Chicken β-globin insulator overcomes variegation of transgenes in *Xenopus* embryos

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ABSTRACT **Chromatin structure and gene transcription regulation are intimately linked, and mosaic expression of randomly integrated transgenes into the genome is frequently observed. This variegation of transgene expression is likely due to the genomic integration site, which can affect the behavior of the integrated DNA sequence in a positive or a negative way. Insulators are a class of DNA elements that can protect genes from inappropriate signals emanating from their environment by acting as boundaries that prevent the spreading of nearby condensed chromatin that may otherwise silence expression. Here we show that transgenes escape this silencing in *Xenopus laevis* and *Xenopus tropicalis* embryos and that a stable, uniform, and heritable expression pattern is obtained when transgenes are flanked with tandem copies of the chicken β-globin 5′HS4 insulator. Our data also indicate that the insulator confers copy-number-dependent transgene expression and can increase transgene expression from weak regulatory elements. Hence, it will be an invaluable tool for generating stable lines expressing different levels of a particular coding sequence.—Sekkali, B., Tran, H. T., Crabbe, E., De Beule, C., Van Roy, F., Vleminckx, K. Chicken β-globin insulator overcomes variegation of transgenes in *Xenopus* embryos. *FASEB J.* 22, 2534–2540 (2008)**

Key Words: transcriptional silencing · heterochromatin · position effect variegation

The ability to stably introduce foreign genes into an organism to generate genetically altered transgenic lines is a major advance in functional genomics. This widely used technology allows investigation of many fundamental biological questions that could not have been tackled by other means. *Xenopus* embryos have been used for decades for elucidating the mechanisms of early development. In recent years, *Xenopus* also become a favored system for the specific manipulation of genes by transgenesis during later stages of development. However, with this procedure, transgenes often integrate randomly in the vertebrate genome, and a mosaic expression pattern is frequently observed in *Xenopus* tadpoles, very similar to what has been well documented in yeast, *Drosophila*, and mice (1–5). This phenomenon of mosaic expression within a cell lineage, called position effect variegation (PEV), usually occurs when a transgene is integrated near heterochromatin regions that are transcriptionally silent (3, 6, 7) or during chromosomal translocations in several human diseases (8, 9).

Several methods for transgenesis have been described for *Xenopus*, but the technique based on the injection of sperm nuclei, classically known as restriction enzyme mediated integration (REMI), is still the most popular (10). Importantly, transgenic embryos and tadpoles can be produced in large numbers in the F_0 generation, which makes it an ideal technique for promoter analysis. Because random integration during REMI transgenesis can lead to undesirable effects, such as heterochromatin silencing or position effects, it has become necessary to search for specialized c*is*-elements that can overcome interference from these chromosomal environments. Insulators, which are cis-elements that can protect transcribed regions from neighboring regulatory influences, are present near chromatin domain boundaries or at sites where they can prevent inappropriate regulation of a promoter region by a nearby heterologous enhancer. One of the best-characterized vertebrate DNA insulators belongs to the chicken β-globin locus. A 1.2 kb cis-element fragment encompassing hypersensitive site 4 at the 5′ end of the chicken β-globin (5′hyper-site/HS4) has positional enhancer-blocking activity and can also overcome chromosomal position effects cross-species (11–14). Here, we investigated whether the 250 bp "core" sequence containing the HS can act as an insulator in *Xenopus* during nuclear transfer-mediated transgenesis.

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doi: 10.1096/fj.07-098111

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MATERIALS AND METHODS

DNA constructs

Plasmid pCMV-DsRed was generated by subcloning the DsRed cDNA from the pDsRed-Express-1 plasmid (Clontech, BD Biosciences, Erembodegem, Belgium) into the pCS2+ expression vector (15). The expression vector containing the *Xenopus* cardiac actin promoter directing expression of green fluorescent protein (GFP) has been described previously (16). The tandem repeat of the 250 bp core fragment of the 5′HS4 insulator was derived from the pNI-CD vector (a gift from Gary Felsenfeld, U.S. National Institutes of Health, Bethesda, MD, USA). We started the construction of the pbinV2 universal vector by amplifying the 5′HS4 core fragment of the 5′HS4 insulator by polymerase chain reaction (PCR) with primers containing *Eco*RI/*Kpn*I or *Pst*I/*Sph*I digestible ends. We used primer pair 5′-GAGTTGGCGCGCCTGTCATTC-3′ and 5′-GGTACCGAGTTGGCGCG-3′, or 5′-AGTTGGCGCGCCTGTCATTCC-3′ and 5′-GAGTTGGCGCG CCTGTCATTC-3′. The resulting 550 bp PCR fragments were first cloned in pGEMTeasy (Promega, Benelux, Leiden, The Netherlands), generating the plasmid pbin1x, sequence verified, and subcloned into pGEM4Z (Promega) on each side of the multiple cloning site, resulting in the vector pbinV2.

To generate pbinCMV-DsRed, the expression cassette CMV-DsRed was cloned as a *Sal*I/blunt-end fragment into the pbinV2 vector digested with *Sal*I/*Eco*RV. For pbinCarGFP, the expression cassette Car-GFP was cloned as *Kpn*I/blunt-end into pbinV2 digested with *Kpn*I/*Eco*RV.

The constructs were analyzed by restriction mapping, and the junctions were verified by sequencing. Plasmids were extracted by alkaline lysis and purified on Qiagen columns (Qiagen, Hilden, Germany).

The expression cassettes were released from the vector backbones by digestion with *Sal*I/*Not*I (for CMV-DsRed), *Not*I (binCMV-DsRed), *Kpn*I/*Sac*II (Car-GFP), or *Sac*II (for binCar-GFP). The StufferCar-GFP fragment was generated by digesting pCar-EGFP with *Sca*I. The fragments were purified on Elutetip minicolumms (Whatman, Schleicher, & Schuell, Hertogenbosch, The Netherlands) before use in transgenesis.

A 600 bp fragment of the *Xenopus tropicalis* cadherin-2 (N-cadherin) promoter was PCR amplified using primer pair 5′-TGTGAGGAGTGACAGGAGCG-′3 and 5′-GGTGCTGCT-GCTGTTAAGGGTCG-′3 and was cloned into pCR2.1-TOPO vector (Invitrogen, Carlsbad, CA, USA). The plasmid pCDH2-DsRed was generated by replacing the CMV promoter of pCMV-DsRed by the *X. tropicalis* cadherin-2 promoter. The pbinV2-ISceI plasmid was generated by cloning the insulator cassette from the pbinV2 vector *Bst*XI-blunt-ended into pBlueSK-ISceI (a generous gift from Jochen Wittbrodt, Developmental Biology Programme, Heidelberg, Germany) digested with *Kpn*I/*Sac*I and blunt-ended. The CDH2-DsRed fragment was then cloned as a *Sal*I/*Kpn*I fragment into pbinV2-ISceI, pbin1x, and pBlueSK-IsceI to generate pbinCDH2-DsRed-ISceI, pbin1xCDH2-DsRed, and pCDH2-DsRed-IsceI, respectively. As a result, pbin1xCDH2-DsRed contains only two copies of the HS4 core element at one side of the CDH2-DsRed cassette. CDH2-DsRed and binCDH2-DsRed were released from the vector backbones with I-SceI meganuclease, and bin1xCDH2-DsRed was released with a *Sal*I/*Nde*I digest.

All constructs containing tandem direct repeats were propagated in recombination-deficient bacteria (SURE cells from Invitrogen).

Generation of founders and F1 transgenics

X. tropicalis frogs were obtained from Nasco (Fort Atkinson, WI, USA; http://www.enasco.com) and *Xenopus laevis* from African Reptile Park, Tokai, South Africa. Transgenesis was performed as described in detail in Hirsch *et al.* (17). Briefly, to induce ovulation in frogs, they were primed with 20 U of human chorionic gonadotropin (HCG; Sigma-Aldrich, Bornem, Belgium) 36–48 h before a boosting dose of 120 U HCG. Eggs were harvested by squeezing the frogs 4 h after boosting. Eggs were semidejellied with 2% cysteine in low-calcium 1× MMR (0.1 M NaCl, 2 mM KCl, 0.2 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM HEPES, pH 7.5); washed 5 times with 1× MMR, and then transferred to agarose-coated dishes containing 8% Ficol in 0.1× MMR for injection. In all experiments, 0.07 pmol of transgene fragment was mixed with the prepared sperm nuclei and 2 μl of oocyte extract, which decondenses the sperm nuclei and facilitates the integration of the transgene. For a detailed description of the preparation of the sperm nuclei and the oocyte extract, see Hirsch *et al.* (17). The reaction mixture was backfilled into a capillary needle and injected into the collected eggs at 1 sperm nucleus/s. Normal dividing embryos were selected at the 4-cell stage (1.5 h after injection), cultured overnight in 6% Ficoll/0.1× MMR, and transferred to 0.1× MMR without Ficoll. Transgenic embryos were screened at the neurula stage for positive GFP or DsRed signals using a Leica MZ FLIII fluorescence stereomicroscope (Leica Microsystems, Wetzlar, Germany). Pictures were taken at stage 45 or later.

Transgenic founder tadpoles were raised to maturity, and at the age of ~6 months, they were crossed with wild-type Nasco frogs to obtain an F_1 generation.

Slot blot

For genomic DNA isolation, a 1 cm piece of tail was cut from each anesthetized transgenic tadpole at stage 54–55. Tail pieces were lysed and treated with proteinase K (200 μg/ml) in lysis buffer (100 mM Tris-HCl, 5 mM EDTA, 0.2% SDS, and 200 mM NaCl) overnight at 55°C. DNA was extracted twice with an equal volume of phenol/chloroform and once with chloroform. Total genomic DNA was precipitated with 0.7 vol of isopropanol, and the pellets were washed twice with cold 70% ethanol and air dried. The DNA was resuspended in 75 μl sterile water.

Probes for GFP, DsRed-express1, and β-catenin were prepared from pCar-GFP, pCMV-DsRed, and pCS2-Xβ-catenin expression plasmids (a gift from Barry Gumbiner, University of Virginia, Charlottesville, VA, USA). The slot blot protocol has been described previously (18). Briefly, 10 μg DNA was denatured with 0.2 vol of 1 N NaOH and heated at 90°C for 10 min. The denatured samples were cooled on ice and immediately applied to prewetted nitrocellulose membranes. The sample-containing membranes were air dried for 30 min and baked in the oven at 80°C for 1–2 h. Membranes were incubated in prehybridization buffer for 2 h at 65°C, and the denatured probes, with equal specific activities, were added together with salmon sperm DNA to the membranes and incubated overnight at 65°C. Excess probe was washed away at 60°C to a stringency of 0.1× saline-sodium citrate/0.1%SDS, and the membranes were air dried and exposed to X-ray film. The films were developed using a Bio-Rad PhosphorImager (Bio-Rad Laboratories, Hercules, CA, USA), and bands were quantified with ImageQuant software (Molecular Dynamics, Inc., Sunnyvale, CA, USA). The ratio between GFP and β-catenin signals was used to estimate the transgene copy number.

Statistical analysis

Statistics were performed using GraphPad Prism version 4.0 (GraphPad Software, San Diego, CA, USA). A χ^2 test was used to evaluate the total numbers of transgenic embryos comparing two different constructs.

RESULTS

To study the effect of the surrounding chromosomal environment on the expression pattern of randomly integrated transgenes, we generated several transgenic *X. tropicalis* and *X. laevis* embryos expressing GFP or DsRed under the control of the *Xenopus* cardiac actin specific promoter (Car-GFP; ref. 16) and the constitutive CMV promoter/enhancer (CMV-DsRed), respectively (**Fig. 1*A***). In the transgenesis procedure (17), restriction enzymes were omitted from the mixture of DNA construct and sperm nuclei, and any residual vector DNA was removed from the expression cassette preparation. To partially decondense sperm nuclei, chromatin oocyte extract was used instead of interphase egg extract. With this procedure, a large proportion of transgenic embryos showed a mosaic expression pattern bearing striking similarity to the PEV described in yeast, *Drosophila*, and mice (3, 19) and with a heterogeneous cell-to-cell intensity (Fig. 1*A*; **Table 1**). It is noteworthy that green fluorescence for the Car-GFP construct was present mainly in the axial myotomes, and only in a few cases was it also observed in the heart and jaw muscles, where the cardiac actin promoter should have been active. We also frequently observed large proportions of isolated differentiated myocytes expressing GFP at different intensities. It is very unlikely that this expression pattern in isolated single cells was due to extrachromosomal expression, because it persisted beyond stage 50 (~15 days postfertilization).

In *Drosophila*, chromosomal position effects and PEV can be overcome by flanking the reporter gene with insulator elements (20, 21). We hypothesized that adding an insulator element to the Car-GFP and CMV-DsRed transgene expression cassettes would suppress the variegation patterns seen in transgenic *X. tropicalis*

TABLE 1. *Transgenesis efficiency of Car-GFP and CMV-DsRed with and without insulators*

Experiment	Total (n)[a]	Transgenic (%)[b]	Homogenous expression (%)[b]
Car-GFP			
Experiment 1			
Car-GFP	23	56.5	8.7
binCar-GFP	196	100.0	56.6
Experiment 2			
Car-GFP	65	47.6	12.3
binCar-GFP	150	90.0	63.3
Experiment 3			
Car-GFP	77	66.2	27.3
binCar-GFP	62	93.6	75.8
Experiment 4			
Car-GFP	65	29.2	4.6
binCar-GFP	15	60.0	40.0
Experiment 5			
Car-GFP	25	28.0	16.0
binCar-GFP	35	74.3	62.9
Experiment 6			
Car-GFP	44	63.6	22.7
binCar-GFP	74	89.2	73.0
CMV-DsRed			
Experiment 1			
CMV-DsRed	44	59.1	18.3
binCMV-DsRed	37	89.2	83.8
Experiment 2			
CMV-DsRed	46	87.0	37.0
binCMV-DsRed	65	96.9	95.4

$P < 0.0001$: Car-GFP *vs.* binCar-GFP, CMV-DsRed *vs.* binCMV-DsRed; χ^2 test. [a] Number of embryos. [b] Percentage of total embryos.

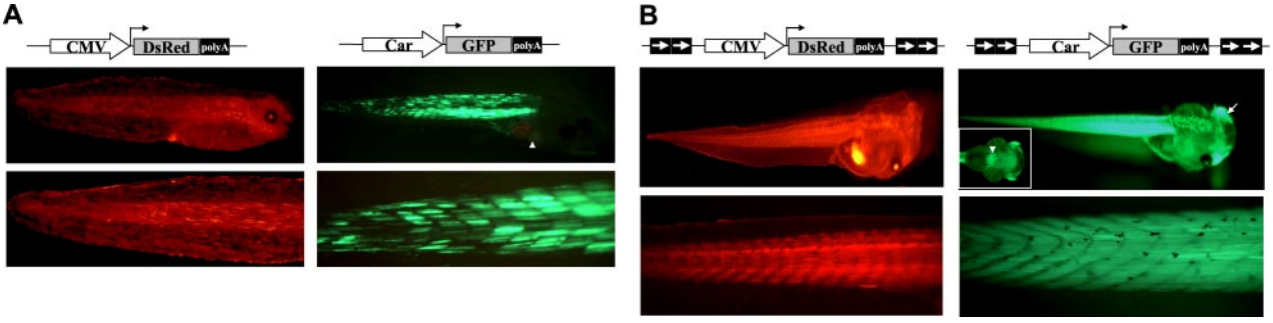

Figure 1. Improved transgene expression and absence of gene silencing in transgenic *X. tropicalis* embryos on addition of 5′HS4 insulator sequences to the transgene constructs. *A*) Left panels: representative stage 40 F_0 tadpole transgenic for CMV-DsRed, showing ubiquitous but patchy red fluorescence. Right panels: a typical stage 45 transgenic tadpole, carrying a cardiac actin-specific transgene, Car-GFP, showing green fluorescence in the axial myotomes but not in the heart (arrowhead) and other muscle tissues. Within the myotomes, GFP intensity in individual muscle cells is variable (bottom right panel). *B*) F_0-generation tadpoles carrying transgenes with insulators show homogeneous expression patterns. Top panels: outline of constructs with transgenes flanked by 2 copies of the chicken 5′HS4 insulator. Red fluorescence is ubiquitous and homogeneous in a representative tadpole carrying the binCMV-DsRed cassette (left). Homogeneous green fluorescence is observed in the axial myotomes and the body, heart (arrowhead), and jaw muscles (arrow) of a transgenic tadpole carrying the binCar-GFP cassette (right). Myocytes within the muscles show no variation in signal intensity.

and *X. laevis* embryos and lead to a more uniform expression pattern. The chicken β-globin insulator element, the best-characterized vertebrate insulator, can suppress PEV of transgenes in cultured erythroid cells (14). Hence, we investigated the ability of the chicken 5′HS4 insulator to suppress PEV of Car-GFP and CMV-DsRed transgene expression cassettes in *Xenopus*. For this purpose, we first generated a universal vector, named pbinV2, containing two copies of the 250 bp "core" sequence of the chicken 5′HS4 insulator on either side of the multiple cloning site. For retroviral vectors, it has been shown that the orientation of the repeated insulators has no effect on transgene expression (22). However, to avoid loss of the transgene by recombination, it may be advisable to put the repeated insulators in the same orientation. The reporter expression cassettes were cloned in the multiple cloning site of the pbinV2 vector. After integration of the cassettes *via* nuclear-transfer-mediated transgenesis, we analyzed the dynamics of the fluorescent reporter genes in live transgenic embryos. Transgenic *Xenopus* embryos with noninsulated transgenes showed various degrees of heterogeneous variegated expression patterns of the GFP and DsRed reporters, but addition of the insulator to the GFP and DsRed reporters rendered the expression of the randomly integrated transgenes independent of the chromosomal integration site. Expression of the DsRed reporter under the control of the constitutive CMV promoter/enhancer was mostly uniformly distributed over the whole embryo (Fig. 1*B*). Similarly, and in contrast to the noninsulated Car-GFP construct, which was expressed mostly in the axial myotomes, expression of the insulated GFP reporter under the control of the cardiac actin promoter was uniform and consistently tissue restricted to the myotomes and heart and jaw muscles (**Figs.** 1*B*, **2*A***). Uniform and strong transgene expression was present in the vast majority of F_0 embryos and tadpoles containing the insulated transgene (**Fig. 3*A*, *B***; Table 1) and was transmitted to the progeny (Fig. 2*B*).

A potential alternative mechanism that would explain our results is that the insulator sequences protect the transgene from an exonuclease activity that may be present in the DNA/nucleus/interphase extract before integration. Hence, we performed transgenesis experiments with a noninsulated Car-GFP transgene that was flanked at its 5′ and 3′ terminus with nonrelevant stuffer DNA (1 and 1.5 kb, respectively). However, this did not result in more homogenous transgene expression (Fig. 3*C*, *D*; **Table 2**); therefore, it is very unlikely that the insulator enhances transgenesis efficiency by protecting the transgene from DNA exonuclease activity.

We wanted to investigate whether addition of the 5′HS4 insulator elements can also enhance the expression of genes driven by weak regulatory sequences. Therefore, we generated constructs in which a DsRed reporter gene was put under control of a fragment of the *X. tropicalis* cadherin-2 promoter. In transgenic embryos and tadpoles that integrated this construct, expression of the reporter gene in the central nervous system was mostly weak or barely detectable. However, flanking this construct with the 5′HS4 insulator greatly increased the percentage of embryos with high DsRed expression (**Table 3**; **Fig. 4**), showing that the insulators can be used to boost transgene expression from weak regulatory elements. The fluorescent signals from the noninsulated constructs were too weak to evaluate potential mosaic expression. We also used the cadherin-2 promoter to investigate whether it is necessary to adjoin the 5′HS4 insulator to both sides of the transgene. The REMI transgenesis method usually results in integration of transgenes in multiple copies. Hence, a tandem integration of a unilaterally insulated

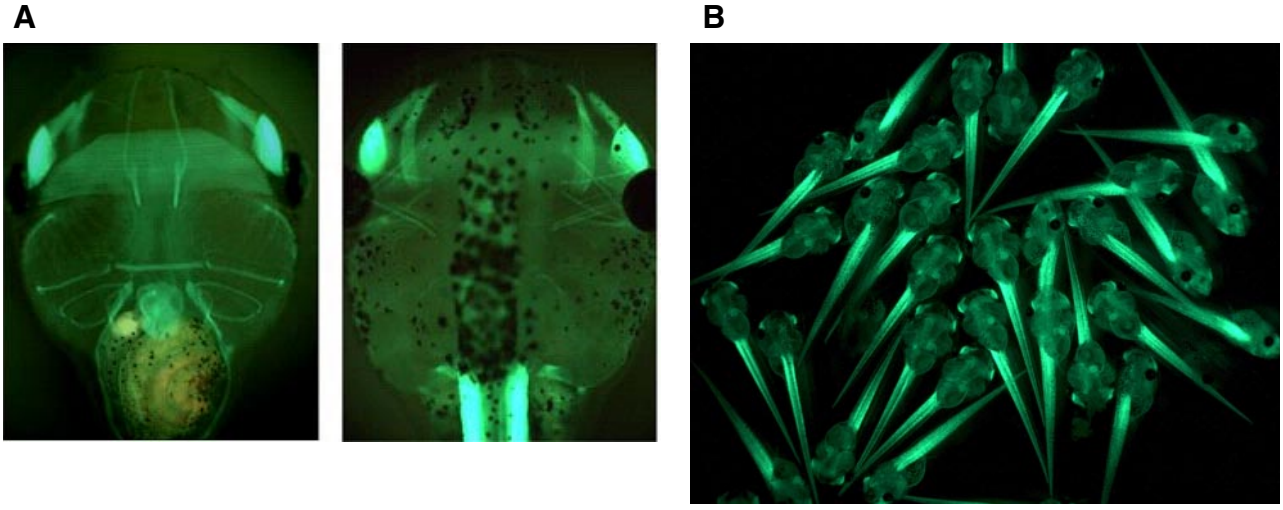

Figure 2. Uniform transgene expression in F_0 and F_1 tadpoles. *A*) F_0-generation tadpole carrying the binCar-GFP cassette: ventral (left panel) and dorsal (right panel) view. Homogeneous green fluorescence is observed in the axial myotomes and the body, heart, and jaw muscles. *B*) F_1 progeny of transgenic tadpoles with homogenous and uniform expression of GFP as a result of a cross between a female transgenic binCar-GFP founder and a wild-type male. Only a fraction (50%) of transgenic tadpoles is shown.

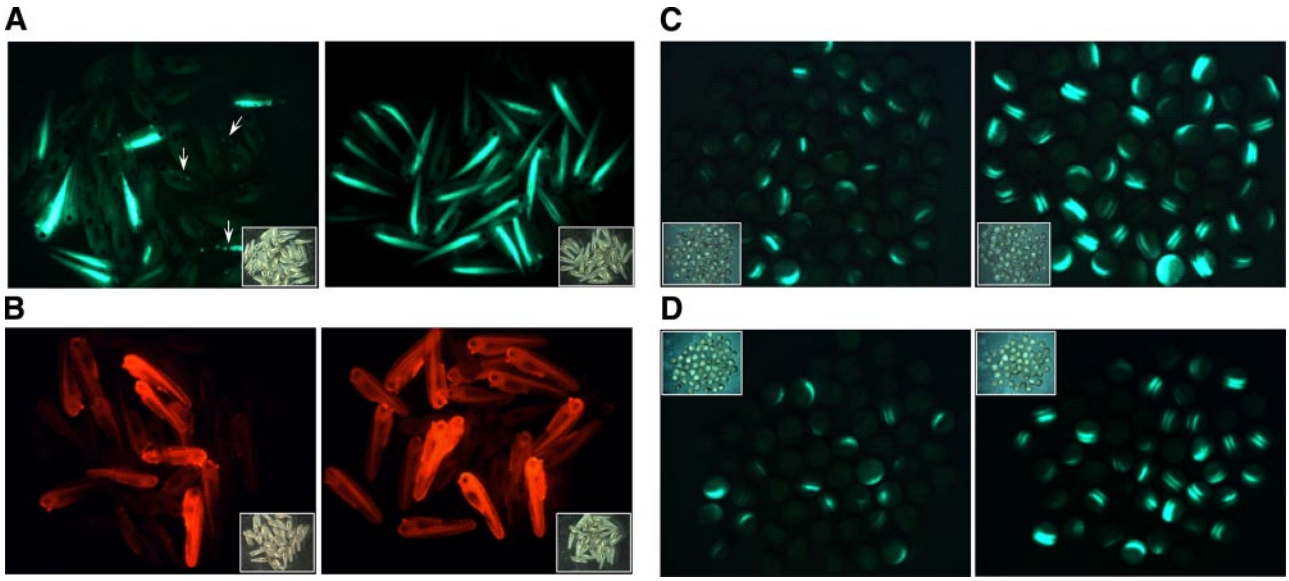

Figure 3. High frequency of transgenic F_0 embryos and tadpoles with constructs flanked by the 5′HS4 insulator. *A*) Results of a representative transgenesis experiment for Car-GFP without (left panel) or with (right panel) insulators. Transgenic embryos with the noninsulated construct have less homogenous and more mosaic expression, especially visible in the somites (arrows). With the insulated construct, most embryos show a strong and homogenous expression pattern. *B*) Embryos from a CMV-DsRed transgenesis experiment without (left panel) and with (right panel) insulators. *C, D*) Fluorescent patterns in embryos containing a transgene cassette flanked by stuffer sequence (stufferCar-GFP; *C*) or harboring a transgene cassette without extra sequences (Car-GFP; *D*). Embryos containing stufferCar-GFP (*C*, left panel) are indistinguishable from those with Car-GFP (*D*, left panel). Both experiments were internally controlled using the insulated transgene cassette (binCarGFP; *C, D*, right panels). Insets are bright-field pictures corresponding to the fluorescent images.

transgene will result in bilateral flanking of the transgene copies, with the exception of the last copy. Indeed, the frequency of strong fluorescent signals from a unilaterally flanked CDH2-DsRed transgene was higher (29% of 102 embryos) than from a noninsulated construct (9% of 82 embryos). Taken together, we have shown that the 5′HS4 cis-element can stably and heritably overcome the variegation of transgene expression in F_0- and F_1-generation tadpoles and induce strong, uniform, and reproducible transgene expression.

The ability to confer copy-number-dependent expression to transgenes has always been considered the hallmark of locus control regions, which have the ability to confer this feature to transgenes by fully establishing and/or maintaining an open chromatin configuration (1, 23). Because we have shown that the chicken 5′HS4 insulator could overcome PEV in *Xenopus* embryos, we focused our attention on copy-number-dependent expression and investigated whether the level of reporter expression was directly related to the number of copies integrated into the genome of *Xenopus* embryos. We therefore generated several transgenic lines harboring the insulated CarGFP cassette construct and examined the intensity of the fluorescent signal in the tadpoles. Several GFP transgenic lines expressed GFP uniformly, although at different levels (**Fig. 5**). We determined the relative transgene copy

TABLE 2. *Transgenesis efficiency of Car-GFP with stuffer sequences or insulators*

Experiment	Total (n)[a]	Transgenic (%)[b]	Homogenous expression (%)[b]
Experiment 1			
stufferCar-GFP	46	52.2	13.0
binCar-GFP	34	76.5	64.7
Experiment 2			
stufferCar-GFP	32	31.3	6.3
binCar-GFP	21	71.4	47.6
Experiment 3			
stufferCar-GFP	61	59.0	21.3
binCar-GFP	67	76.9	68.7

$P < 0.0001$: stufferCar-GFP *vs.* binCar-GFP; χ^2 test. [a] Number of embryos. [b] Percentage of total embryos.

TABLE 3. *Transgenesis efficiency of CDH2-DsRed with and without insulators*

Experiment	Total (n)[a]	Transgenic (%)[b]	Strong expression (%)[c]
Experiment 1			
CDH2-DsRed	33	30.3	9.1
binCDH2-DsRed	38	44.7	39.5
Experiment 2			
CDH2-DsRed	22	36.4	9.1
binCDH2-DsRed	27	29.6	29.6

$P = 0.0173$: CDH2-DsRed *vs.* binCDH2-DsRed; χ^2 test. [a] Number of embryos. [b] Percentage of total embryos. [c] Percentage of total embryos that show strong expression of the reporter gene (scored by 2 independent investigators).

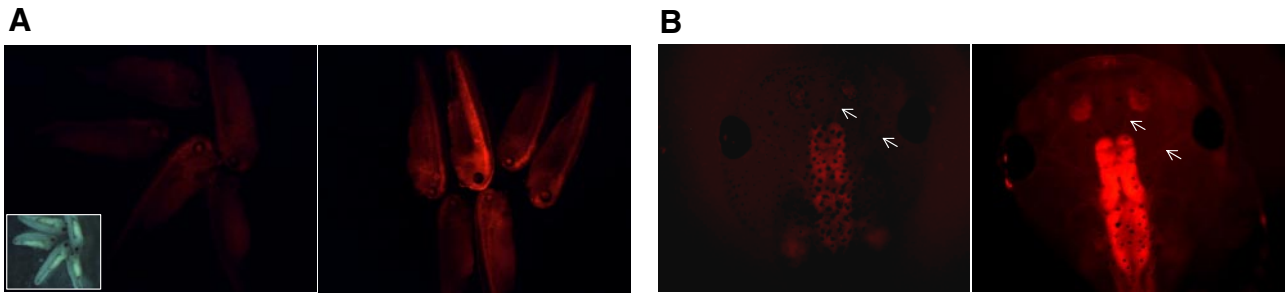

Figure 4. Transgene expression from a weak *X. tropicalis* cadherin-2 promoter is increased by addition of chromosomal insulators. Representative transgenic F_0 tadpoles are shown at stages 36 (*A*) and 45 (*B*). Pictures in the left and right panels were taken with the same camera setting. Expression of DsRed from the cadherin-2 promoter was much weaker in tadpoles containing transgene cassette without insulators (CDH2-DsRed; *A*, *B*, left panels) compared with those having insulators (binCDH2-DsRed; *A*, *B*, right panels). Arrow in *B* shows the position of the optic and olfactory nerves, which can be easily discerned in the transgenic tadpole containing the insulated transgene.

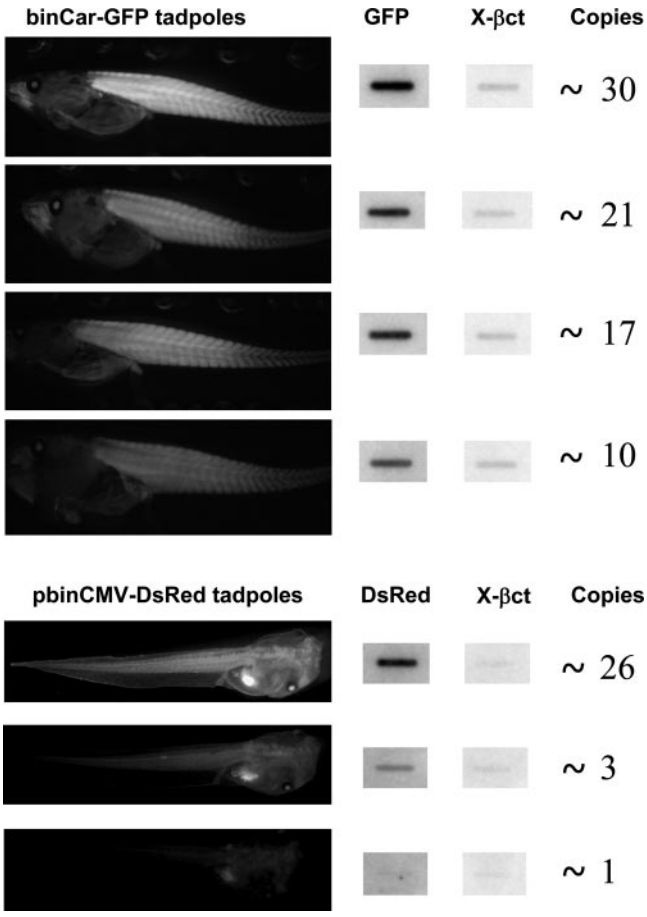

Figure 5. The 5′HS4 insulator can confer copy-number-dependent expression to the insulated transgenic cassette in *X. tropicalis*. Different transgenic F_0 tadpoles for binCar-GFP or binCMV-DsRed with various degrees of GFP or DsRed intensity were selected for analysis of transgene copy number by a slot blot assay. Total genomic DNA was extracted and hybridized with a GFP or DsRed probe together with a *Xenopus* β-catenin probe as reference. The copy number of the transgene (GFP/DsRed) was estimated by directly comparing the signal intensities with that of β-catenin (Xβct), which is set at 2 copies. The GFP or DsRed intensity of the transgenic lines was directly related to their estimated transgene copy numbers.

number in each transgenic line by a slot-blotting experiment. A strong correlation was observed between transgene copy number and intensity of the GFP signal. Similar results were obtained with transgenic lines harboring the DsRed reporter under the control of the CMV promoter/enhancer (Fig. 5), which indicates that this feature is likely to function in any type of cell in *Xenopus* embryos. Together, these results indicate that the chicken β-globin 5′HS4 insulator can confer copy-number-dependent expression to transgenes in *Xenopus*, although it has been assumed that it does not do so when pre-B or B cell lines are transfected with a V_H promoter-GFP reporter gene that was linked to the 3′ and/or 5′ IgH regulatory elements (24).

DISCUSSION

Our results provide strong support for the use of chromosomal insulators to improve transgene expression in F_0- and F_1-generation *Xenopus* tadpoles. Silencing typically occurs in a proportion of cells and is heritable, leading to mosaic patterns of gene expression known as PEV. This inefficient and nonuniform gene expression, which has been reported for several expression vectors and in different species (1–5), seriously hampers interpretation of results, *e.g.*, of *in vivo* promoter analysis. Here, we convincingly show that the 250 bp core sequence of the chicken β-globin 5′HS4 insulator can overcome chromatin silencing during REMI transgenesis in *Xenopus* embryos. The use of this insulator has been described before as a prerequisite for obtaining efficient transgene expression in *X. laevis* embryos *via* the PhiC31 integrase system (25). However, establishment of transgenic lines has not yet been described with this method, and, in contrast to REMI transgenesis, the PhiC31 integrase preferentially generates single-copy integrations and may not be suitable when used with weak promoter or enhancer elements. Our results clearly demonstrate that for transgenesis *via* REMI, which is still the most commonly used method in *Xenopus*, the 250 bp core sequence of the chicken β-globin 5′HS4 insulator is invaluable for obtaining a

reproducible, faithful, and heritable expression pattern of transgenes and for achieving more efficient expression levels. Our transgenesis vector will be exceedingly useful for increasing the efficiency and ease of transgenesis in *X. laevis* and *X. tropicalis* and will further advance the use of these important model organisms. FJ

We thank Dr. Gary Felsenfeld (U.S. National Institutes of Health, Bethesda, MD, USA), Dr. Jochen Wittbrodt (Developmental Biology Programme, Heidelberg, Germany), and Dr. B. Gumbiner (University of Virginia, Charlottesville, VA, USA) for the gift of plasmids. We thank A. Bredan for editing the manuscript and T. Roose for excellent animal care. Research was supported by grants from the Belgian Federation against Cancer and the Institute for the Promotion of Innovation through Science and Technology, Flanders (IWT).

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Received for publication September 18, 2007.
Accepted for publication February 21, 2008.