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Inducing Segmental Aneuploid Mosaicism in the Mouse Through Targeted Asymmetric Sister Chromatid Event of Recombination

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ABSTRACT

Loss or gain of whole chromosomes, or parts of chromosomes, is found in various pathological conditions, such as cancer and aneuploidy, and results from the missegregation of chromosomes during cellular division or abnormal mitotic recombination. We introduce a novel strategy for determining the consequences of segmental aneuploid mosaicism, called targeted asymmetric sister chromatin event of recombination (TASCER). We took advantage of the Cre/loxP system, used extensively in embryonic stem cells for generating deletions and duplications of regions of interest, to induce recombination during the G2 phase. Using two loxP sites in a *Cis* configuration, we generated *in vivo* cells harboring microdeletions and microduplications for regions of interest covering up to 2.2 Mb. Using this approach in the mouse provides insight into the consequences of segmental aneuploidy for homologous regions of the human chromosome 21 on cell survival. Furthermore, TASCER shows that Cre-induced recombination is more efficient after DNA replication *in vivo* and provides an opportunity to evaluate, through genetic mosaics, the outcome of copy number variation and segmental aneuploidy in the mouse.

VARIATIONS in copy number are an important source of modifications in the genome, contributing to the diversity of phenotypes and to disease (Shaw and Lupski 2004; Gonzalez *et al.* 2005; Aitman *et al.* 2006; Fellermann *et al.* 2006; Sebat *et al.* 2007; Sharp *et al.* 2007, 2008; Hollox *et al.* 2008). They correspond to segmental duplications or deletions and represent rare variants or polymorphic events in the population (Feuk *et al.* 2006a; Redon *et al.* 2006; Kidd *et al.* 2008). As a consequence, genetic expression could change either directly, for genes located inside the region, or indirectly through positional effects for genes located outside (Lupski and Stankiewicz 2005; Feuk *et al.* 2006a,b; Freeman *et al.* 2006).

Complete loss of a chromosome often induces more severe consequences with cellular dysfunction, such as tumorigenesis, impaired viability, such as spontaneous abortion, or aneuploid syndromes such as Down syndrome (DS) (Robinson *et al.* 1999, 2001; Hassold and Hunt 2001; Hassold *et al.* 2007). Nevertheless, aneuploid mosaicism has been described for almost all the chromosomes in humans and confined chromosomal mosaicism affects ~2% of viable pregnancies (Hahnemann and Vejerslev 1997; Kalousek 2000). It originates during cell division with the missegregation of a chromosome; while one cell will receive an additional chromosome leading to trisomy, the second daughter cell will be monosomic. Somehow, the distribution of mosaicism depends on the timing, the cell lineage, the viability of the aneuploid cells, and the chromosome or the region involved. Generalized chromosomal mosaicisms, induced early during embryogenesis, exist but are less frequent and often linked to pathological conditions. For example, in DS, mosaicism represents 1–2% of cases (Mikkelsen 1977), whereas the complete absence of the HSA21 is almost incompatible with life (Katz-Jaffe *et al.* 2004, 2005). Somatic aneuploid mosaicism has been described in blood cells (Cheung *et al.* 2007) and in normal brain with a frequency of 1.25–1.45% per chromosome and a percentage of aneuploid cells >30% (Rehen *et al.* 2001, 2005; Kingsbury *et al.* 2005, 2006; Yurov *et al.* 2008). Several hypotheses have been drawn to explain the presence and the outcome of such aneuploid cells (Kingsbury *et al.* 2006) but no clear evidence has been documented.

In vivo, the induction of segmental aneuploidy has been achieved by using the Cre/loxP recombinase system in several species (for review see Lewandoski and Martin 1997; Brault *et al.* 2006). In the mouse, a large panel of Cre transgenes are described to generate segmental deletion, duplication, inversion, and translocation (Kmita *et al.* 2002; Spitz *et al.* 2005; Wu *et al.*

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2007). For such large fragments, specific promoters have been used to direct the expression of the recombinase during meiosis in the *ta*rgeted *me*iotic *re*combination (TAMERE), (Herault *et al.* 1998; Kmita *et al.* 2002; Genoud *et al.* 2004; Zakany *et al.* 2004; Tarchini and Duboule 2006), in different tissues (Mao *et al.* 2005; Spitz *et al.* 2005), or in specific cell lineages (Zheng *et al.* 2000). The improvement of the chromosomal engineering technique is definitely based on the availability of a powerful Cre-expressing construct such as the *Hprt1*$^{tm1(Cre)Mnn}$ mouse line (Tang *et al.* 2002; Wu *et al.* 2007).

To go further into the analysis of the aneuploid mosaicism consequences, we used the property of the Cre recombinase to react on G2 phase of the cell cycle to develop a strategy for generating segmental aneuploid mosaicism in mouse tissues, allowing the study of the survival and the function of cells carrying microdeletion and microduplication. This strategy is based on a targeted asymmetric sister chromatid event of recombination (TASCER), due to a Cre-dependent recombination induced after the replication of DNA. In this article, we reveal for the first time the utility of TASCER to determine the consequences of segmental aneuploid mosaicism for two specific regions homologous to the telomeric part of HSA21 in the mouse.

MATERIALS AND METHODS

Gene targeting and mouse lines: The targeting vectors for *Prmt2* and *U2af1* were isolated from the *3′HPRT* library, whereas the *Col6a1*, *Cstb*, and *Abcg1* targeting vectors were obtained from the *5′HPRT* library (Zheng *et al.* 1999). LoxP sites were inserted in the same relative orientation by two successive homologous recombination events in HM-1 embryonic stem (ES) cells, as described in (Besson *et al.* 2007). For each targeting event, ES clones were selected for homologous recombination at the defined loci and injected into blastocysts to obtain chimeras. By crossing with C57BL/6J (B6) mice, we established the *Cis(Prmt2-Col6a1)*^{tm1Yah}, *Cis(Prmt2-Cstb)*^{tm1Yah}, and *Cis(Abcg1-U2af1)*^{tm1Yah} mouse lines called *Cis1* (Besson *et al.* 2007), *Cis2*, and *Cis3*, respectively, which carry the two loxP sites in a *Cis* configuration on *Mus musculus* (MMU) chromosome 10 (MMU10) for the first two lines and 17 (MMU17) for the last one.

Four transgenic mouse lines, expressing the CRE recombinase were used. They are either transgenic or site-targeted derived and express the *Cre* gene from various promoters: ubiquitously [*Tg(CMV-Cre)1Pcn*] (Dupe *et al.* 1997), during female ovogenesis [*Tg(Zp3-Cre)93Knw*] (De Vries *et al.* 2000), in specific tissues, such as neural progenitors [*Tg(Nes-Cre)1Kln*] (Tronche *et al.* 1999), or in specific cell types from the myeloid lineage [*Tg(MLys1-Cre)Cgn*] (Clausen *et al.* 1999). Cre mouse lines were crossed with the "*Cis*" lines described above to generate double heterozygote individuals, which were studied further. All animals were bred under specific pathogen-free (SPF) conditions and were treated in compliance with animal welfare policies from the French Ministry of Agriculture (law 87 848 and YH accreditation 45-31).

Genotyping and sequencing: Genomic DNA was extracted from tails, ES cells, and mouse organs, including testis, intestine, liver, kidney, muscles (gastrocnemius and biceps brachialis), heart, lung, brain, cerebellum, tongue, eyes, salivary glands, spleen, skin, stomach, and thymus. The analysis was carried out by PCR or Southern blot using standard operating procedures (Besson *et al.* 2007). Specific primer pairs (available upon request) were used to generate PCR-derived probes for the ampicillin and the neomycin resistance genes included in the targeting vectors.

In addition, PCR was performed to amplify specific 2-kb and 3-kb DNA fragments from the chromosomes carrying the deletion and the segmental duplication, respectively. To check the integrity of the recombined sequences, sequencing was performed with the BigDye Terminator V3.1 chemistry and analyzed with an ABI PRISM 310 apparatus (Applied Biosystem, Foster City, CA), as described previously (Besson *et al.* 2005).

Fluorescence *in situ* hybridization: Interphase nuclei were recovered by affixing a sample of frozen/defrosted organs onto a slide. The mouse BACs were chosen from the RPCI-24 Mouse BAC Library (C57BL/6J Male, http://bacpac.chori.org/mmouse24.htm). BAC DNAs were purified with Nucleobond BAC 100 (Macherey-Nalgen, Hoerdt, France). One microgram of mouse BAC located inside (RPCI24-445L22) and outside (RPCI24-247G13) of the deleted region was used to generate DNA probes labeled by nick translation with DIG-dUTP (for 445L22) and biotin-dUTP (for 247G13) using ROCHE nick translation mix for *in situ* probe. The detection was realized by the use of both antidigoxigenin-rhodamine antibody and avidin-fluorescein (Roche Molecular Biochemicals, Mannheim, Germany) according to the manufacturer's instruction manual. The slides were mounted and images were captured as described previously (Besson *et al.* 2007).

RESULTS

Generating targeted asymmetric sister chromatid event of recombination: To generate segmental mosaic aneuploidies on the two distinct chromosomes MMU10 (*Cis1* and *Cis2*; Figure 1) and MMU17 (*Cis3*; Figure 1), we used several Cre-expressing transgenes. As a control experiment, we checked whether we were able to create the corresponding deletion from the *Cis* configuration (*Cis/+*). We crossed the *Cis/+* mice with a general Cre deleter line, the *Tg(CMV-Cre)1Pcn* (Dupe *et al.* 1997). We were successful in generating the deletion *Del1* of a 0.5-Mb genetic interval, as reported in 30% of newborns ($n = 18$) obtained by breeding the *Cis1/+*, *Tg(CMV-Cre)1Pcn/0* mosaic mice, which carry the *Cis* configuration plus the deletion of the targeted region, with wild-type mice (Besson *et al.* 2007). Similarly, we tried with the *Cis2* configuration, in which the two loxP sites are located 2.2 Mb apart. However, analysis of 183 progeny revealed that we did not get the transmission of the *Del2* chromosomes from a transheterozygous animal carrying the *Cis2/+*, *Tg(CMV-Cre)1Pcn/0*. Nevertheless, we succeeded *in vivo* to recover *Del2* using the *Tg(Zp3-Cre)93Knw* transgene in 3 of 83 animals. Furthermore, we detected the deleted band from the tail of such a *Cis2/+*, *Tg(CMV-Cre)1Pcn/0* animal using a specific PCR assay (data not shown). At this point, we investigated whether the transheterozygous animal carrying the *Cis2/+* chromosome and the *Tg(CMV-Cre)1Pcn* trans-

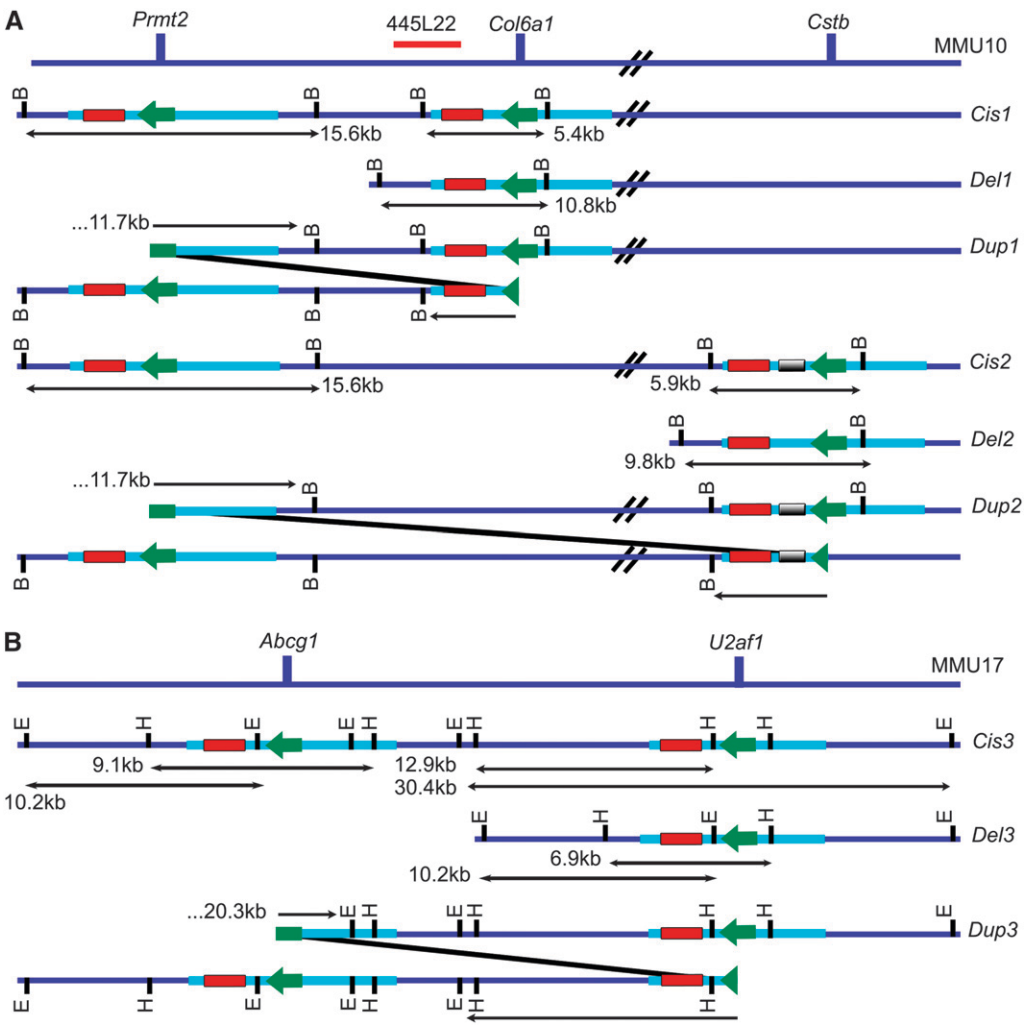

Figure 1.—Schematic of the different genetic configurations used in the study located on mouse chromosome 10 (A) or 17 (B). (A) The loxP sites were inserted in a *Cis* configuration by gene targeting in ES cells in the *Prmt2* and *Col6a1* loci (*Cis1*). The Cre expression generates the corresponding deletion (*Del1*) and duplication (*Dup1*). *Cis2* corresponds to the insertion of a loxP site in a *Cis* configuration in the *Prmt2* and *Cstb loci* while *Del2* and *Dup2* are the respective deletion and duplication of the 2.2-Mb-long genetic interval. The localization of the BAC used for the FISH analysis is shown (orange). (B) An additional *Cis* configuration (*Cis3*) with the corresponding deletion and duplication, respectively *Del3* and *Dup3*, was used in the study on MMU17 with two loxP sites targeted at the level of the *Abcg1* and *U2af1* genes. The positions of the targeting vectors are shown in blue with the loxP sites represented as a green arrow. The size of the restriction bands identified by probe A (red box) or probe B (black box) are indicated in kilobases. Bg, *Bgl*II; H, *Hin*dIII; E, *Eco*RI.

gene was mosaic for the partial deletion of the corresponding region.

The CMV promoter is known to be expressed ubiquitously and early during embryogenesis (Zakany and Duboule 1996; Dupe *et al.* 1997; Spitz *et al.* 2005). Consequently, the Cre recombinase should be active in a variety of cell types using the *Tg(CMV-Cre)1Pcn* transgene (Dupe *et al.* 1997). We isolated DNA from 18 different organs from ~20 *Cis2*/+, *Tg(CMV-Cre)1Pcn*/0 mice to detect the corresponding *Del2* deletion. Southern blot analysis, as shown in Figure 2A, unraveled a more complex situation than initially expected. The parental *Cis2* alleles were detected in all organs (Figure 2A). Surprisingly, additional bands could be associated with the presence of both the deletion (*Del2*) and the tandem duplication (*Dup2*) in a variety of organs, such as the kidney, different muscles, the tongue, the lung, or the cerebellum. The identification of the *Dup2* was confirmed on the same blot by using a neomycin-specific probe that revealed the expected restriction pattern of the *Dup2*, the recombinant *Cstb* locus, and the integrated *Cre* transgene (Figure 2B).

To produce both the deleted and the duplicated configurations, there should be a TASCER. This event takes place between the two loxP sites placed in a *Cis* configuration after the replication of cellular DNA when loxP are present on both sister chromatids (Figure 3). A direct consequence of such a TASCER event is the generation of cells carrying partial monosomy and trisomy in a diploid context. Thus, to check this hypothesis, we performed a fluorescence *in situ* hybridization (FISH) analysis on nuclei isolated from gastrocnemius muscles of mutant animals, using a BAC probe located in the *Prmt2-Cstb* genetic interval and a control one outside the region (Figures 1 and 2D). The number of detected trisomic (Ts) and monosomic (Ms) aneuploid nuclei for the *Prmt2-Cstb* interval in this tissue was relatively stable between the four independent individuals and reached 46.5 ± 6.4% of the observed nuclei (Table 1). Similar aneuploid mosaicism was observed with the *Prmt2-Col6a1* interval, which is a MMU10 *Prmt2-Cstb* subregion (Figure 1). The combination of the *Cis1* allele with the *Cre* transgene leads to an equivalent distribution of *Del1*, the corresponding deletion, and *Dup1*, the respective dupli-

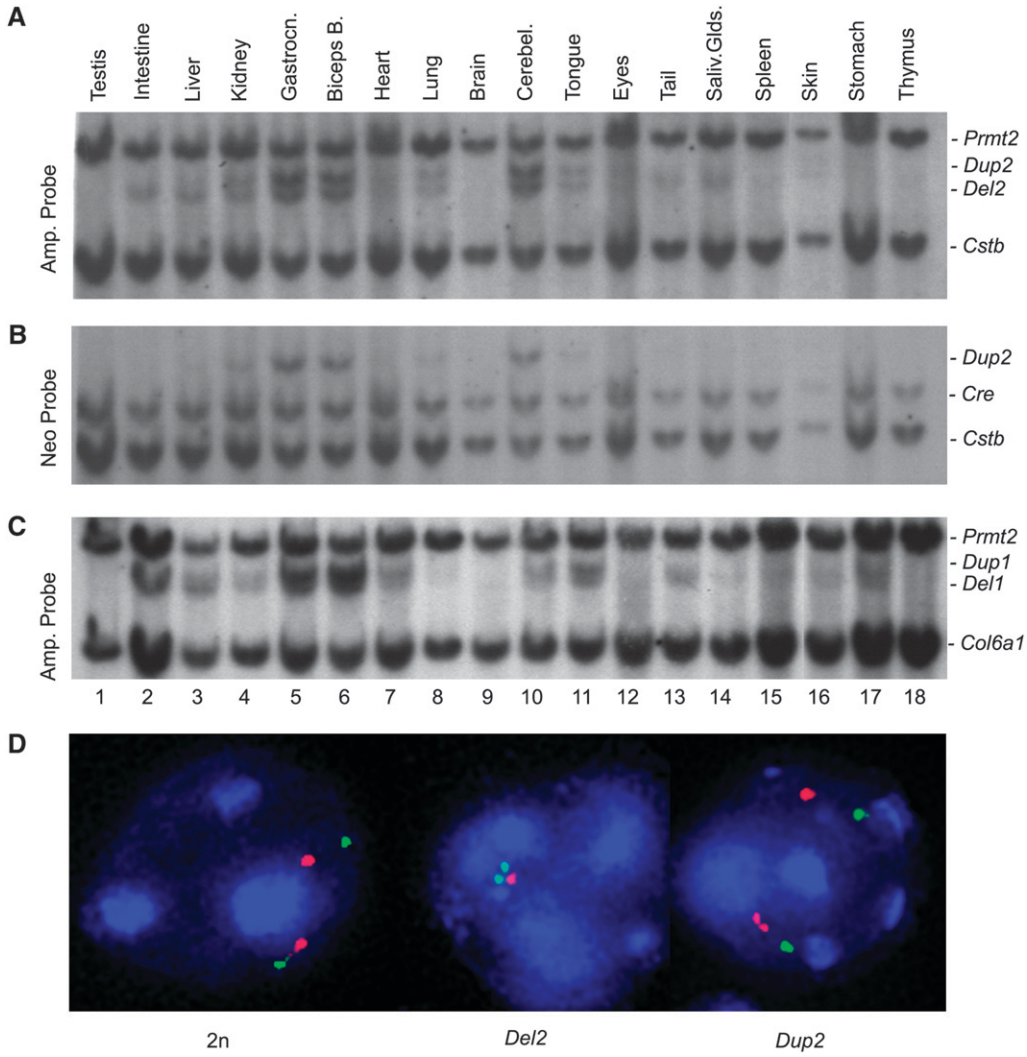

FIGURE 2.—Detection of TASCER. (A–C) Southern blot analysis of targeted asymmetric chromatid event of recombination for the *Prmt2-Ctsb* and *Prmt2-Col6a1* genetic intervals located on MMU10. Lanes 1–18 are DNA extracted from various tissues of one tested male carrying the *Cis2* (A and B) or the *Cis1* (C) alleles and hybridized with the Amp (A and C) or Neo (B) probes, as indicated. (A) Bands corresponding to the bordering locus of *Cis2* are found in all the lanes. No Cre-dependent recombination is induced in the testis, brain, or stomach, whereas the deletion *Del2* is observed in the intestine, tail, and salivary gland. Moreover, balanced *Dup2* and *Del2* are detected in the kidney, muscles (gastrocnemius and biceps brachialis), lung, cerebellum, tongue, eyes, and skin. Accordingly, tissues where the balanced *Del2* and *Dup2* were detected (A) displayed a specific band for the duplication when hybridized with the Neo probe. The Neo probe hybridized also with the *Cstb* locus and the Cre transgene in all the lanes (B). (C) Similar tissues were isolated from a *Cis1*/+, *Tg(CMV-Cre)Pcn*/0 individual and bands corresponding to the *Del1* and the *Dup1* were detected in the same panel of tissues (*Del1*: lanes 2–8, 10, 11, 13–17; *Dup1*: lanes 3–8, 10, 11, 16, 17). (D) Interphase FISH analysis with BAC probes that map in the region of the deletion and duplication (red). The wild-type (2*n*) nuclei showed two red signals whereas those carrying the *Del2* or the *Dup2* displayed, respectively, one or three red signals for the *Prmt2-Cstb* region.

cation in the same panel of organs (Figure 2C). Further investigation using muscles isolated from four *Cis2*/+, *Tg(CMV-Cre)1Pcn*/0 animals, aged from 28 days postpartum (dpp) to 156 dpp, revealed a stable level of aneuploid cells. However, we noticed little variation with aging (data not shown). In addition, we tested the variability of the TASCER event in five different tissues and we estimated the percentage of aneuploid nuclei using FISH analysis. As shown in Table 2, the level of aneuploid cells was very low in the kidney (19.0 ± 6.3%), the lung (25.0 ± 2.7%), and the brain (18.9 ± 6.4%). In addition, the percentage of monosomic cells was higher in these tissues, as observed from the Southern blot analysis. Both segmental trisomic and monosomic nuclei were found in equal amounts within muscles (18.3 ± 3.8% and 28.2 ± 3.4%, respectively) and cerebellum (19.6 ± 3.5% and 21.0 ± 4.7%, respectively) at similar levels in both populations. Altogether, these data demonstrated that the aneuploid mosaicism induced by the *Tg(CMV-Cre)1Pcn*/0 is stable between individuals for a given tissue.

We next analyzed the border of the new loci by direct sequencing of specific PCR products generated from the duplicated and the deleted regions. No modification of the sequence was observed and we confirmed that the recombination takes place as expected (Figure 4). However, this absence of modification in the loxP sequence cannot explain why the duplicated chromosome, carrying three loxP sites in tandem, is still found in some cells. On the contrary, we would have expected that the loxP sites were the target of the Cre recombinase leading to the previous *Cis* configuration and ultimately to the deletion with a unique loxP site. Despite the fact that it is impossible to have germline transmission of the rearranged chromosome from the *Cis2*/+, *Tg(CMV-Cre)1Pcn*/0, as described above, we were able to detect the new genetic configuration in a few somatic tissues. These results definitely show that the CMV pro-

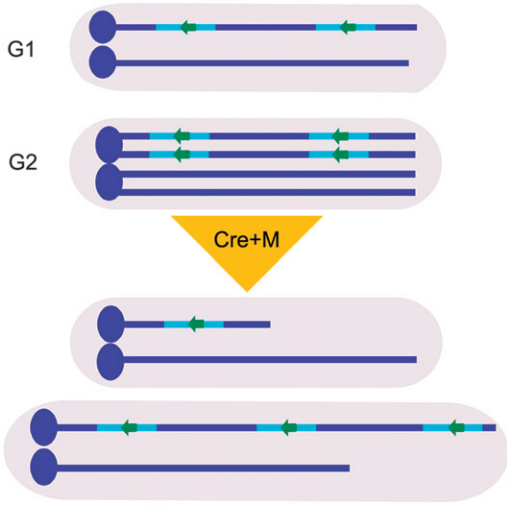

FIGURE 3.—The targeted asymmetric sister chromatid event of recombination (TASCER). Schematic of the TASCER that should take place in the cells carrying a *Cis* configuration with two loxP sites. After the S phase the *Cis* configuration has been replicated and could be the target of the Cre recombinase in an asymmetric way leading to monosomic and trisomic daughter cells after mitosis.

moter is able to drive the Cre recombinase expression in a tissue-specific manner with the ability to generate a deletion and a duplication of the targeted region of interest. Moreover, the recombination event seems to be as accurate in this experiment, using long-range loxP sites spacing, as in more classical *Cis* configurations.

Thus, starting from a *Cis* configuration with two loxP sites in the same relative orientation, located either 0.5 or 2.2 Mb apart, the expression of the Cre does not produce solely a complete deletion, but a more complex situation with monosomic and trisomic cells due to an efficient recombination after the G2 phase. This event should result from a higher activity of the Cre recombinase in dividing cells, but raises the question whether the TASCER is dependent on the tissue or on the Cre-expressing transgene.

Tissue-specific aneuploid mosaicism: Although the TASCER event occurs in tissues from different embryonic origins, such as muscle and cerebellum, the segmental aneuploid mosaicism shows a large variability between tissues. Some tissues, such as the cerebellum and muscle,

TABLE 1

Percentage of aneuploid nuclei detected in muscles (*gastrocnemius*) of four *Cis2/+; Tg(CMV-Cre)*^{Pcn}/0 animals

Individual	2*n*	Ms	Ts
N1	47.1	31.8	21.2
N2	59.8	27.2	13.0
N3	49.0	30.0	21.0
N4	58.0	24.0	18.0

2*n*, wild type; Ms, monosomic; Ts, trisomic.

TABLE 2

Percentage of aneuploid nuclei found in different tissues derived from four *Cis2/+; Tg(CMV-Cre)*^{Pcn}/0 animals (mean ± SD)

Tissue	2*n*	Ms	Ts
Kidney	81.0 ± 6.3	16.3 ± 5.6	2.8 ± 1.0
Lung	75.0 ± 2.7	20.1 ± 2.3	4.9 ± 3.4
Brain	81.1 ± 6.4	15.5 ± 4.1	3.5 ± 2.4
Muscle	53.5 ± 6.4	28.2 ± 3.4	18.3 ± 3.8
Cerebellum	59.4 ± 8.0	21.0 ± 4.7	19.6 ± 3.5

2*n*, wild type; Ms, monosomic; Ts, trisomic.

sustain or maintain the aneuploid conditions, while in others the level of aneuploid cells is reduced. In addition, the duplicated configuration is normally unstable with three unmodified loxP sites (HOLZENBERGER *et al.* 2000). Consequently, duplication should be observed only (1) if the expression or the activity of the Cre recombinase is reduced or (2) if the local and tissue-specific structure of the chromosome does not allow the Cre to act on the loxP sites. Definitely the level of the Cre recombinase should reach a level that allows the recombination to occur in the tissue. Nevertheless the variability of aneuploid cells between different tissues may be a direct consequence of the Cre expression level or may result from the lethal effect induced by the aneuploidy during cellular development. Indeed, it is known that complete monosomy 21 often leads to premature death of the patient. Thus, we explored further the potential of the TASCER strategy by comparison with classical deletion induced by the Cre. We used additional transgenic lines expressing constitutive Cre in specific tissues, such as neural progenitors [*Tg(Nes-Cre)1Kln*] (TRONCHE *et al.* 1999), in cell types derived from the myeloid lineage [*Tg(MLys1-Cre)Cgn*] (CLAUSEN *et al.* 1999), and in another cell line carrying two loxP sites located 0.6 Mb apart on MMU17 (*Cis3*; Figure 1). In a series of experiments we tested the induction of the partial monosomy and trisomy in cells from different tissues that were not affected or analyzed previously, such as the brain, bone marrow, and bone-marrow-derived macrophages (BMDM) in culture. As a result, we observed similar recombination events leading to *Del3* and *Dup3* in different parts of the brain of *Cis3/+; Tg(Nes-Cre)1Kln/0* mice but not in tissues where the Cre is not expressed with this transgene, such as liver, kidney, and muscle. These results show that TASCER could be used for another region of the mouse genome on MMU17 (Figures 1 and 5A). Similarly, TASCER events were detected in the subregions of the brain isolated from the *Cis2/+; Tg(Nes-Cre)1Kln/0* animals (Figure 5A). TASCER could be induced both on MMU10 and MMU17 during neural development in progenitor cells while the *Nestin* promoter is expressed (TRONCHE *et al.* 1999), thus TASCER could be induced

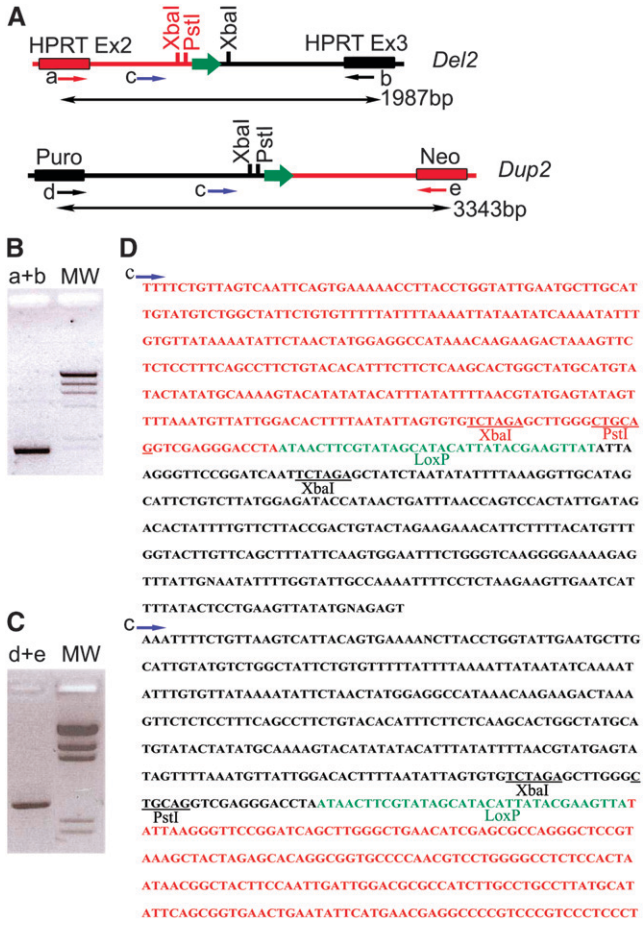

FIGURE 4.—Controlling the TASCER event. (A) Detection of recombinant alleles. A PCR fragment encompassing the deletion was amplified as a specific 1987-bp DNA fragment using primer a located in the 3′HRPT vector and primer b in the 5′HPRT vector. For the duplication, a PCR was performed to amplify a specific 3343-bp fragment using primer d located in the puromycin-resistant gene and primer e located in the neomycin-resistant gene. (B) PCR amplification for deletion using genomic DNA isolated from the muscle of a mosaic animal, using both primers (a + b). The fragments (a + b) were purified and sequenced using primers a, b, and c. (C) PCR amplification for duplication using the same animal tissue with both primers d and e. The fragment (d + e) was purified and sequenced using primers d, e, and c. (D) DNA sequence of fragment amplified in B and C with primer c. Color of the sister chromatid DNA source is similar to A. Restriction sites are underlined and the sequence of the loxP sites is shown in green.

in different genomic contexts and in most organs. Finally, we investigated whether TASCER could be used to generate monosomic or trisomic cells *in vitro*. We prepared BMDM from four independent individuals with the *Cis1/+*, *Tg(MLys1-Cre)Cgn/0* genotype. The *Tg(MLys1-Cre)Cgn/0* transgene is known to be preferentially expressed in neutrophils and macrophages *in vivo*. A rapid analysis of several hematopoietic tissues from the double mutants (Figure 5C) pinpointed that a few monosomic and trisomic cells were present in the bone marrow and to a lesser extent in lung. However, these two populations were nearly absent from the tissue, except for a few cells carrying the *Del1* in the thymus or the spleen. On the contrary, the specific *Del1* and *Dup1* bands were observed *in vitro* in BMDM prepared from such *Cis1/+*, *Tg(MLys1-Cre)Cgn/0* animals (Figure 5C). This series of experiments using two new Cre expressing transgenes clearly demonstrates that TASCER can be widely and specifically induced for different genomic regions depending upon the specificity of the Cre driver transgene. Furthermore, it definitely confirms that both aneuploid conditions are compatible with cellular viability at least in several tissues.

DISCUSSION

In this article we report a strategy, called TASCER, for engineering segmental aneuploidy mosaicism *in vivo*. We show, on two distinct chromosomes and with three different Cre-expressing transgenes, how TASCER can efficiently recover cells with partial monosomy and trisomy with a relatively constant level, in a variety of tissues. We took advantage of the post-S phase-induced Cre recombination on loxP sites inserted on the same chromosome in a *Cis* configuration separated by up to 2.2 Mb. Such an event has not been previously reported, presumably because the Cre is very active on loxP sites that are located nearby, such as in most Cre/loxP applications. At this point, the duplicated configuration that contains three loxP sites may happen but should react again to get a *Cis* or a deletion and could not be observed. In our *Cis* configuration where the two loxP sites are located far away, the distances tend to decrease from the Cre-mediated recombination frequency (Liu *et al.* 1998; Zheng *et al.* 2000) somehow preserving the chromosome with a large duplication. Although duplications were commonly produced to obtain germline transmission of new configurations (Puech *et al.* 2000; Zheng *et al.* 2000), an *in vivo* generated duplication coming from a *Cis* configuration has not been published to date.

The Cre-mediated recombination event that takes place in between loxP sites after the S phase is crucial to the TASCER process. TASCER clearly depends on the capacity of the targeted cells to proliferate and on the ability of the Cre transgene to be expressed during proliferation. Results obtained with the three distinct *Cis* configurations tested in this report demonstrate that in the mouse, the Cre recombinase is much more active during the G2 phase than in G1, a phenomenon already described *in vitro* (Liu *et al.* 1998, 2002; Yu and Bradley 2001) and discussed *in vivo* (Zong *et al.* 2005). Nevertheless, the *in vitro* efficiency was so low that the restoration of a selectable marker, such as the *Hprt* minigene, is required to select the event (Ramirez-Solis *et al.* 1995; Adams *et al.* 2004). In our experiment, the rate of recombination in TASCER is far more efficient than the *in vitro* induction in embryonic stem cells (Liu *et al.*

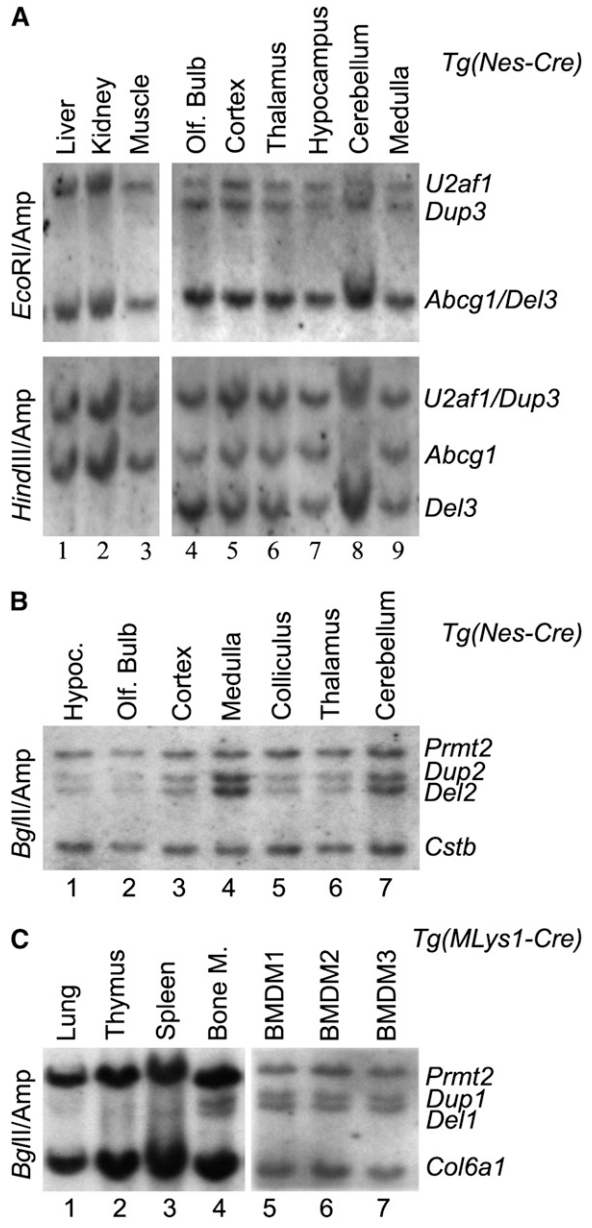

FIGURE 5.—Various Cre-expressing transgenes can induce TASCER *in vivo* or *ex vivo*. Different *Cis* configurations (A, *Cis 3*; B, *Cis2*, and C, *Cis1*) were associated with tissue-specific Cre-expressing lines [A and B, *Tg*(*Nes-Cre*) *1Kln*; C, *Tg(MLys1-Cre)Cgn*]. (A) Southern blot analysis of DNA isolated from the tissues of *Cis3/+*, *Tg(Nes-Cre)1Kln/0* animals that do not express the Cre recombinase (lanes 1, 2, and 3: liver, kidney, and muscle) or are expressing the Cre (different parts of the brain: lanes 4–9) revealed with the Amp probes and different restriction enzymes (top, *Eco*RI; bottom, *Hin*dIII), the bordering loci of the *Cis3* regions (*Abcg1* and *U2af1*), and the recombined fragment either *Del3* (bottom, lanes 4–9) or *Dup3* (top, lanes 4–9) resulting from TASCER. (B) Similar results were obtained during the analysis of several subparts of the brain [hippocampus (1), olfactory bulbs (2), cortex (3), *medulla oblongata* (4), colliculus (5), thalamus (6), cerebellum (7)] of a *Cis2/+*, *Tg(Nes-Cre)1Kln/0* individual showing that TASCER was induced efficiently in the different parts of the brain with the *Dup2* and *Del2* specific bands. (C) The *Tg(MLys1-Cre)Cgn* expressing line in macrophages and neutrophils was combined with the *Cis1* locus. Specific bands for *Cis1*, *Del1*, and *Dup1* were strongly detected *ex vivo* in bone-marrow-derived macrophages (BMDM: 5, 6, and 7) and in bone marrow (lane 4) whereas the recombination seems to be less efficient in the lung (lane 1), thymus (lane 2), and spleen (lane 3) from *Cis1/+*, *Tg(MLys1-Cre)Cgn* animals.

2002). Somehow, the Cre recombinase is more active *in vivo,* presumably due to stable conditions of expression *in vivo*, after the replication of DNA, in the G2 phase, while the chromatin structure is changing to prepare the future mitosis (LIU *et al.* 1998; WU *et al.* 2007). We could also assume that the *in vivo* condition is more permissive to support the aneuploid cells due to the variety of tissue conditions. The physical connection of the sister chromatids due to structure, such as the cohesin complex that is found until anaphase, should facilitate the induced event of mitotic recombination between DNA (LOSADA *et al.* 2002; TAKATA *et al.* 2007). In addition, the TASCER observation suggests that the Cre is preferentially active *in vivo* while cells are proliferating.

As reported in previous studies, the Cre recombinase activity is dose dependent and varies with the driving promoter. The human cytomegalovirus sequence is a powerful promoter that directs Cre expression in early embryogenesis. Thus it has been used to completely knock out genes of interest or to generate large rearrangements (ZAKANY and DUBOULE 1996; SPITZ *et al.* 2005). Here, we were able to induce up to 2.2 Mb long chromosomal rearrangements with a set of three promoters driving the expression of the Cre recombinase. Remarkably, although the duplicated configuration should be unstable due to the presence of three loxP sites in the same orientation (HOLZENBERGER *et al.* 2000), trisomic cells were found in a variety of organs showing that trisomy might be beneficial in some tissues. We tried without success to generate larger aneuploid mosaicisms with the *Tg(CMV-Cre)1Pcn* transgene for a region of ~9.2 Mb. The recombination of a larger genetic interval might require a stronger promoter such as the CAG promoter inserted in the *Hprt* locus of the *Hprt1*$^{tm1(Cre)Mnn}$ mouse line, which has been used successfully in a recent study (TANG *et al.* 2002; WU *et al.* 2007). As a consequence, inducing mosaic conditions in an adult animal will require preliminary experiments to balance the size of the targeted region with the expression level of the *Cre* gene. In addition, TASCER should be taken into account when trying to generate deletions *in vivo*. Additional controls to check for the duplication event should be carried out to avoid interference in the analysis of conditional knockouts for large genes or chromosomal regions. Thus, the degree of mosaicism generated through TASCER could vary in different tissues depending on the Cre transgene and on the proliferative capacities of the different cell types of each tissue.

Chromosomal mosaicism has been observed in pathological conditions, such as mosaic trisomy 21 (MODI

et al. 2003) or in cancer (Weaver and Cleveland 2007). In this report, we followed the impact of time on the survival of cells that are trisomic and monosomic for small regions homologous to HSA21 and we did not find any dramatic changes. Thus, the presence of trisomic and monosomic cells for the analyzed regions does not affect the cell survival. Due to the cellular organization of muscle cells, any trisomic or monosomic consequences should be compensated by the plurinucleated syncitia of the muscle fiber. On the contrary, in the cerebellum the consequence of aneuploidy on cell survival could only be compensated through noncell autonomous effect.

To date, little evidence supports a functional consequence of partial trisomy or copy number variation in nonpathological conditions, even though aneuploidies have been described in normal lineages, such as neurons (Iourov *et al.* 2006; Kingsbury *et al.* 2006; Muotri and Gage 2006; Yurov *et al.* 2007a,b, 2008) but one could expect to use TASCER to study such impact. More importantly, TASCER allows us to induce partial aneuploid conditions that will mimic the aneuploidy detected in cancer cells and to study further the consequence of loss or gain of copy number for chromosomal regions during tumorigenesis (Weaver and Cleveland 2007). For example, looking for tumor suppressor genes in a region identified as an aneuploidy-induced loss of heterozygosity in cancer cells could be done on a larger scale with TASCER compared to classical loss-of-function studies that need gene-by-gene inactivation or ES-cell-derived technology such as Micer (Adams *et al.* 2004). In conclusion, we believe that TASCER opens new perspectives to assess the consequence of aneuploid conditions on viability and to decipher the impact of copy number variation during cell fate determination and tumorigenesis.

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