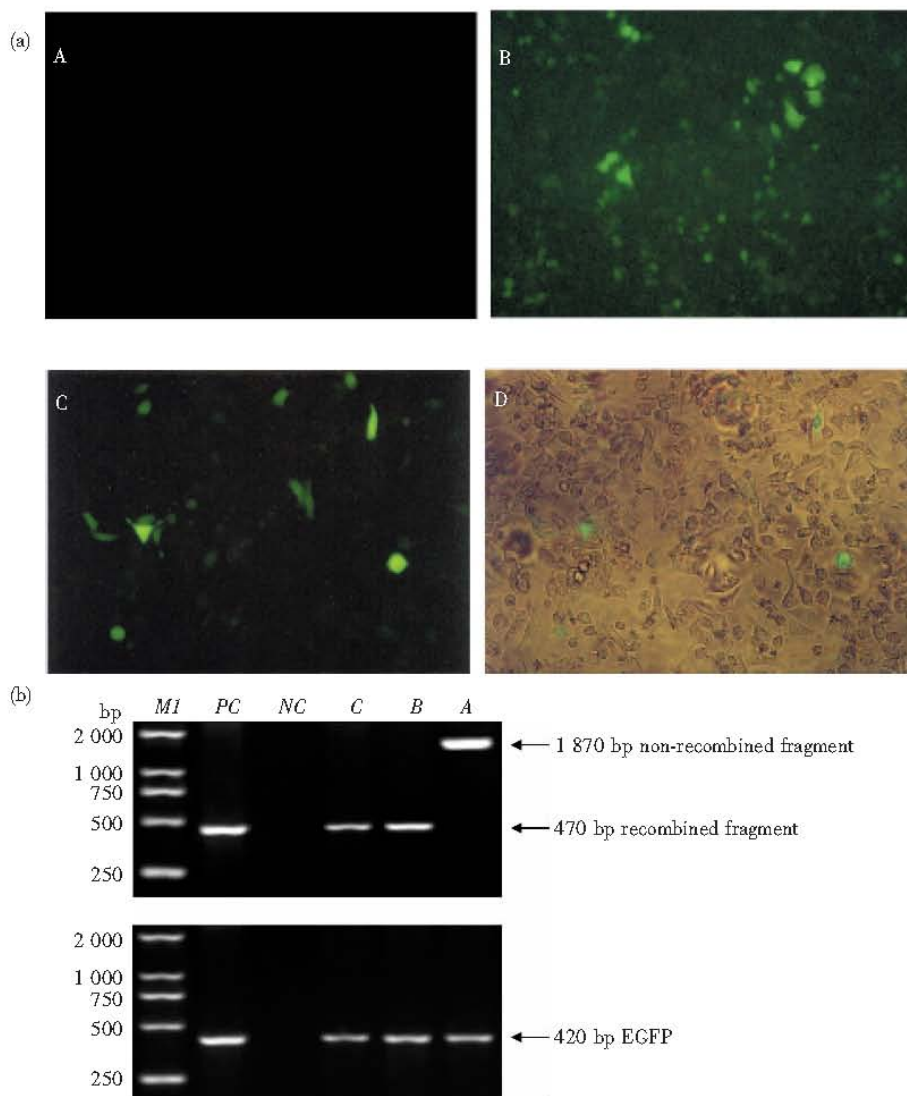
In the *lox* P-modified BEL-7402 cells, Cre-mediated recombination activates expression of EGFP gene. The control cells showed no background under the fluorescence microscope (Figure 5a-A) or phase contrast microscopy (data not shown). 72 h after a 2 h exposure to His6-NLS-Cre-MTS, recombination was observed in most of the cells by EGFP assay (Figure 5a-B) and weak EGFP fluorescence was visible by phase contrast microscopy (data not shown). Similarly, 72 h after the cells were infected with Ad5CMVCre virus, strong EGFP fluorescence was visible by fluorescence microscopy (Figure 5a-C) and weak EGFP fluorescence by phase contrast microscopy (Figure 5a-D).

Finally, PCR analysis confirmed that EGFP expression accurately reflected the extent of template recombination (Figure 5b). The *lox* P-modified BEL-7402 cells contain a single *lox* P-modified SCS gene.

Cre-mediated recombination generates a unique template that can be detected by PCR (Figure 5b). In control cells, a 1 870 bp non-recombined fragment was detected, while cells infected with Ad5CMVCre virus or exposed to His6-NLS-Cre-MTS generated a 470 bp recombined fragment (Figure 5b). Absence of the 1 870 bp fragment does not indicate that recombination occurred in every cell: non-recombined transgenes were detected in Southern blot analysis (data not shown), and this confirmed observations on EGFP (+) in partial cells and by the EGFP and *lacZ* reporter assays<sup>[5,18]</sup>. The non-recombined fragment is not amplified because PCR kinetics favor amplification of the shorter target sequence. Furthermore, treatment with 4  $\mu$ mol/L His6-NLS-Cre-MTS for 2 h induced recombination in 50% of cells (e. g., Tex. *lox*P. EG); this increased to 69% following exposure to 10  $\mu$ mol/L Cre<sup>[6]</sup>. Recombination was also observed in 50% of cells exposed for 30 min to 10  $\mu$ mol/L His6-NLS-Cre-MTS, increasing to 75% after 2 h and 82% after three consecutive 2 h treatments<sup>[5]</sup>. Exposure of *lox* P-modified ES cells to 5 ~ 10  $\mu$ mol/L His6-NLS-Cre-MTS for 2 h was sufficient to induce recombination in 33% ~ 100% of templates.

Overall, these data demonstrate the functionality of His6-NLS-Cre-MTS in cells containing floxed genes.





**Fig. 5** *In vitro* activation of EGFP expression by the fusion cell-permeable Cre recombinase

Conditional expression of EGFP in the *lox P*-modified BEL-7402 cells (carrying the dormant transgene EGFP) was employed to assay the activity of Cre produced from pDJHisCre. (a) EGFP assay under fluorescence microscopy. (A) Blank control; (B) Cells treated with the cell-permeable Cre; (C) Cells infected with AdCre virus (under fluorescence microscopy); (D) Cells infected with AdCre virus (under phase contrast microscopy). (b) Identification of Cre-mediated DNA recombination product by PCR. PCR was performed on genomic DNA isolated from *lox P*-modified BEL-7402 cells of different groups to analyze the recombination events mediated by the cell-permeable Cre and a Cre-producing recombinant adenovirus. A typical analysis of the PCR products by agarose gel electrophoresis from a positive-control [pApoE-*lox P*-EGFP (resulted from removal of SCS from pApoE-SCS-EGFP *in vitro* digested with commercially available Cre) for EGFP and recombined DNA] (lane PC), genomic DNAs from A, B and C groups [for details, see legends to Figure 5] was shown. The arrows indicated the positions of predicted PCR products. The 470 bp and 1 870 bp fragments were diagnostic for the recombined DNA and non-recombined DNA, respectively. The 420 bp PCR product was a result of EGFP amplification. See Figure 3 for further details. Lane NC, PCR without template DNA input. Lane M1, PCR marker (DL2 000, TaKaRa).

## 2.4 Characteristics and advantages of fusion cell-permeable Cre

Cultured cells and a wide variety of non-proliferating, terminally differentiated cell types can be transduced with His6-NLS-Cre-MTS and are competent to undergo recombination soon after exposure to the enzyme (compare Jo *et al.* [5]). When used on cells or in mice containing *lox P*-modified genes, the cell-permeable Cre permits rapid ablation or activation of gene expression both *in vivo* and *ex vivo* (compare Jo *et*

*al.* [5]). This circumvents the need to express Cre on cell type-specific promoters in order to induce recombination in those cell types. Moreover, it is simpler than other methods of temporal control [1~5], and recombination is achieved soon after exposure to the enzyme. This is particularly important in mice, where mutations induced by expressing Cre on a tissue-specific promoter may interfere with cell differentiation or survival, precluding studies of gene function in terminally differentiated cells. Systemic delivery of

His6-NLS-Cre-MTS is remarkably efficient, crossing even the blood-brain barrier. Protein transduction provides a new paradigm for the analysis of mammalian gene functions in cultured cells and mice. Jo *et al.* [5] and the present study illustrate several advantages of protein transduction over conventional gene-based approaches to quantifying the direct effects of an enzyme on processes in living cells under non-steady-state conditions.

## 2.5 Summary and conclusions

In summary, a purified His6-NLS-Cre-MTS, which contains (1) a 12-amino acid MTS facilitating direct delivery into mammalian cells, (2) a NLS from SV40 large-T antigen, and (3) an N-terminal His6 tag, displays the best combination of yield, solubility, *in vitro* specific activity, transferability (into cells), nuclear localization, and intracellular activity (compare Jo *et al.*, 2001) for conditional gene knockout/activation studies. In a word, the cell-permeable Cre recombinase provides an alternative approach to genetically manipulating cells in culture and in live mice.

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# 细胞可透过性 Cre 重组酶表达、纯化及生物活性检测\*

郭中敏<sup>1)</sup>\*\* 徐康<sup>2)</sup>\*\* 岳颖<sup>1)</sup> 黄冰<sup>1)</sup> 唐欢<sup>3)</sup>  
马芸<sup>1)</sup> 洪迅<sup>1)</sup> 陈系古<sup>1)</sup> 肖东<sup>1)</sup>\*\*\*

(<sup>1)</sup> 中山大学实验动物中心, 广州 510080; (<sup>2)</sup> 中山大学第一附属医院, 广州 510080; (<sup>3)</sup> 第三军医大学, 重庆 400010)

**摘要** Cre/*lox* P 系统由 Cre 位点特异重组酶和可被 Cre 特异性识别的 *lox* P 位点构成, 该系统广泛用于条件性基因敲除和表达, 以研究基因功能。为了表达和纯化一种细胞可透过性 Cre 重组酶(即 His6-NLS-Cre-MTS); 经 IPTG 诱导, 在 BL21(DE3) 宿主菌成功表达 His6-NLS-Cre-MTS 融合蛋白, 通过 His-Bond Ni-NTA 树脂分离并纯化了该蛋白质, 随后借助细胞和非细胞的重组系统成功检测了 His6-NLS-Cre-MTS 的生物活性。细胞可透过性 Cre 重组酶提供了一种快捷而高效的在细胞和在体水平进行遗传操作的新工具。

**关键词** Cre/*lox* P 系统, 细胞可透过性 Cre 重组酶, 表达, 纯化, 生物活性检测, 细胞和非细胞的重组系统

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\*\* 共同第一作者。\*\*\* 通讯联系人。Tel: 020-87330450, Fax: 020-87331230, E-mail: Xiao\_d@hotmail.com

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