J Med Dent Sci 2008; 55: 247–254

Original Article

Cre complementation with variable dimerizers for inducible expression in neurons

Tomohiko Maruo[1,2], Tatsuhiko Ebihara[4], Emi Sato[2,5], Satoru Kondo[3] and Shigeo Okabe[2,3,4]

1) Department of Cell Biology, School of Medicine, Tokyo Medical and Dental University, Tokyo, Japan
2) COE Program for Brain Integration and its Disorders, Tokyo Medical and Dental University, Tokyo, Japan
3) Department of Cellular Neurobiology, Graduate School of Medicine and Faculty of Medicine, The University of Tokyo, Tokyo, Japan
4) Molecular Neurophysiology Group, Neuroscience Research Institute, National Institute of Advanced Industrial Science and Technology, Ibaraki, Japan
5) Institute of Applied Beam Science, Ibaraki University, Ibaraki, Japan

Cre complementation is a process of reconstitution of the activity of DNA recombinase by non-covalent association of multiple segments of Cre recombinase, which are enzymatically inactive by themselves. Cre complementation is potentially useful in restriction of Cre activity in a specific subset of cells, with temporal regulation, by limiting overlap in expression of Cre fragments. We analyzed the efficiency of Cre complementation using three different dimerizing modules in the context of non-neuronal cells and found differential Cre complementation efficiency. We further tested the efficiency of Cre complementation in primary hippocampal neurons derived from transgenic mice harboring a reporter gene flanked by loxP sites and confirmed differential activity of dimerization modules in Cre-dependent recombination of the transgene. These results suggest possible application of dimerizer-based Cre complementation in inducible expression/inactivation of target genes in a specific subset of neurons in the complex environment of nervous tissue *in vivo*.

Key words: Cre recombinase, complementation, leucine zippers, hippocampal neurons, adenovirus

Corresponding Author: Shigeo Okabe,
COE Program for Brain Integration and its Disorders, Tokyo Medical and Dental University, Tokyo 113-8519, Japan.
Fax: +81-3-5841-1930,
E-mail address: okabe@m.u-tokyo.ac.jp
Received April 16; Accepted May 15, 2008

Introduction

Cre recombinase derived from bacteriophage P1 is a site-specific DNA recombinase, which catalyzes excision, inversion, or recombination of DNA fragments flanked by 34-bp sequences called loxPs. Cre has been widely utilized in region-specific inactivation of specific genes in combination with generation of mutant mice engineered to place loxP sequences in specific genomic sites[1,2].

Protein engineering of Cre recombinase is a promising approach toward regulating gene expression spatially and temporally. Recently, it has been shown that the cleavage of Cre protein into two independent polypeptides, their re-association and the restoration of recombinase activity are possible[3,4,5,6,7]. However, the potential of Cre complementation has not been fully explored in terms of its efficiency and possible variation in fragment design. One possible way to facilitate the efficiency of Cre complementation is to utilize the dimerizing modules fused to Cre moieties[4,5,7]. Therefore we analyzed the efficiency of Cre complementation systematically using multiple dimerizing modules covalently attached to Cre moieties[8,9,10] in the context of non-neuronal cells. We further tested the feasibility of this approach in modification of genomic DNA using primary hippocampal neurons derived from transgenic mice.

Materials and Methods

Plasmid construction

DNA sequences encoding each Cre fragment were generated by PCR with pBS185 (Invitrogen, Carlsbad, CA) as templates. Amplified DNA was cloned into pGEM-Teasy vector (Promega, Madison, WI, USA). DNA sequences encoding nuclear localization signal (NLS), peptide-binding peptide, GCN4pIL/pLI and F2 linker were also generated by PCR with synthesized oligos as templates. All inserts derived from PCR products were verified for their DNA sequences. DNA sequences encoding Zip(+)/(-) were artificially synthesized (GenScript, Piscataway, NJ). DNA fragments were fused with each other to be for in-frame generation of fusion proteins (Table 1) on pGEM-Teasy or expression vectors with standard DNA recombination techniques. Finally, DNA fragments encoding all fusion proteins were cloned into mammalian expression vectors having either the CMV, β-actin or CAG promoter.

Recombinant adenoviral vectors

Recombinant adenoviral vectors containing the loxP-stop-loxP-EGFP unit under the control of the CAG promoter (Fig. 1B) were as described previously[11]. Translational/transcriptional termination signals and stuffer fragment sequences were included in floxed stop sequences. Adenoviral vectors for the expression of Cre recombinase were provided by Dr. Okado[12]. Adenoviral vectors for the expression of NLS-Zip(-)-F2-CreL(60-343) and NLS-ICS-F2-CreL(60-343) were generated with the ViraPower Adenoviral Expression System according to the manufacture's instructions (Invitrogen) and further purification was conducted[13].

Cre activity assay with Cos7 cells

Cos7 cells were cultured using a standard method. For the Cre activity assay, a defined number of cells (7 x 10^4 cells / well) were plated in 24 well-plates containing 12-mm coverslips (Fisher Scientific, Pittsburgh, PA) coated with 50 μg/ml poly-l-lysine (Sigma, St. Louis, MO). On the next day (14-16 hours later), cells were transfected with 0.3 μg respectively of pCMV plasmids for the expression of CreS and CreL using lipofectamine2000 (Invitrogen). Twenty four hours later, they were infected with reporter adenoviruses (containing pCAG-loxP-stop-loxP-EGFP), at a multiplicity of infection of 50. Two days after adenoviral infection, Cos7 cells were fixed with 2% paraformaldehyde in phosphate buffered saline (PBS) for 25 min at room temperature and washed twice with PBS. They were then permeabilized with 0.1% TritonX-100 in PBS for 10 min, washed 3 times with PBS, and mounted on glass slides and sealed. The preparations were photographed on a Zeiss Axiovert microscope equipped with a Micromax CCD camera (Roper Scientific, Trenton, NJ). Several images were taken per coverslip with the identical illumination and camera setting throughout

Table1 Summary of constructs

No.	Constructs	Binding module	Cre fragment
		(1) Peptide binding peptide pair	
1	NLS(*1)-LEPB-F2-CreS	LEPB(*2)	CreS (19-59)
2	NLS-ICS-F2-CreS	ICS(*3)	CreS (19-59)
3	NLS-LEPB-F2-CreL	LEPB	CreL (60-343)
4	NLS-ICS-F2-CreL	ICS	CreL(60-343)
		(2) Multimerizing GCN4 modules	
5	NLS-GCN4pLI-F2-CreS	GCN4pLI(4mer)(*4)	CreS(19-59)
6	NLS-GCN4pIL-F2-CreS	GCN4pIL(2mer)(*5)	CreS(19-59)
7	NLS-GCN4pLI-F2-CreL	GCN4pLI(4mer)	CreL(60-343)
8	NLS-GCN4pIL-F2-CreL	GCN4pIL(2mer)	CreL(60-343)
		(3) Artificial heterodimer Zip(+)/(-)	
9	NLS-Zip(+)-F2-CreS	Zip(+)(*6)	CreS(19-59)
10	NLS-Zip(-)-F2-CreS	Zip(-)(*7)	CreS (19-59)
11	NLS-Zip(+)-F2-CreL	Zip(+)	CreL(60-343)
12	NLS-Zip(-)-F2-CreL	Zip(-)	CreL(60-343)

(*1) NLS = GPPKKKRKVEDP
(*2) LEPB = KARKEAELAAATAEQ
(*3) ICS = NEAYVHDGPVRSLN
(*4) GCN4pLI(4mer) = RMKQIEDKLEEILSKLYHIENELARIKKLLGER
(*5) GCN4pIL(2mer) = RMKQLEQKIEELLSKIYHLENEIARLKKLIGER
(*6) Zip(+) = LEIEAAFLERENTALETRVAELRQRVQRLRNRVSQYRTRYGPLGGGK
(*7) Zip(-) = LEIRAAFLRQRNTALRTEVAELEQEVQRLENEVSQYETRYGPLGGGK

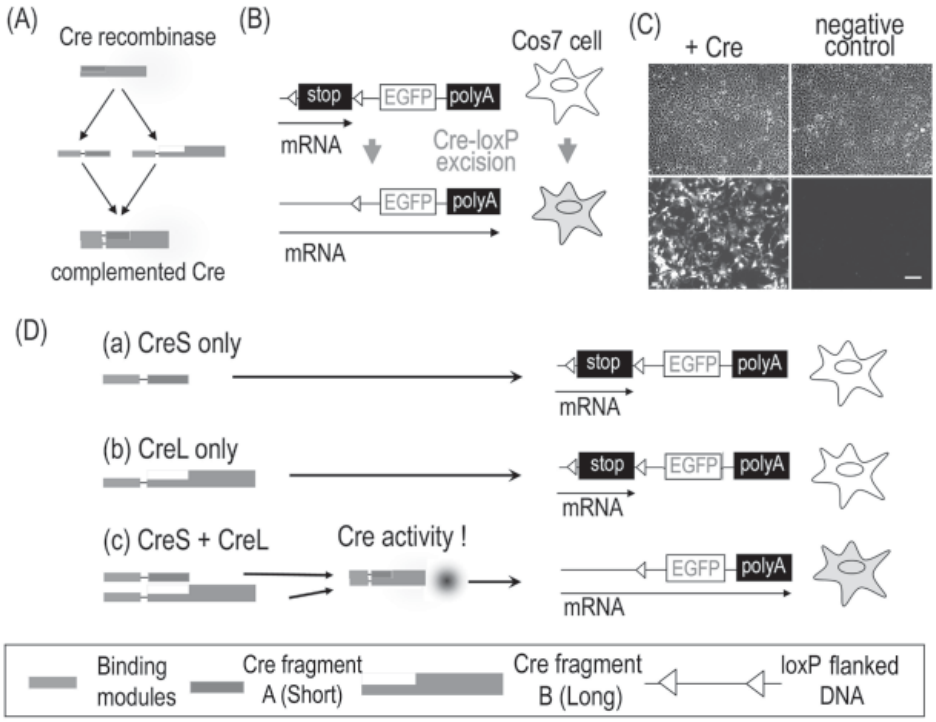

Fig. 1. Cre complementation system
(A) Principles of Cre complementation. Cre complementation requires adequate design of Cre fragments A and B attached to binding modules. Subsequent co-expression of two fragments induces self-association of two proteins, leading to recovery of Cre activity.
(B) Design of the reporter adenovirus. When loxP-stop-loxP-EGFP units are introduced into cells without Cre activity, transcription is terminated by a polyA addition signal in stop sequences flanked by two loxP sites. After loxP excision by Cre, EGFP-encoding sequences become transcriptionally active (shadowed Cos7 cells).
(C) Cos7 cells transfected with either Cre expression plasmid or control plasmid expressing β-galactosidase, followed by infection of reporter adenoviruses. Phase contrast images (upper row) and EGFP fluorescence (lower row) are shown. Only cells transfected with Cre expression plasmid showed EGFP fluorescence, indicating reliable monitoring of Cre activity by EGFP in this Cos7 expression system. Scale bar, 100 μm.
(D) Assay of Cre complementation. Transfection of individual Cre fragments (fragment A (a) or B (b)) into Cos7 cells and subsequent infection of the reporter adenoviruses do not induce EGFP fluorescence. Only after complementation of Cre by co-expression of fragments A and B (c), do Cos7 cells become fluorescent.

the experiments. Each imaging field was chosen randomly.

Hippocampal cultures from transgenic embryos

Male mice heterozygous for the loxP STOP-GFP (pβ-actin-loxP-stop-loxP-EGFP unit transgene[12]) were mated with wild-type females, and the embryos at embryonic day 17 (E17) were dissected separately. Preparation of hippocampal culture and identification of transgenic embryos were conducted as described previously[12,13,14]. Transfection into transgenic neurons was done at 3-4 days in vitro. A total of 0.3-0.6 µg of plasmid DNA was used to transfect cells on several coverslips coated with 1 mg/ml poly-l-lysine placed in a 35-mm culture dish using lipofectamine2000. Cells were infected with CreL adenoviruses at 17 days in vitro and fixed at 24 days in vitro. To unambiguously identify cells with weak EGFP fluorescence, the EGFP signal was amplified by immunostaining with rabbit anti-EGFP antiserum (Invitrogen) and anti-rabbit IgG antibody conjugated to Alexa488 (Invitrogen). Observation was conducted as described in the Cos7 assay system.

Image analysis

Image analysis was performed using Metamorph software (Universal Imaging Corporation, Downingtown, PA). Multiple images from each condition were compared after identical image processing was conducted. Digital images from 9-10 different fields were selected and the absence of pixels with saturated signal intensity was confirmed. The average total fluorescence intensities under different conditions were calculated. For the statistical analysis, one-way ANOVA and Scheffe's post hoc test were done using Origin4.1 software (MicroCal Software, Northampton, MA).

Abbreviations

ANOVA, analysis of variance; CAG, AG promoter (modified chicken β-actin promoter) with CMV immediate-early enhancer; CCD, charge coupled device; CMV, cytomegalovirus; EGFP, enhanced green fluorescent protein; GCN4, General Control Nondepressible 4; ICS, interleukin converting enzyme cleavage site epitope; LEPB, library encoded peptide B; mRFP-1, monomeric red fluorescent protein-1; NLS, nuclear localization signal; PBS, phosphate buffered saline; polyA, polyadenosine.

Results

Assay of Cre activity in non-neuronal cells

The first step in developing an optimal Cre complementation system is a survey of candidate pairs of Cre fragments and assessment of their efficiency to re-associate and restore enzymatic activity (Fig. 1A). For this purpose, it is necessary to establish a reliable assay system for the detection of reconstituted Cre activity in living cells.

An effective approach toward real-time detection of Cre recombinase in culture cells is detection of DNA fragment excision between two loxP sites by induction of reporter genes, such as EGFP. Previous analyses on Cre complementation also utilized EGFP-based reporter plasmid constructs to successfully monitor reconstituted Cre recombinase activity within single cells, even more accurately than a system with genomic reporter-bearing cells[5]. To improve detection sensitivity, we generated recombinant adenoviruses containing a loxP-stop-loxP-EGFP unit under the control of the CAG promoter (a chimeric promoter of β-actin and CMV)[11] (Fig. 1B). The reporter adenoviruses showed no cell toxicity and inhibition of cell growth two days after infection. To estimate infection efficiency, we infected the reporter adenoviruses together with another type of adenoviruses containing Cre recombinase sequences under the control of the CAG promoter. We observed EGFP fluorescence in more than 90% of Cos7 cells two days after co-infection, indicating successful introduction of CAG promoter-loxP-stop-loxP-EGFP sequences into the majority of cells under this condition.

To efficiently screen multiple Cre fragments for their ability to restore Cre activity, we combined transient chemical transfection of multiple Cre fragments with adenovirus-mediated introduction of the reporter gene. We transfected candidate pairs of expression plasmids of Cre fragments, and on the next day the same cell preparations were exposed to the reporter adenoviruses. The cells were examined for EGFP expression using a fluorescence microscope two days after infection of the adenoviruses (Fig. 1D). We assessed the number of EGFP-positive cells after transient transfection of either authentic Cre recombinase or control β-galactosidase under the same CMV promoter. In Cos7 cells transfected with CMV-Cre, more than 80% of cells were EGFP fluorescence positive. In contrast, no EGFP-positive cells were present in Cos7 cells expressing β-galactosidase (Fig. 1C). These results collectively indicate reliable and efficient detection of Cre recombinase activity using reporter adenoviruses and Cos7 cells.

Artificial binding modules for Cre complementation

To design constructs of Cre complementation, two factors should be taken into account. First, Cre breakpoints should be appropriately designed. Second, fusion constructs with additional dimerization modules may increase the efficiency of complementation.

Cre complementation has been reported by several researchers. The reported successful fragments pairs are 1-190G/191G-343D[5], 19T-59A/60A-343D[4], 19T-104L/106R-343D[4], and 1-196H/182V-343D[3]. From these possibilities, we selected a breakpoint between 59A and 60A[4]. This site is in the N-terminal domain, thus we can leave intact the larger C-terminal moiety, which includes major active sites for DNA recombination. Addition of dimerizing modules has been reported to increase the efficiency of Cre recombination. One report utilized a rapamycin-induced hetero-dimerizing system, which is beneficial for temporal regulation of Cre activity, but may not be optimized for recombination efficiency[4]. The other group reported application of anti-parallel hetero-leucine zipper peptides[5]. This dimerizing module is not compatible with re-association of Cre moieties with the 59A/60A breakpoint, thus

we predicted parallel alignment of the two N-terminal ends of Cre moieties, where dimerization modules should be attached (Fig. 2A).

For these reasons, we searched other interacting peptide motifs and selected the following three known artificially designed parallel binding modules. (1) Peptide-binding peptide pair[9]. Random screening of peptides which bind to 14-amino acid peptides from immature interleukin-1β (ICS peptide) resulted in identification of a 15-amino acid peptide (LEPB peptide). (2) Multimerizing GCN4 modules[8]. These modules were derived from the DNA-binding transcription factor of yeast. The leucine zipper region was artificially modified and multimerized as a tetramer or dimer. (3) Artificial heterodimer Zip(+)/(-)[10]. This is a pair of synthetic leucine zippers based on vitellogenin-binding protein, a member of the chicken b-ZIP family. This pair shows high heterodimerization ability and low homodimerization ability, and is thus regarded as a suitable binding module. Previous studies showed that both multimerizing GCN4 modules and the artificial heterodimer Zip(+)/(-) exhibited strong binding ability and could be applied to the studies of neuronal cells[15,16].

We designed 12 plasmid constructs with different combinations of artificial binding modules and Cre fragments linked with F2 linker (Table1). Because these binding modules lack NLS, we added NLS to the N-termini to facilitate nuclear localization[17].

We examined EGFP expression in Cos7 cells transfected with binding module-Cre fragments followed by reporter virus infection. Single transfection of binding module-CreS or binding module-CreL fragments did not induce EGFP expression, confirming the inactivity of split Cre. However when CreS(19-59) and CreL(60-343) fused to binding modules were co-transfected, all of the combinations showed EGFP expression in cells, but the number of cells expressing EGFP and the intensity of EGFP signals differed among the combinations. We compared these indexes within each type of binding module and selected the following appropriate combinations for detailed characterization: LEPB-CreS(19-59)/ICS-CreL(60-343), pIL(2mer)-CreS(19-59)/pIL(2mer)-CreL(60-343), and Zip(+)-CreS(19-59)/Zip(-)-CreL(60-343) (Fig. 2B).

To further compare complementation efficacy, we evaluated the expression of EGFP intensity for LEPB-CreS/ICS-CreL, pIL(2mer)-CreS/pIL(2mer)-CreL, and Zip(+)-CreS/Zip(-)-CreL in Cos7 cells transfected simultaneously. The intensity of EGFP was measured by summing the signals in the fields where similar numbers of cells existed. From this analysis, we con-

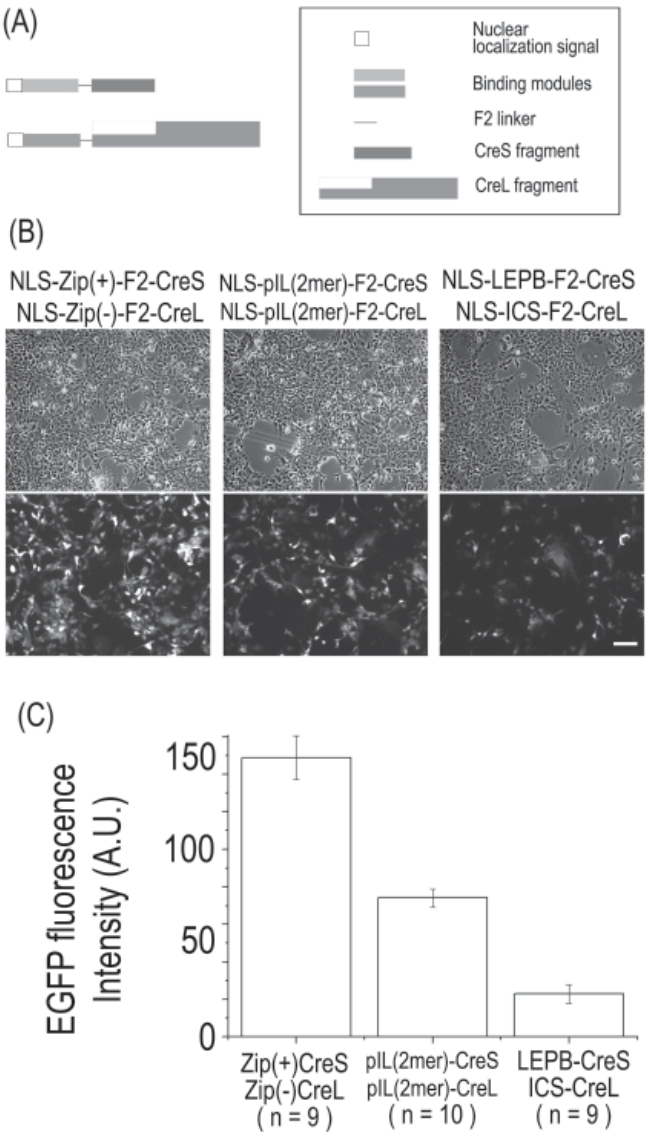

Fig. 2. Differential levels of EGFP fluorescence induced by Cre-complementation in Cos7 cells transfected with three distinct sets of re-associable Cre fragment constructs

(A) Modular design of Cre fragment constructs. The details of binding modules are shown in Table 1 and described in the Materials and Methods section. The squares in the box on the right indicate their properties.

(B) Phase contrast and fluorescence images of Cos7 cells transfected with Cre fragment pairs fused with different binding modules. Scale bar, 100 μm.

(C) Quantification of EGFP fluorescence. The ordinate represents the total EGFP fluorescence intensity (arbitrary unit) within randomly selected fields. Total numbers of fields examined are indicated under the names of binding module-Cre fragment pairs. Two independent experiments were performed. The difference between conditions was statistically significant, determined by one-way ANOVA followed by post-hoc analysis of Scheffe's test. Error bars represent the S.E.M.

cluded the following order of efficiency of binding modules for Cre reconstitution; Zip(+)-CreS/Zip(-)-CreL > pIL(2mer)-CreS/pIL(2mer)-CreL > LEPB-CreS/ICS-CreL (Fig. 2C).

Application of Cre complementation in cultured neurons

Finally, we tested two pairs of artificial binding modules for their ability to achieve excision of floxed stopper sequences and induction of a transgene in primary hippocampal neurons. We designed the sequential gene delivery system to initiate expression of individual Cre fragments at different stages of neuronal maturation and tested whether a subset of neurons expressing both Cre fragments could restore Cre activity after the second gene delivery.

We previously reported Cre-dependent induction of EGFP expression in hippocampal neurons by using transgenic mice containing a loxP-stop-loxP-EGFP unit under the control of the β-actin promoter[12,14]. Strong expression of EGFP was observed in pyramidal neurons, with less prominent expression in interneurons. By preparing dissociated neurons from this transgenic embryo, we expect to detect Cre-complementation with high fidelity. To achieve sequential expression of Cre fragment pairs, we utilized a combination of chemical transfection into immature neurons, generally resulting in prolonged expression of reporter genes over several weeks, and subsequently recombinant adenovirus-mediated gene transfer at a later stage.

We introduced pCMV-LEPB-CreS or pCMV-Zip(+)-CreS into immature neurons together with an expression construct of mRFP-1 under the control of β-actin promoter using a chemical transfection method. Two weeks after transfection, culture preparations were infected with recombinant adenoviruses containing pCAG-ICS-CreL or pCAG-Zip(-)-CreL sequences. We examined Cre-dependent EGFP expression by staining with anti-EGFP antiserum. Transfection efficacy estimated by the number of mRFP-positive cells was very low with this protocol. The proportion of transfected neurons was less than 1% of total cells.

We identified EGFP-positive neurons at an estimated frequency of 1 per 10^3 surviving neurons when the Zip(+)-CreS/Zip(-)-CreL pair was utilized (Fig. 3A and B). We observed partial overlap of mRFP- and EGFP-expressing neurons. The efficiency of Cre-dependent complementation was estimated to be lower in the case of the LEPB-CreS/ICS-CreL pair, in the range of 1 per 10^4 surviving neurons. As expected, no EGFP-positive neurons were detected in preparations either transfected with CreS constructs without adenovirus infection (Fig. 3A), or expressing only CreL fragments by adenoviruses (data not shown). We obtained suc-

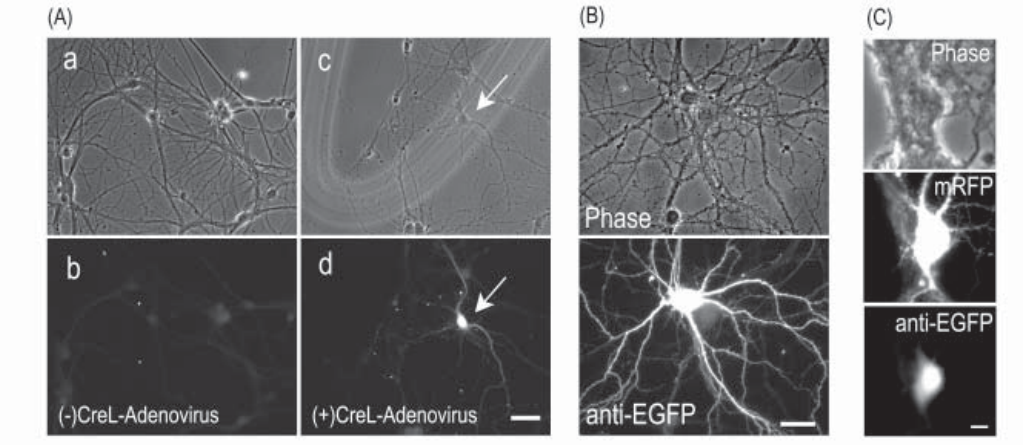

Fig. 3. Cre complementation in mature hippocampal neurons derived from mice containing the β-actin promoter- loxP-stop-loxP-EGFP transgene
(A) Phase contrast images and fluorescence images of EGFP-positive neurons derived from transgenic mice, transfected with a plasmid encoding Zip(+)-CreS(19-59), followed by infection of adenoviruses for the expression of Zip(-)-CreL(60-343) (c, d). Control cultures without adenovirus infection show no EGFP fluorescence (a, b). Scale bar, 50 μm.
(B) Higher magnification images of another EGFP-positive neuron. Scale bar, 30 μm.
(C) An mRFP-1 and EGFP double-positive neuron in culture derived from transgenic mice, co-transfected with plasmids encoding mRFP-1 and LEPB-CreS(19-59), and subsequently infected with adenoviruses for the expression of ICS-CreL(60-343). Scale bar, 15 μm.

cessful Cre complementation in two major subtypes of hippocampal neurons, namely pyramidal neurons and interneurons.

Discussion

Cre complementation is a strategy with several advantages over conventional Cre-dependent gene rearrangement. Its advantages in biological applications rely on both restriction of cell types by combination of promoters with different activities and temporal control of Cre activity by sequential expression of Cre fragments.

Possibility of Cre complementation has been explored recently by several laboratories and their results collectively indicate efficient restoration of Cre activity after co-expression of two partners with or without additional dimerization modules. Structural similarities between Cre and topoisomerase I, as well as the crystal structure of Cre, are informative in systematic determination of breakpoints. The structural information of Cre helped to achieve complementation without additional dimerization modules when two fragments with overlapping amino acid sequences 182V-196H were used, with reduced but modest recombinase activity[3]. Addition of interacting modules may further enhance recovery of Cre activity after co-expression[5], and may also increase the speed of Cre re-association, which is important in regulating rapid biological phenomena during development. Jullien et al. reported efficient recovery of Cre activity when a Cre breakpoint was introduced between 59A and 60A, and rapamycin-dependent dimerization modules were attached to the N-terminals of two fragments[4]. As previously described in the Results section, we used the design of a Cre breakpoint between 59A and 60A. Instead of using a drug inducible system, we took an approach to optimize complementation efficacy by screening multiple dimerizing modules. Thus, we selected three artificial dimerization modules that bind in parallel configuration[8,9,10,15,16]. Among them, Zip(+)/Zip(-) binding partners showed the highest activity when they are attached to CreS(19-59) and CreL(60-343) fragments. The other two binding partners (peptide-biding peptides and GCN4pIL(2mer)S/pIL(2mer)L) showed relatively weak enhancement in Cre complementation. This difference may be explained by their weak affinity, low expression level or differential intracellular localization. Although we have not yet tested the latter two possibilities by western blotting and immunocytochemistry of transfected cells, as expected, we found different efficacies of Cre enzymatic activities in both Cos7 cells and primary neurons using several pairs of divided Cre moieties, which are inactive by themselves.

What is the advantage of Cre complementation in neurobiology? One possible application is precise control of cell types by differential expression of two Cre fragments with distinct gene delivery methods. For example, introduction of adenoviruses expressing CreS(19-59) into the neocortex, which already expresses CreL(60-343) under forebrain-specific promoters such as CaMKIIα, will restrict Cre activity in pyramidal neurons to within the tissue volume exposed to the adenoviruses. Another possibility is temporal control of Cre activity by complementation. A major advantage here is preceding genetic marking of cells expressing the first Cre fragment by co-expression of EGFP. Cells at this stage are positive for both EGFP and the first Cre fragment but negative for Cre activity. Subsequent introduction of the second Cre fragment will induce Cre activity, and direct comparison of cell morphology and dynamic behavior before and after gene modification can be achieved. Indeed, our experiments with primary neurons derived from β-actin promoter-loxP-stop-loxP-EGFP mice clearly indicated the feasibility of this approach (Fig. 3C).

In summary, our results demonstrate that optimization of Cre complementation can be achieved by testing multiple dimerization modules in combination with appropriate nuclear localization signals and structure-based design of Cre breakpoints. Appropriate selection of cell type-specific promoters for driving individual Cre fragments in transgenic mice will facilitate precise tuning of Cre expression patterns *in vivo*. In addition, delayed expression of the second Cre fragment in cells already expressing the first Cre fragment and fluorescent protein markers will facilitate repetitive imaging studies of identical neurons before and after Cre-dependent gene modifications.

Acknowledgements

This work was supported by grants from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, the Japan Society for the Promotion of Science, and the Japan Science and Technology Corporation. We thank Dr. H. Okado for providing recombinant adenovirus vectors.

References

1. Sauer B. Inducible gene targeting in mice using the Cre/lox system. Methods 14 (1998) 381-392.
2. Nagy A. Cre recombinase: the universal reagent for genome tailoring. Genesis 26 (2000) 99-109.
3. Casanova E, Lemberger T, Fehsenfeld S, et al. Alpha complementation in the Cre recombinase enzyme. Genesis 37 (2003) 25-29.
4. Jullien N, Sampieri F, Enjalbert A, et al. Regulation of Cre recombinase by ligand-induced complementation of inactive fragments. Nucleic Acids Res. 31 (2003) e131.
5 Xu Y, Xu G, Liu B, et al. Cre reconstitution allows for DNA recombination selectively in dual-marker-expressing cells in transgenic mice. Nucleic Acids Res. 35 (2007) e126.
6. Seidi A, Mie M, Kobatake E. Novel recombination system using Cre recombinase alpha complementation. Biotechnol Lett. 29 (2007) 1315-1322.
7. Jullien N, Goddard I, Selmi-Ruby S, et al. Conditional transgenesis using dimerizable Cre (DiCre). PLoS ONE. (2007) e1355.
8. Harbury PB, Zhang T, Kim PS, et al. A switch between two-, three-, and four-stranded coiled coils in GCN4 leucine zipper mutants. Science 262 (1993) 1401-1407.
9. Zhang Z, Zhu W, Kodadek T. Selection and application of peptide-binding peptides. Nat Biotechnol. 18 (2000) 71-74.
10. Moll JR, Ruvinov SB, Pastan I, et al. Designed heterodimerizing leucine zippers with a range of pIs and stabilities up to 10(-15) M. Protein Sci. 10 (2001) 649–655.
11. Nishida H, Okabe S. Direct astrocytic contacts regulate local maturation of dendritic spines. J. Neurosci. 27 (2007) 331-340.
12. Umeda T, Ebihara T, Okabe S. Simultaneous observation of stably associated presynaptic varicosities and postsynaptic spines: morphological alterations of CA3-CA1 synapses in hippocampal slice cultures. Mol. Cell. Neurosci. 28 (2005) 264-274.
13. Okabe S, Kim HD, Miwa A, et al. Continual remodeling of postsynaptic density and its regulation by synaptic activity. Nat Neurosci. 2 (1999) 804-811.
14. Ebihara T, Kawabata I, Usui S, et al. Synchronized formation and remodeling of postsynaptic densities: long-term visualization of hippocampal neurons expressing postsynaptic density proteins tagged with green fluorescent protein. J. Neurosci. 23 (2003) 2170-2181.
15. Hayashi MK, Ames HM, Hayashi Y. Tetrameric hub structure of postsynaptic scaffolding protein homer. J. Neurosci. 26 (2006) 8492-8501.
16. Luan H, Peabody NC, Vinson CR, et al. Refined spatial manipulation of neuronal function by combinatorial restriction of transgene expression. Neuron 52 (2006) 425-36.
17. Kalderon D, Roberts BL, Richardson WD, et al. A short amino acid sequence able to specify nuclear location. Cell 39 (1984) 499-509.