

Cell to cell transfer of the chromatin-packaged human β -globin gene cluster

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ABSTRACT

Cell type-specific gene expression is regulated by chromatin structure and the transcription factors provided by the cells. In the present study, we introduced genes packaged into chromatin into target cells using a human artificial chromosome (HAC) and analyzed regulation of gene expression. The human β -globin gene cluster was built into an HAC (globin-HAC) and introduced into mouse embryonic stem (ES) cells using microcell-mediated chromosome transfer (MMCT); the adult-type human β -globin gene was expressed in bone marrow and spleen cells of the transgenic mice. *In vitro* differentiation of ES cells into mouse erythrocytes indicated that the natural sequential expression of ϵ , γ and β -globin genes was reproduced on the globin-HAC. Combination of MMCT and a novel chromosome transfection technique allowed transfer of globin-HAC from HT1080 cells into the human leukemia cell line K562, and from K562 cells back into HT1080 cells. Expression of the γ -globin gene, repressed in HT1080 cells, was activated in K562 cells without any processes of differentiation into adult erythroid cells, and was completely repressed again in HT1080 cells when transferred back from K562 cells. Thus, transfer of target genes packaged into chromatin using a HAC was useful for functional analyses of gene regulation.

INTRODUCTION

The potency of transcriptional activation can be regulated by re-organization of chromatin structure and the association of transcriptional activators in the regulatory regions of the genes. It has been assumed that the DNA of transgenes introduced into cells could be functionally formed into active chromatin in the cells and that transgenes introduced into mouse embryonic stem (ES) cells could be activated through differentiation. However, the expression of transgenes integrated into chromosomes in cultured cells and in transgenic mice is often subject to position effects.

In previous work, chromosome manipulation technology in combination with microcell-mediated chromosome transfer (MMCT) enabled us to introduce chromosome fragments into target cells and to reproduce tissue-specific gene expression (1–4). Human artificial chromosomes (HACs) were constructed using a bottom-up strategy based on the transfection of cloned or synthetic centromeric alphoid DNA precursors with CENP-B boxes into a cultured human cell line, HT1080 (5–10). The HACs were built up to megabase size (1–10 Mb) by multimerization of alphoid precursors. The generation of bottom-up HACs via multimerization resulted in the development of HACs into mini-chromosomes carrying large genomic regions that contain genes and their regulatory elements, such as the human guanosine triphosphate cyclohydrolase I (GCH1) and the globin cluster (11,12). Recently, we developed a chromosome vector that allowed the introduction of transgenes into several cell lines and the reproduction of tissue-specific expression according to the genetic sequences (13). Due to the

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chromosomal structure and megabase size of bottom-up HACs, the method for their transfer into target cells was limited to MMCT (14,15). HACs have been transferred successfully into many vertebrate cell lines by MMCT and are stably transferred during mitosis (13). We have used MMCT to establish mouse ES cell lines carrying the HAC, and then created mice that harbour the HAC (12). The HACs carrying GCH1 or the globin cluster (globin-HAC) were mitotically stable in mouse ES cells and the HACs were transmittable in mice.

Considerable amounts of data on regulation of the human β -globin locus are available. The human β -globin gene cluster is composed of five functional genes (ϵ , $G\gamma$, $A\gamma$, δ and β) that are arrayed on chromosome 11 in the order in which they are developmentally expressed. The genes are flanked by a group of DNase I hypersensitive sites known as the locus control region (LCR) upstream and a downstream hypersensitive site (3'HS1). A number of groups have reproduced the architecture of the human β -globin locus in transgenic mice using cosmid, yeast artificial chromosome (YAC) and bacterial artificial chromosome (BAC) constructs (16–19). Using these large constructs, the high-level tissue and developmental stage-specific globin gene expression of the human β -globin locus were recapitulated in mice. Integration of the entire β -globin locus containing the LCR into the mouse genome resulted in authentic expression of the globin genes independent of the site of integration and dependent on the number of integrated copies (20–22). Thus, the β -globin gene locus is a good model for manipulating and evaluating gene expression and the reproduction of functional chromatin on a HAC.

In the present study, we established a technology that can be used to analyze the regulation of expression of genes built into a HAC. Reproduction of the tissue-specific and development-specific expression of human globin genes was demonstrated using globin-HAC in transgenic mice and *in vitro*-differentiated mouse erythrocytes. The regulation of globin genes was further analyzed by transferring globin-HAC from HT1080 cells into K562 cells, and from K562 cells back into HT1080 cells.

MATERIALS AND METHODS

Cell culture

HT1080, HeLa and A9 cells were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS). K562 cells were grown in RPMI1640 supplemented with 10% FBS. The ES cell lines TT2 and TT2F were maintained on feeder cells in ES cell medium consisting of DMEM supplemented with 20% Knockout SR (Invitrogen), 1000 U/ml ESGRO (Chemicon) and 0.1 mM β -mercaptoethanol.

Microcell-mediated chromosome transfer

MMCT from HT1080 to K562 or ES cells was carried out as described previously (12). Microcells from HT-A9/globin29 were recovered and fused with K562 cells. K562 cells containing the HAC were selected in RPMI1640 supplemented with 6 μ g/ml blasticidin S.

Preparation of chromosomes

Metaphase chromosomes from K562/globin29 cells were prepared by a polyamine (PA) procedure according to (23). K562/globin29 cells were arrested in metaphase for 18 h using 0.1 μ g/ml colcemid in culture medium. Mitotic cells were then swelled in 75 mM KCl for 10 min at room temperature. The cells were then washed in PA buffer (15 mM Tris-HCl, pH 7.4; 0.2 mM spermine; 0.5 mM spermidine; 2 mM EDTA; 0.5 mM EGTA; 80 mM KCl; and 20 mM NaCl) and then resuspended in cold PA buffer containing 0.1% digitonin. To release the chromosomes, the swollen cells were passed through a 27-gauge needle attached to a 10 ml syringe.

Chromosome transfection

HT1080 and HeLa cells were transfected using FuGENE HD (Roche) according to the instructions. At the time of transfection, the number of recipient cells per well was $1.0\text{--}1.2 \times 10^6$ cells. One million HACs in 100 μ l PA buffer were combined with the transfection reagents. Stable transfectants were screened by G418 resistance at 400 μ g/ml.

Fluorescent *in situ* hybridization

Fluorescent *in situ* hybridization (FISH) analysis was carried out according to conventional procedures. To detect HACs, biotin-labeled α 21-I alphoid DNA (11-4) (24) and digoxigenin-labeled pYAC2 were used as probes. Globin genes were detected with biotin-labeled PCR products amplified using the following primers: globin1: 5'-AAGACCAGATAGTACAGGGCCTGGCTAC-3', 5'-AAGATTATTCAAGGTTACTATGAACAC C-3'; globin2: 5'-TGCTAATGCTTCATTACAACTTATATC-3', 5'-ATCAGCCAACCAGGAATTCGGCAGT TAC-3'; globin3: 5'-GTGTAAGAAGGTTCTCCTGAGGC TCTACAGATAGGGAG-3', 5'-AAGCAGCACTTGAC TAGAGTATTTTTATACATGCTCTAC-3'.

For dual FISH, biotin-labeled DNA was visualized with FITC-conjugated avidin (Vector), and digoxigenin-labeled DNA was visualized with TRITC-conjugated anti-digoxigenin antibody (Roche Applied Science).

Pulsed-field gel electrophoresis and Southern analysis

Agarose-embedded DNA was prepared as previously described (24). DNA in agarose blocks was digested with KpnI for 2 h and size separated by 1% GTG agarose gel (Cambrex) using the CHEF mapper system (Bio-Rad). The running conditions were dependent on the auto algorithm for 5–70 kb. Genomic DNA was digested with EcoRI and size separated in a 1% GTG agarose gel in conventional gel electrophoresis. DNA in the gel was transferred to a nylon membrane and hybridized with 32 P-labeled DNA probes prepared from human globin gene sequences (5'HS5 and introns of ϵ , $G\gamma$, and β -globin DNA) produced by PCR using the following primers: 5'HS5, 5'-GCATCCTGACACAT ATGACA-3' and 5'-ATCCAGTCATAAGCACAG AC-3'; ϵ globin, 5'-GGTGAGTTCAGGTGCTGGTGA TGTG-3' and 5'-CAGGCATGTTGAGTAGAAGTTTC

CG-3'; G γ globin, 5'-GTGAGTCCAGGAGATGTTTCA GCAC-3' and 5'-GATGAACCCATAGTGAGCTGAGA GC-3'; and β -globin, 5'-GGGTGAGTCTATGGGACGC TTGATG-3' and 5'-CTGTGGGAGGAAGATAAGAG GTATG-3'.

RT-PCR

Total RNA (25 ng) was used for reverse transcriptase-PCR (RT-PCR) using the One Step RNA PCR Kit (AMV) (TaKaRa). For human ϵ -globin, the primers were h ϵ (sen) 5'-CAATCACTAGCAAGCTCTCAGG-3' and h ϵ (anti) 5'-GGGCTTGAGGTTGTCCATGTTT-3'. For human γ -globin, the primers were h γ (sen) 5'-GATG CCATAAAGCACCTGGATG-3' and h γ (anti) 5'-TTGC AGAATAAAGCCTATCCTTGA-3'. For human β -globin, the primers were h β (sen) 5'-AACTGTGTTCA CTAGCAACCTCAA-3' and h β (anti) 5'-GAGTGGACA GATCCCCAAAGGA-3'. The amplification conditions were 50°C for 30 min, 94°C for 2 min, followed by 25 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 30 s. The primers for $\epsilon\gamma$, β -major and GATA-1 were reported previously (25); the samples were amplified at 50°C for 30 min and 94°C for 2 min, followed by 25 cycles of 94°C for 20 s, with a ramp time of 1 min, 55°C for 1 s and 72°C for 1 min.

Real-time PCR

Genomic DNA from HAC-containing cells was extracted according to standard procedures. Total RNA was prepared using SV Total RNA Isolation System (Promega). cDNA was synthesized using Transcriptor First Strand cDNA Synthesis Kit (Roche Applied Science). Real-time PCR was carried out using ABI PRISM 7700 Sequence Detection System (Applied Biosystems). PCR reactions were carried out using the QuantiTect SYBR Green PCR kit (Qiagen) and Power SYBR Green PCR Master Mix (Applied Biosystems). The primers were ϵ -globin: 5'-TTTGCAA GGAGTTCACCCCTGAA-3' and 5'-GAACACCTGCA AACTGGAAGAGAA-3'; G γ -globin: 5'-ATCCTGAGA ACTTCAGGGCTCCTG-3' and 5'-AAAGCTCTGCAT CATGGGCAGTGA-3'; A γ -globin: 5'-ATCCTGAGAA CTTCAGGGCTCCTG-3' and 5'-AAAGCTCTGCATC ATGGGCAAGAG-3'; β -globin: 5'-GAAAGTGGTGG CTGGTGTGGCTAA-3' and 5'-AGGCCCTTCATAAT ATCCCCAGT-3'; $\epsilon\gamma$: 5'-TCGGCAATGAATTCACA GCTGAGA-3' and 5'-ATTGCTGGACAGCTAGAGA GAGGG-3'; β -major: 5'-AGAAGGTGGTGGCTGGA GTGGCTG-3' and 5'-AAGTCTTCATCATTTTCCCC ACAA-3'; and β -actin: 5'-ATCTGGCACACACCTTC TACAATGAGCTGCG-3' and 5'-GTCATCTTTTTCAC GGTTGGCCTTAGGGTTTCAG-3'.

Threshold cycles of PCR reactions were determined in triplicate using the following conditions: 40 cycles of 94°C for 15 s, 60°C for 30 s and 72°C for 30 s.

In vitro differentiation of embryonic stem cells

Mouse ES cells were differentiated into hematopoietic cells *in vitro* using methods reported previously, with some modifications (25). The ES cells were seeded at 4×10^4

onto confluent OP9 cells (RIKEN cell bank) in a 6-cm dish and cultured in α -MEM medium (Invitrogen) supplemented with 20% FBS, 2 U/ml erythropoietin (Roche Applied Science), and 100 ng/ml recombinant mouse stem cell factor (R&D Systems). After 5 days in culture, the cells were dissociated and seeded onto fresh OP9 layers in the same culture medium. Both floating and adherent cells were harvested after 3, 6 and 9 days.

RESULTS

Transfer of the human β -globin gene cluster using an HAC

The human β -globin gene cluster packaged into chromatin (globin-HAC) was previously constructed by HAC technology using YACs 7C5hTEL and A201F4.3 in HT1080 cells (Figure 1a) (12). The globin-HAC in HT1080 cells (HT/globin29) was mitotically stable (Figure 1b). To analyze cell type-specific expression of the globin gene cluster packaged into chromatin, the globin-HAC was transferred into human K562 leukemia cells by MMCT; in addition, the globin-HAC was previously transferred into mouse ES cells (TT2 and TT2F) (12). The presence of globin-HAC in the K562 cell lines was confirmed by FISH analysis (Figure 1b).

The integrity of the globin gene clusters in TT2/globin29-2 and K562/globin29 was analyzed by Southern hybridization and real-time PCR (Figure 1c). Genomic DNA from HT/globin29, TT2/globin29-2 and K562/globin29 was digested with EcoRI or KpnI and hybridized using 5'HS5, ϵ , A γ , G γ and β -globin as probes (Figure 1c). The EcoRI 3.4-kb fragment, 5'HS5 and KpnI 14-kb and 40-kb fragments were detected as added signal intensities relative to the signals from HT1080 cells. Quantitative real-time PCR analysis indicated that the globin genes on the HACs existed in 12 copies in K562/globin29, which is about the same as the number of copies in HT/globin29 cells (12). These results indicated that after transfer, the globin-HAC maintained its internal structure in K562 and mouse ES cells.

Using the MMCT technique, it is not possible to transfer HACs from donor cells that cannot form microcells, including K562 cells. We established an alternative transfer technology for isolation and transfection of HACs into target cell lines. We isolated HACs from metaphase cells by simple 25% sucrose cushion centrifugation under polyamine buffer conditions. Transfer of HACs into target cell lines was achieved using conventional transfection reagents, which enabled direct transfer of the HAC from a variety of donor cells, including those that could not form microcells (Figure 2a). The efficiency of chromosome transfection (number of transformants/number of recipient cells) was $1.1\text{--}8.7 \times 10^{-5}$. Using this procedure, we successfully transferred the globin-HAC from K562 cells into target cells (Figure 2b).

Tissue-specific expression of human globin genes from the HAC in mice

To examine tissue-specific gene expression of globin genes from the globin-HAC in mice, we produced chimeric mice

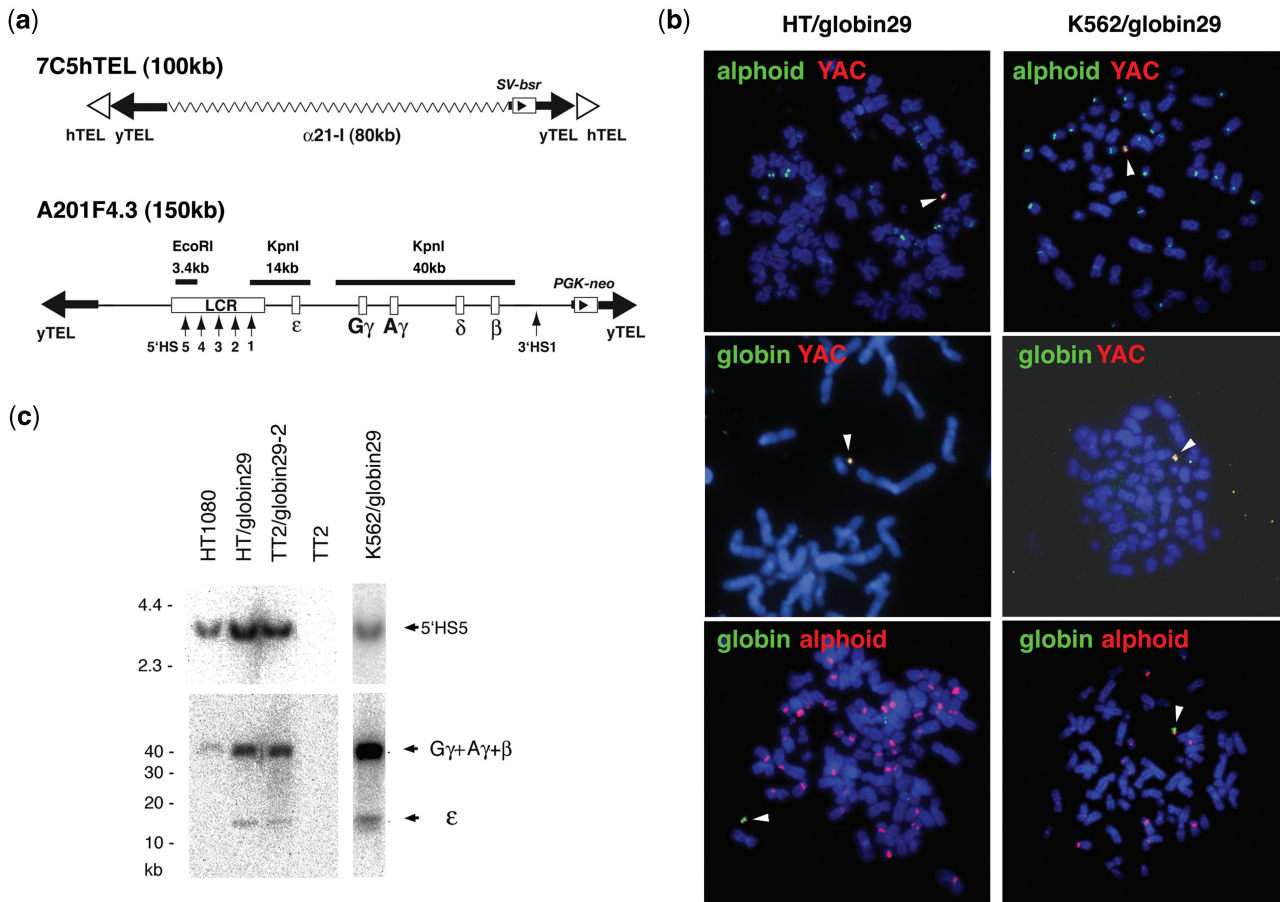


Figure 1. Properties of the globin-HAC. (a) Schematic representation of the precursor YACs. The 7C5hTEL is composed of an 80-kb $\alpha 21$ -I array, the selection marker SV-bsr, and human telomeres. The A201F4.3 contains 130 kb of the entire human β -globin locus (ϵ , $G\gamma$, $A\gamma$, δ and β) and the locus control region (LCR). The bars indicate the EcoRI and KpnI restriction fragments and their sizes. (b) Characterization of the globin-HAC in HT1080 and K562 cells. HT1080 cells carrying the globin-HAC (HT/globin29) and K562 cells carrying the globin-HAC (K562/globin29) were analyzed by FISH using YAC vector, alphoid DNA and globin genes as probes. Arrowhead indicates the globin-HAC. (c) Integrity analysis of the globin locus in the globin-HAC by Southern hybridization. Genomic DNA from HT1080, HT/globin29, TT2/globin29-2, TT2 and K562/globin29 cells was digested with EcoRI or KpnI; 5'HS5, ϵ -, $G\gamma$ -, β -globin were used as probes.

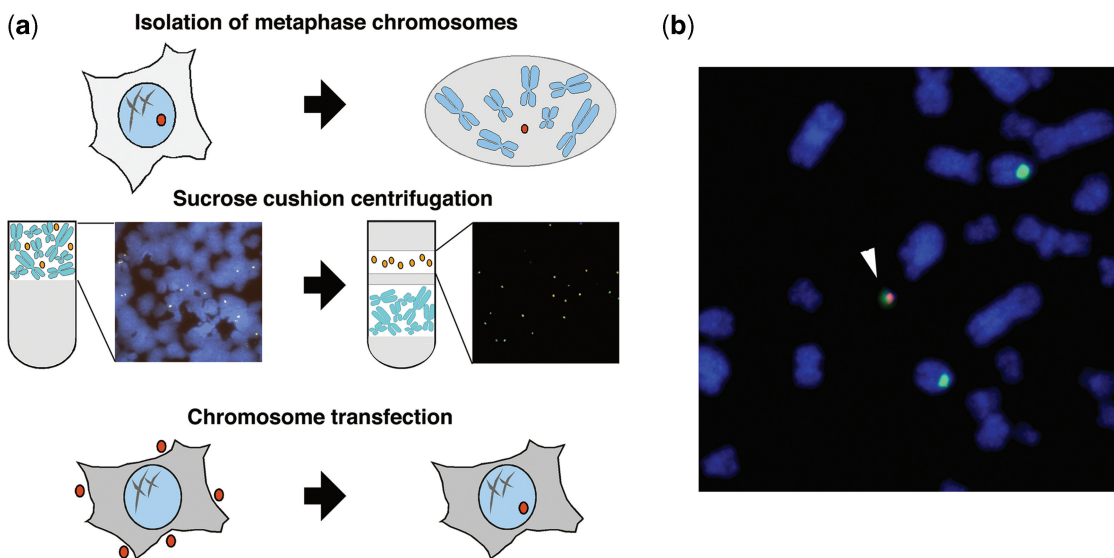


Figure 2. Transfer of the HAC by chromosome transfection. (a) Scheme of chromosome transfection. HACs were collected from metaphase cells, isolated by centrifugation using sucrose cushion and transfected into target cells using lipofection reagents. The red shapes show HACs and blue shapes show nuclei or metaphase cellular chromosomes. (b) FISH analysis of the globin-HAC in HeLa/globin-vK cells. Metaphase chromosomes of HeLa/globin-vK cells were probed using alphoid DNA (green) and YAC vector DNA (red).

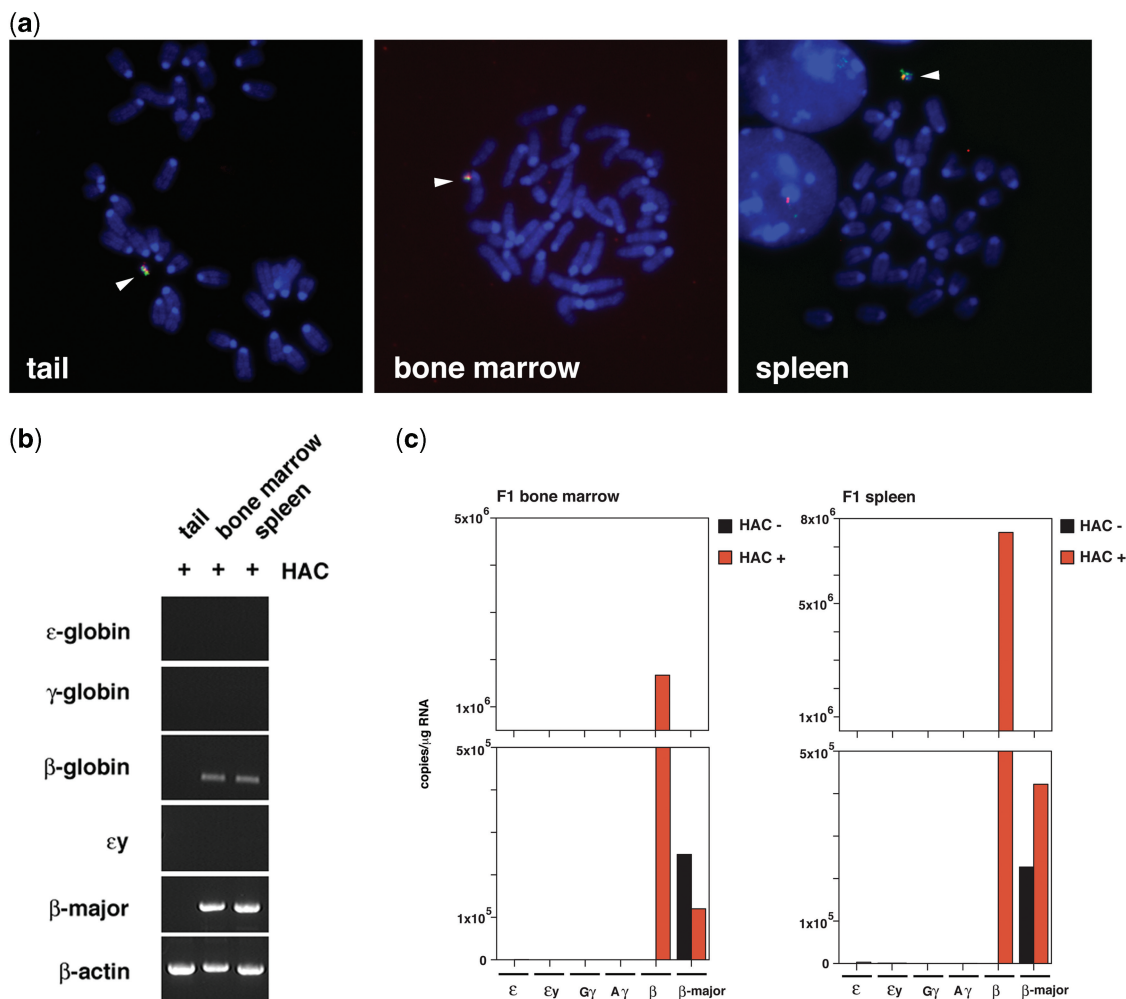


Figure 3. Expression of globin genes from the globin-HAC in mice. (a) FISH analysis of the globin-HAC in tissues (tail, bone marrow and spleen) from an F1 mouse. The red signal shows YAC DNA and the green signal shows alphoid DNA. Arrowheads indicate the globin-HAC. (b) Tissue-specific expression of globin genes from the HAC. The globin transcripts from the human and mouse genes were detected by RT-PCR using total RNA derived from adult mouse tail, bone marrow and spleen. (c) Quantification of globin transcripts in an F1 mouse. Expression of globin genes from bone marrow and spleen was determined by quantitative real-time PCR. Histograms show globin gene transcript quantities in the presence (+) or absence (–) of the globin-HAC.

from TT2/globin29-2 and TT2F/globin29 cells. Mice carrying the globin-HAC were obtained by crossing the chimeric mice created using TT2/globin29-2 cells (12). FISH analysis using metaphase chromosomes from the F1 mice revealed that the globin-HAC was maintained in the tail, bone marrow, and spleen cells at 90%, 98% and 96%, respectively (Figure 3a). RT-PCR and quantitative real-time PCR analyses showed that the human adult-type β -globin gene was expressed in bone marrow and spleen, but not in the tail, and that the human ϵ - and γ -globin genes were not expressed in adult tissues (Figure 3b and c). The expression level of the human adult-type β -globin gene in an F1 mouse was 14.3- or 15.2-fold higher than that of the mouse β -major globin in bone marrow or spleen, indicating that high-level expression was dependent on the globin gene copy number, as previously reported (Figure 3c) (22). These results indicate that the natural patterns of tissue-specific expression of the human globin genes on the HAC were mimicked in mice.

Differentiation-specific responses of human globin genes in maturing mouse erythrocytes

Differentiation-specific expression patterns of globin genes could be reproduced by inducing *in vitro* differentiation of ES cells harbouring the globin-HAC into blood cells (Figure 4) (25). During differentiation of TT2/globin29-2 and TT2F/globin29 cells into erythrocytes, the mouse and human globin gene transcripts ($\epsilon\gamma$, β -major, ϵ , γ and β) were detected by RT-PCR (Figure 4a), and the expression levels were determined by quantitative real-time PCR (Figure 4b). Expression of endogenous mouse $\epsilon\gamma$ and β -major globin genes in TT2 and TT2F cells was examined at 5, 8, 11 and 14 days after onset of differentiation. Peak expression of the $\epsilon\gamma$ -globin occurred during days 8–11, whereas that of the β -major globin occurred during days 11–14. Under differentiation conditions, expression of the human ϵ - and γ -globin genes from the HAC in TT2/globin29-2 and TT2F/globin29 cells also began at day 8 and peaked after 8–11 days

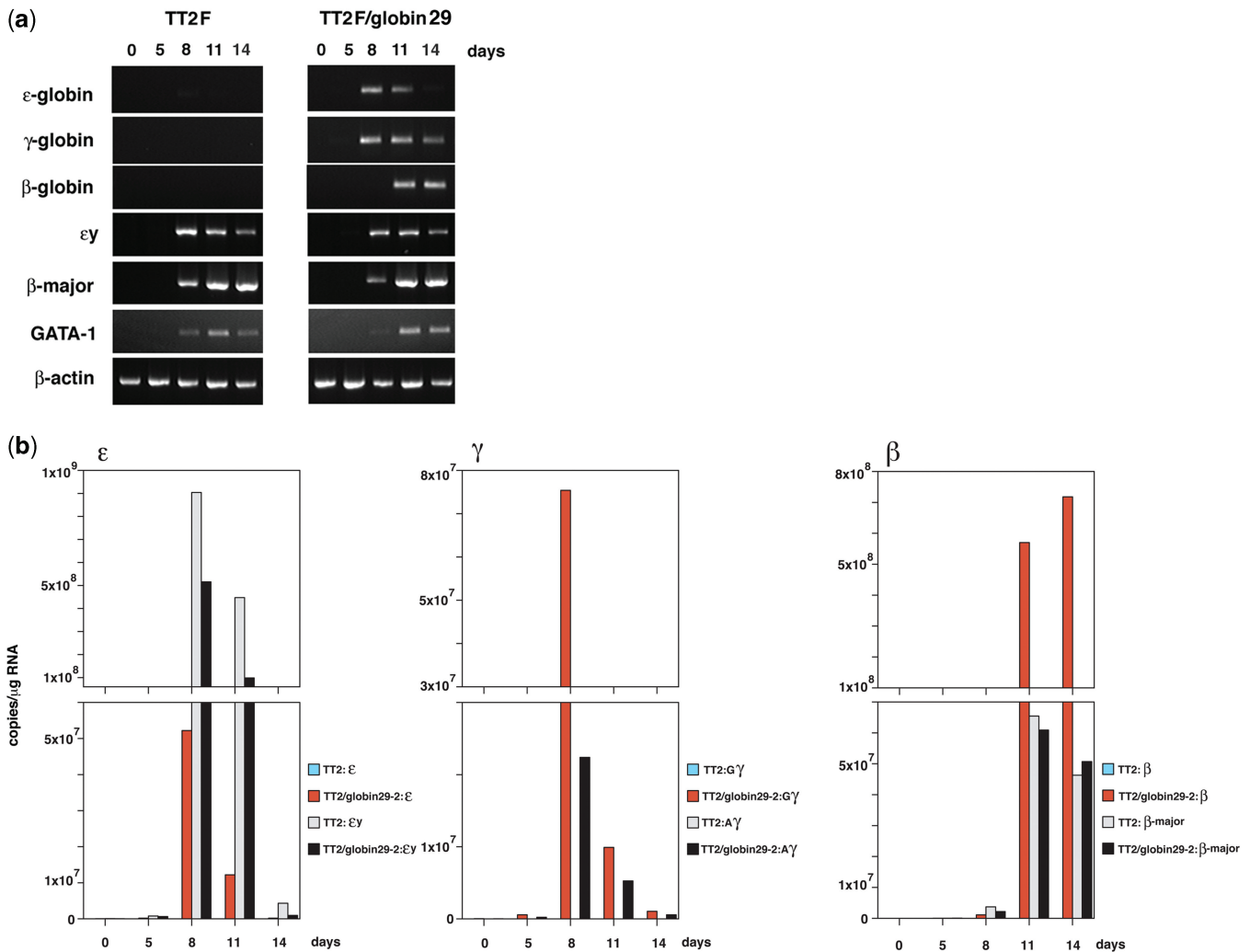


Figure 4. Expression pattern of globin genes from the globin-HAC in mouse blood cells differentiated from ES cells. (a) Expression pattern of human globin genes in differentiating ES cells. The floating cells were recovered from TT2F and TT2F/globin29 cells at days 0, 5, 8, 11 and 14 after induction of differentiation of ES cells into blood cells. The expression of human ϵ , $A\gamma$, $G\gamma$ and β -globin genes, and mouse $\epsilon\gamma$, β -major, GATA-1 and β -actin was detected by RT-PCR using total RNA. (b) Expression level of globin genes determined by quantitative real-time PCR. The histogram shows the quantities (copies/ μg RNA) of ϵ -, $G\gamma$ -, $A\gamma$ - and β -globin transcripts in TT2 and TT2/globin29-2 cells.

(Figure 4a and b). In contrast, expression of human β -globin began on day 11 and peaked during days 11–14 (Figure 4a and b). These data indicate that the expression pattern of human ϵ , γ and β -globin genes from the HAC switched in an appropriate stage-specific manner during differentiation into blood cells and correlated with the expression pattern of the mouse globin genes.

Responses of globin genes to a drastic change in cell type

To analyze the regulation of gene expression in response to drastic changes in cell type, the expression of globin genes was compared before and after globin-HAC transfer (Figure 5a). mRNA transcripts of the $G\gamma$ - and $A\gamma$ -globin genes were detected by RT-PCR using total RNA from cell lines with or without globin-HACs (Figure 5b). In the case of transfer from HT1080 to K562 cells, $G\gamma$ - and $A\gamma$ -globin transcripts were not

detected in HT/globin29 cells or in HT1080 cells. The $G\gamma$ -globin and $A\gamma$ -globin transcripts in K562/globin29 cells were 10.7- and 20.7-fold greater than those in the K562 cells without globin-HAC, as shown by quantitative real-time RT-PCR (Figure 5b). To analyze the expression response when transferring globin-HAC from erythrocyte cells to non-erythrocyte cells, we transferred the globin-HAC from K562 cells back into HT1080 or HeLa cells by chromosome transfection. We established eleven HT1080 and seven HeLa clones carrying globin-HAC (HT/globin-vK, HeLa/globin-vK). In the case of transfer from K562 to HT1080 or HeLa, $G\gamma$ - and $A\gamma$ -globin transcripts were not detected in HT/globin-vK or HeLa/globin-vK cell lines (Figure 5c). These results indicated that expression of the $G\gamma$ - and $A\gamma$ -globin genes from the HAC was activated in the K562 cells and was reproducibly repressed in non-erythrocyte cell lines.

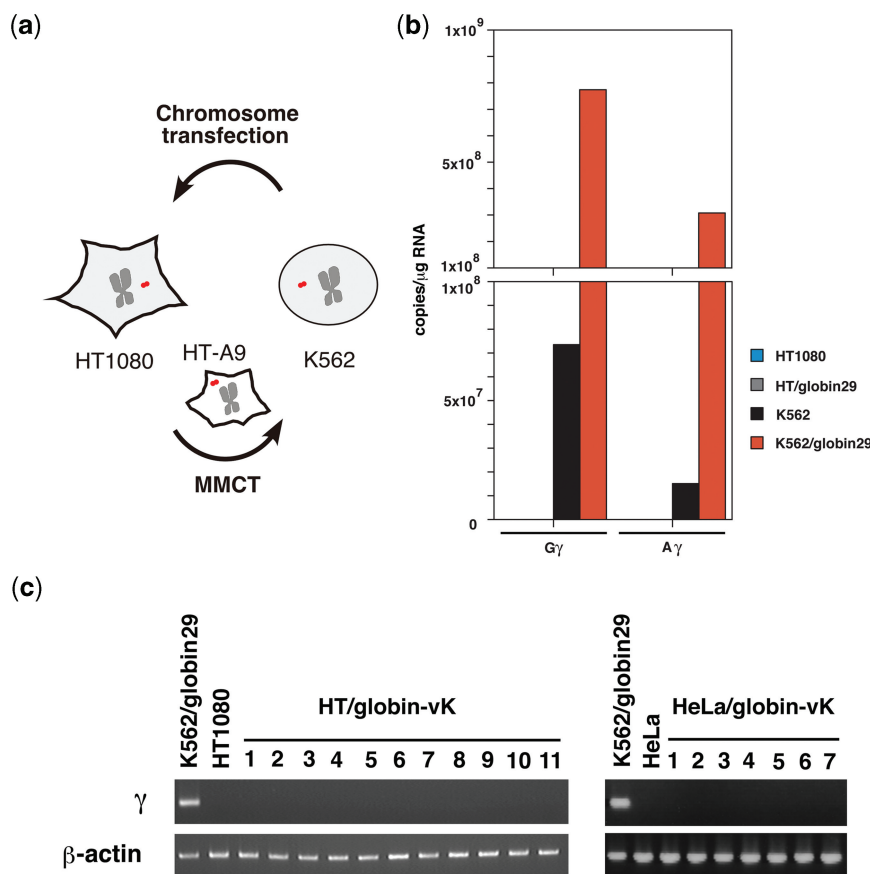


Figure 5. Expression of the globin genes after a drastic change in host cell type. (a) Scheme for HAC transfer. The globin-HAC was originally constructed in HT1080 cells, and then transferred to K562 cells by microcell-mediated chromosome transfer via a fusion cell line (HT-A9/globin29). The globin-HAC in K562/globin29 cells was transferred again to HT1080 and HeLa by transfection. (b) Quantification of globin transcripts by real-time PCR analysis. The histogram shows the quantities (copies/ μ g RNA) of G γ -globin and A γ -globin transcripts in HT1080 (blue), HT/globin29 (gray), K562 (black) and K562/globin29 (red) cells. (c) Expression of γ -globin gene from the globin-HAC in non-erythrocyte cells. Expression of γ -globin genes in 11 cell lines of HT/globin-vK and 7 cell lines of HeLa/globin-vK was analyzed by RT-PCR.

DISCUSSION

Genes can be packaged into chromatin using HAC technology in human HT1080 cells. However, one problem in manipulating HACs has been the introduction of the HAC into target cells. The general technique for introducing chromosomes has been MMCT, in which donor cells are limited to the minority of cultured cells that can form microcells. Previously, another technique for introducing chromosomes using murine satellite DNA-based artificial chromosomes (SATAC) ranging from 60 to 400 Mb was reported (26). SATACs could be purified by flow cytometry due to their characteristic base-pair composition (a greater AT:GC ratio), and they could be transferred to mammalian cell lines and primary cells using transfection reagents (27,28). This purification strategy by flow cytometry was specialized for SATAC and is not applicable to other chromosome materials, including HACs. The isolation and transfection strategy using metaphase chromosomes described herein is likely to be applicable to a variety of cell types, both as donors and recipients. Transfection efficiencies using this method are comparable to those of MMCT, indicating the utility of this strategy for the introduction of transgenes.

The mitotic and meiotic stability of human chromosomes in mice was previously demonstrated using the natural chromosome 21 and truncated mini-chromosomes that retained native chromosome function (1,29,30). We have established mouse ES cell lines carrying globin-HAC using a bottom-up strategy; we then created mice harbouring the HAC (12). In the current study, we first demonstrated the authentic expression of the transgenes from the HAC in mice. The behaviour of the human globin gene locus in transgenic mice is well-studied (16–22). The natural stage- and tissue-specific expression of the ϵ , γ and β -globin genes was reproduced from the human globin cluster on the HAC in hematopoietic differentiated cells, mouse embryos, and adult mouse tissue. In this study, the number of β -globin transgenes was 12 copies that was not physiological number. While high copy number of transgenes was useful for detection of the transgene expression, we need to examine the expression of β -globin from physiological copy number using HAC vector system in which we can put one or two copies of transgenes (13).

We could also manipulate the same globin-HAC in a human cell culture system. Expression of the γ -globin

gene on the globin-HAC was repressed in HT/globin29 cells, whereas the γ -globin gene was activated in a cell type-specific manner when the globin-HAC was transferred into K562. The γ -globin genes both on original HAC (HT/globin29) and transferred HAC (HT/globin-vK and HeLa/globin-vK) were repressed as the same level of endogenous genes in HT1080 and HeLa cells. However, the β -globin gene, which was repressed in HT1080 and expressed at low level in K562, barely expressed in small portion of HT/globin-vK cell lines (2 lines out of 11) and HeLa/globin-vK cell lines (1 line out of 7) at the lower level of that in K562 (data not shown). The low level transcript of β -globin gene in non-erythrocyte cells could be also detected in case of transiently transfected DNA or chromatin packaged HAC, as reported previously (31,32). Expression of the globin gene locus, repressed in HT1080 cells, was activated in K562 cells in the absence of the process of differentiation into adult erythrocyte cells, and was again repressed in HT1080 and HeLa cells when the HAC was transferred back from K562 cells. These data suggest that these HACs could be useful tools to correlate gene regulation with chromatin epigenetics, by examining DNaseI sensitive sites, histone modification and DNA methylation in the LCR formed in erythrocyte (K562) and non-erythrocyte (HT1080 and HeLa) cells. Full control of transgene expression in mammalian cell systems is very important, but this is not widely possible bioscience research because transgene expression is often influenced by chromatin structure and there is little methodological control over the number, integration location, and chromatin structure of transgenes in host cell chromosomes. The HAC developed in these studies allowed us to control the expression of transgenes in various cell lines. The technology for manipulating genes packaged into chromatin using HACs could find wide-spread application in basic science, applied biology, and in regeneration medicine.

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Conflict of interest statement. None declared.

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