A Synthetic Maternal-Effect Selfish Genetic Element Drives Population Replacement in Drosophila

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A Synthetic Maternal-Effect Selfish Genetic Element Drives Population Replacement in *Drosophila*

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One proposed strategy for controlling the transmission of insect-borne pathogens uses a drive mechanism to ensure the rapid spread of transgenes conferring disease refractoriness throughout wild populations. Here, we report the creation of maternal-effect selfish genetic elements in *Drosophila* that drive population replacement and are resistant to recombination-mediated dissociation of drive and disease refractoriness functions. These selfish elements use microRNA-mediated silencing of a maternally expressed gene essential for embryogenesis, which is coupled with early zygotic expression of a rescuing transgene.

Mosquitoes with a diminished capacity to transmit malaria or dengue have been identified in the wild and/or created in the laboratory, demonstrating that endogenous or engineered mosquito immunity can be harnessed to attack these pathogens (*1*–*5*). However, it will be necessary to replace a large percentage of the wild mosquito population with refractory insects to achieve substantial levels of disease control (*6*–*8*). Mosquitoes carrying genes that confer disease refractoriness are not expected to have a higher fitness than native mosquitoes, implying that Mendelian transmission is unlikely to result in an increase in the frequency of transgene-bearing individuals after their initial release into the wild (*4*, *9*). Thus, effective population replacement will require the coupling of genes conferring disease refractoriness with a genetic mechanism for driving these genes through the wild population at greater than Mendelian frequencies (*10*, *11*).

Maternal-effect selfish genetic elements [first described in the flour beetle *Tribolium castaneum* and known by the acronym *Medea* (maternal-effect dominant embryonic arrest)] select for their own survival by inducing maternal-effect lethality of all offspring not inheriting the element-bearing chromosome from the maternal and/or paternal genome (*12*) (Fig. 1A). Current models predict that if *Medea* elements are introduced into a population above a threshold frequency, determined by any associated fitness cost, they will spread within the population (*12*–*14*) (Fig. 1, C and D). When introduced into a population at relatively high frequencies, *Medea* elements are predicted to rapidly convert the entire population into element-bearing heterozygotes and homozygotes (Fig. 1C). *Medea* in *Tribolium* is hypothesized to consist of a maternal lethal activity (a toxin) that kills non-*Medea*–bearing progeny and a zygotic rescue activity (an antidote) that protects *Medea*-bearing progeny from this maternal lethal effect (*12*, *15*) (Fig. 1A).

To create a *Medea*-like maternal-effect selfish genetic element in *Drosophila*, we generated a P transposable element vector in which the maternal germline–specific *bicoid* (*bic*) promoter drives the expression of a polycistronic transcript encoding two microRNAs (miRNAs) designed to silence expression of *myd88* (the gene producing the toxin) [Fig. 1B and (*16*)]. Maternal Myd88 is required for dorsal-ventral pattern formation in early embryo development. Females with germline loss-of-function mutations for *myd88* give rise to embryos that lack ventral structures and thus fail to hatch, even when a wild-type (WT) paternal allele is present (*17*). This vector (known as *Medea*^{myd88}) also carries a maternal miRNA–insensitive *myd88* transgene expressed under the control of the early embryo–specific *bottleneck* (*bnk*) promoter (the gene producing the zygotic antidote) (Fig. 1B). Our analysis focused on flies carrying a single autosomal insertion of this element, *Medea*$^{myd88-1}$.

Matings between heterozygous *Medea*$^{myd88-1}$/+ males (where + indicates a chromosome that does not carry *Medea*$^{myd88-1}$) and homozygous +/+ females resulted in high levels of embryo viability, similar to those for the w^{1118} strain used for transformation (Table 1). In addition, 50% of the adult progeny carried *Medea*$^{myd88-1}$, as expected for Mendelian segregation without dominance. Matings among homozygous *Medea*$^{myd88-1}$ flies also resulted in high levels of egg viability. In contrast, when heterozygous *Medea*$^{myd88-1}$/+ females were mated with homozygous +/+ males, ~50% of progeny embryos had ventral patterning defects (fig. S1) and did not hatch (Table 1). All adult progeny (n > 12,000 flies) carried *Medea*$^{myd88-1}$ (Table 1). On the basis of these data and the results of several other crosses (Table 1), we inferred that a single copy of *bic*-driven miRNAs targeting maternal *myd88* expression was sufficient to induce maternal-effect lethality and a single copy of zygotic *bnk*-driven *myd88* expression was sufficient for rescue.

The above observations, in conjunction with the lack of any obvious fitness effects (lethality) in individuals carrying one or two copies of *Medea*$^{myd88-1}$, suggested that *Medea*$^{myd88-1}$ should be able to drive population replacement. To test this prediction, we mated equal numbers of WT (+/+) and *Medea*$^{myd88-1}$/*Medea*$^{myd88-1}$ males with homozygous +/+ females, giving rise to a progeny population with *Medea*$^{myd88-1}$ present at an allele frequency of ~25% (*16*). This level of introduction, although high, is not unreasonable, given previous insect population suppression programs (*18*). Replicate population cage experiments, carried out in a darkened incubator to prevent *Medea*$^{myd88-1}$–bearing flies (which are P^{w+} and thus red-eyed) from obtaining any vision-dependent advantage over their +/+ counterparts (which are w^{1118} and white-eyed) (*19*), followed three replicates for 20 generations. A second set of four replicates, which were initiated by crossing heterozygous *Medea*$^{myd88-1}$/+ males with homozygous +/+ females, was followed for 15 generations. In both experiments, non-*Medea*$^{myd88-1}$–bearing flies permanently disappeared from the population between generations 10 and 12 (Fig. 1E), without a loss of non-*Medea*–bearing + chromosomes (in *Medea*$^{myd88-1}$/+ individuals) in the population (Fig. 1F). The observed changes in *Medea*$^{myd88-1}$ were not significantly different from the null hypothesis that the element had no fitness cost [(*16*) and fig. S2], although we cannot exclude the possibility that a *Medea*$^{myd88-1}$–associated cost is counterbalanced by an unknown negative effect associated with

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the w^{1118} mutation in +/+ individuals. Finally, we carried out three further replicate population cage experiments in which the *Medea*$^{myd88\text{-}1}$ transgene was introduced at a frequency of 25% into the Oregon-R strain, which is WT with respect to the endogenous *w* locus (and thus members of which are red-eyed). Evidence for population replacement by generation 12 was observed in this context as well (*16*), suggesting that *Medea*$^{myd88\text{-}1}$–associated P^{w+} expression is unlikely to be a major contributor to the ability of *Medea*$^{myd88\text{-}1}$ to drive population replacement.

For any gene-drive mechanism to be successful in reducing parasite transmission, there must be tight linkage between the genes that mediate drive and effector functions (*10*). If the driver becomes separated from the effector gene through chromosome breakage and nonhomologous end joining (as in a reciprocal translocation) (Fig. 2A), and the effector gene carries a fitness cost, selection will favor and promote the spread of individuals carrying *Medea* elements that lack the effector (*Medea*$^{\Delta eff}$). Locating the effector gene between the toxin and antidote prevents a single chromosome breakage and end joining event from creating a *Medea*$^{\Delta eff}$-bearing chromosome (Fig. 2B). However, it does not prevent the creation of *Medea*^{ins}-bearing chromosomes that carry the antidote, and perhaps the effector, but not the toxin (Fig. 2B). *Medea*^{ins}-bearing chromosomes are insensitive to *Medea*-dependent killing. If the presence of the toxin and/or the effector results in a fitness cost, then *Medea*^{ins}-bearing chromosomes gain a fitness advantage with respect to those carrying the complete *Medea* element, thereby promoting spread of the former. This outcome can lead to the reappearance of pathogen-transmitting insects (*14*).

One way to prevent chromosome breakage and end joining–mediated formation of *Medea*$^{\Delta eff}$ and *Medea*^{ins} elements is to put the toxin and effector genes into an intron of the antidote (Fig. 2C). To test this approach, we generated flies carrying P^{w+}*Medea*$^{myd88\text{-}int}$, in which the toxin, a transcript generating maternally expressed miRNAs targeting *myd88*, was placed in an intron of the zygotically expressed antidote, *bnk*-driven *myd88*, in the opposite orientation (Fig. 2D). We characterized the behavior of one autosomal insertion of this element *Medea*$^{myd88\text{-}int\text{-}1}$, which behaved as a maternal-effect selfish genetic element (Table 2). Females heterozygous for *Medea*$^{myd88\text{-}int\text{-}1}$ gave rise to a high frequency of *Medea*$^{myd88\text{-}int\text{-}1}$–carrying progeny (>99%), and the maternal-effect lethality associated with a single copy of P^{w+}*Medea*$^{myd88\text{-}int\text{-}1}$ was rescued by zygotic expression of the antidote from either the maternal or paternal genome (Table 2). However, when homozygous element-bearing females were crossed with non–element-bearing males, progeny embryo survival was very poor (~20%), suggesting an inefficient zygotic rescue, perhaps resulting from inefficient splicing of the *myd88* artificial intron. Population replacement for an element with these fitness characteristics is still expected to occur, though with some delay (fig. S3) as compared to that for an element in which the fitness costs are a simple function of copy number in either sex (Fig. 1, C and D).

Medea^{ins}-bearing chromosomes can also arise if the toxin mutates to inactivity. Although toxin mutation cannot be prevented, the use of

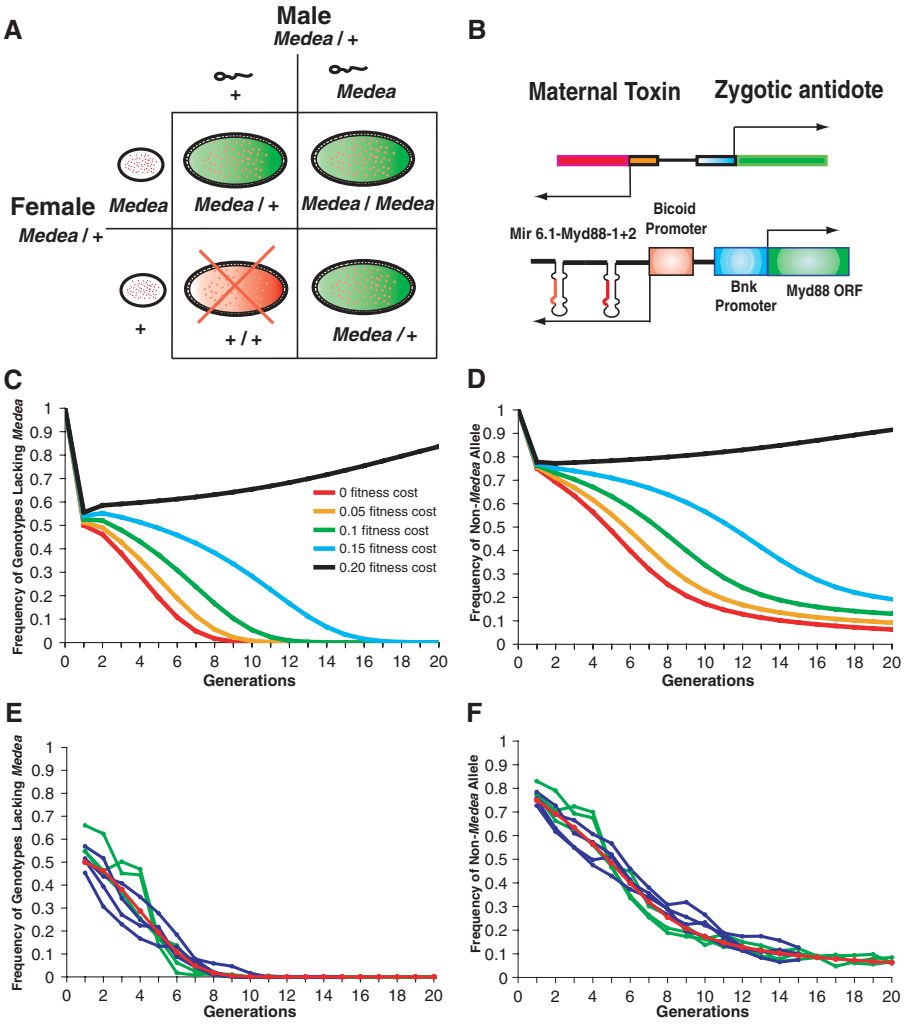

Fig. 1. Characteristics of a maternal-effect selfish genetic element (*Medea*) and a synthetic *Medea* element in *Drosophila*. (**A**) It is postulated that females heterozygous for *Medea* (*Medea*/+) deposit a protoxin or toxin (red dots) into all oocytes. Embryos that do not inherit a *Medea*-bearing chromosome die because toxin activation or activity is unimpeded (bottom left square). Embryos that inherit *Medea* from the maternal genome (top left square), the paternal genome (bottom right square), or both (top right square) survive because zygotic expression of a *Medea*-associated antidote (green background) neutralizes toxin activity. (**B**) (Top) Schematic of a simple molecular model that accounts for *Medea* behavior postulates the existence of two tightly linked loci. One locus consists of a maternal germline–specific promoter that drives the expression of RNA or protein that is toxic to the embryo. The second locus consists of a zygotic promoter that drives the expression of an antidote. (Bottom) Schematic of *Medea*^{myd88} is shown. ORF, open reading frame; Mir 6.1-Myd88-1+2, transcript encoding two copies of *Drosophila* miRNA 6.1 modified to target the myd88 5′ untranslated region. (**C**) Frequency of genotypes lacking *Medea* for an element carrying the additive fitness costs indicated, over generations, with *Medea* introduced at a 1:1:2 ratio of homozygous *Medea*-bearing males, non-*Medea*–bearing males, and females lacking *Medea*. Generation zero refers to the wild population (non-*Medea*/non-*Medea* = 1) before population seeding. Generation one refers to the progeny of crosses between these individuals and homozygous *Medea*-bearing males. (**D**) Frequency of the non-*Medea*–bearing chromosome for the populations described in (C). (**E** and **F**) *Medea*$^{myd88\text{-}1}$ drives population replacement in *Drosophila*. *Medea*$^{myd88\text{-}1}$ was introduced into seven population cages at an allele frequency of ~25% (*16*). (E) The frequency of genotypes lacking *Medea* (+/+) over generations is indicated for two separate sets of population cage experiments, involving three (green lines; 20 generations) or four (blue lines; 15 generations) population cages each. The predicted frequency of genotypes lacking *Medea*, for a *Medea* element with zero fitness cost (introduced at an allele frequency of 25%) is indicated by the red line. (F) The frequency of the non-*Medea*$^{myd88\text{-}1}$–bearing chromosome (+/*Medea* and +/+) over generations from the population cage experiments in (E) is indicated as above, as is the predicted frequency for an element with zero fitness cost.

Table 1. *Medea*$^{myd88\text{-}1}$ shows maternal-effect selfish behavior. Progeny of crosses between parents of several different genotypes (M refers to the *Medea*$^{myd88\text{-}1}$–bearing chromosome; + refers to the non–element-bearing homolog) are shown. The maternal copy number (0 to 2) of *bic*-driven miRNAs targeting the endogenous *myd88* transcript (maternal toxin) and zygote copy number (0 to 2) and percentage of embryos inheriting *bnk*-driven *myd88* (zygotic antidote) are indicated, as are the adult progeny genotypes predicted for Mendelian inheritance of *Medea*$^{myd88\text{-}1}$ and the percent embryo survival. -, not measured. The asterisk denotes that embryo survival was normalized with respect to percent survival (± SD) observed in the w^{1118} stock used for transgenesis (97.1 ± 0.7%).

Parental genotype		Inherited by the		Adult M progeny (%)		Embryo survival (%)*
		Oocyte	Embryo			
Male	Female	Maternal toxin	Zygotic antidote (*n*, %)	Predicted	Observed	
M/+	+/+	0	0, 50 1, 50	50	50 (*n* > 7000)	99.6 ± 1
M/M	M/M	2	2, 100	100	-	98.1 ± 0.4
+/+	M/+	1	0, 50 1, 50	50	100 (*n* > 12,000)	48.3 ± 2
M/M	M/+	1	1, 50 2, 50	100	-	98 ± 1
M/+	M/+	1	0, 25 1, 50 2, 25	75	-	74.3 ± 0.5
M/+	M/M	2	1, 50 2, 50	100	-	98.3 ± 1
+/+	M/M	2	1, 100	100	-	99.1 ± 0.4
M/M	+/+	0	1, 100	100	-	98.8 ± 0.5

miRNAs as toxins can provide a degree of redundant protection because multiple miRNAs, each processed and functioning as an independent unit, can be linked in a polycistronic transcript [(Fig. 1B and (*16*)]. The use of miRNAs as toxins also provides a basis by which selfish genetic element drive can be limited to the target species. *Medea* elements only show drive when maternal-effect lethality creates an opportunity for zygotic rescue of progeny that inherit the element. Therefore, drive can be limited to a single species by the use of miRNAs that are species-specific in their ability to target the maternally expressed gene of interest.

Perhaps the most likely point of failure in any population-replacement strategy involves the effector. Effector genes can mutate to inactivity, creating *Medea*$^{\Delta eff}$-bearing chromosomes. In addition, parasites may undergo selection for resistance to these effectors. These events, as well as the possible appearance of *Medea*^{ins}-bearing chromosomes discussed above, will lead to the reappearance of permissive conditions for disease transmission. Therefore, it is important that strategies be available for removal of an element from the population, followed by its replacement with a new element. One potential strategy for achieving this goal, in which different *Medea* elements located at a common site in the genome compete with each other for germline transmis-

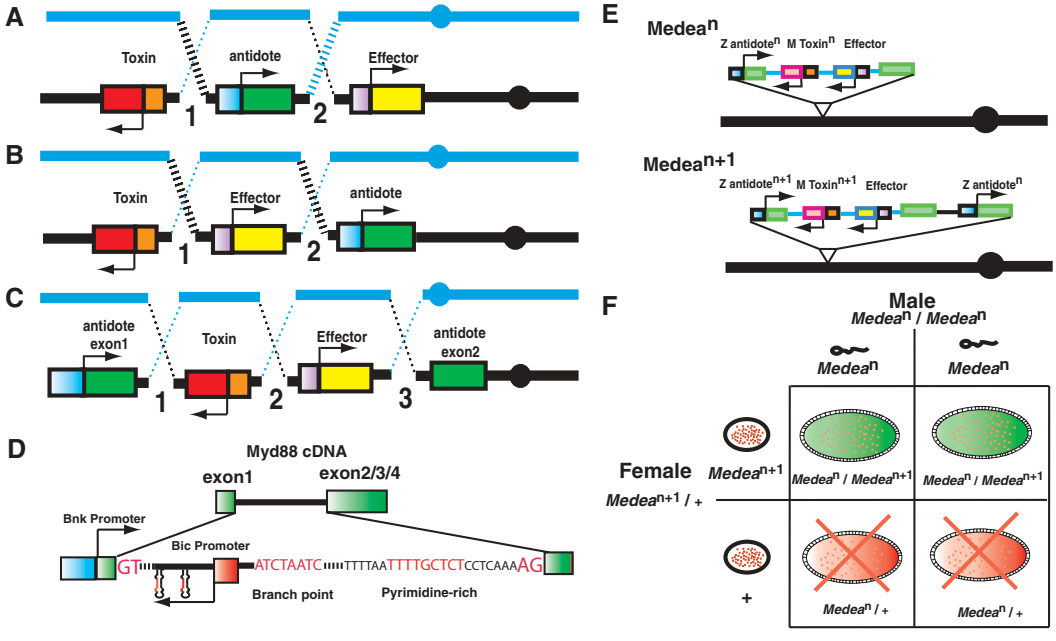

Fig. 2. A strategy for enhancing the functional lifetime of *Medea* elements in the wild and for carrying out cycles of population replacement. (**A** to **D**) Locating *Medea* toxin and effector genes in an intron of the antidote prevents chromosome breakage and end joining–mediated separation of drive and effector genes and creation of *Medea*^{ins}-bearing chromosomes. [(A) to (C)] *Medea* constructs with different gene arrangements are shown. Sites of chromosome breakage and end joining with a second nonhomologous chromosome are indicated by the crossed lines. Recombinant products referred to in the text are indicated by thick lines, the color of which indicates the centromere (solid circles) involved. (A) Recombination at site 1 generates a *Medea*^{ins}-bearing chromosome that carries the effector. Recombination at site 2 generates a *Medea*$^{\Delta eff}$-bearing chromosome. (B) Recombination at site 1 or site 2 generates a *Medea*^{ins}-bearing chromosome. (C) Recombination at sites 1 to 3 generates benign chromosomes that cannot show *Medea*^{ins} or *Medea*$^{\Delta eff}$ behavior. (D) Schematic of *Medea*$^{myd88\text{-}int}$. Splice donor and acceptor sites are indicated in large red letters, with the branchpoint and polypyrimidine stretch in small red letters. (**E** and **F**) A strategy for carrying out cycles of population replacement with *Medea*. (E) A first-generation *Medea* element (*Medea*^{n}), driven by Toxin^{n} and Antidote^{n}, is integrated into the chromosome [thick black line with centromere (solid circle) at the right] at a specific position (triangle). A second-generation *Medea* element (*Medea*$^{n+1}$), driven by Toxin^{n+1} and Antidote^{n+1}, can be integrated at the same position using site-specific recombination (*24*). Locating both elements at the same position limits the possibility of homologous recombination creating chromosomes that carry both elements. (F) Because progeny carrying *Medea*^{n} are sensitive to Toxin^{n+1}, the only progeny of females heterozygous for *Medea*$^{n+1}$ that survive are those that inherit *Medea*$^{n+1}$, regardless of their status with respect to *Medea*^{n}. In contrast, the progeny of *Medea*^{n} females that fail to inherit *Medea*^{n} survive if they inherit Antidote^{n} as a part of *Medea*$^{n+1}$.

Table 2. *Medea*$^{myd88\text{-}int\text{-}1}$ shows maternal-effect selfish behavior. Progeny of crosses between parents of several different genotypes are shown, and notations are the same as those in Table 1.

Parental genotype		Inherited by the		Adult M progeny (%)		Embryo survival (%)*
		Oocyte	Embryo			
Male	Female	Maternal toxin	Zygotic antidote (*n*, %)	Predicted	Observed	
M/+	+/+	0	0, 50 1, 50	50	51 (*n* = 5000)	98.4 ± 0.6
M/M	M/M	2	2, 100	100	-	98.6 ± 0.8
+/+	M/+	1	0, 50 1, 50	50	99.5 (*n* = 5000)	48.7 ± 0.6
M/M	M/+	1	1, 50 2, 50	100	-	98.4 ± 0.7
M/+	M/+	1	0, 25 1, 50 2, 25	75	-	73.6 ± 1.2
M/+	M/M	2	1, 50 2, 50	100	-	57.2 ± 1.5
+/+	M/M	2	1, 100	100	-	20.2 ± 1.1
M/M	+/+	0	1, 100	100	-	98.5 ± 0.7

sion in transheterozygous females, is illustrated in Fig. 2, E and F.

Our data show de novo synthesis of a selfish genetic element able to drive itself into a population. This laboratory demonstration notwithstanding, several obstacles remain to the implementation of *Medea*-based population replacement in the wild. First, for pests such as mosquito species, there is little genetic or molecular information regarding genes and promoters used during oogenesis and early embryogenesis. This information is straightforward to generate, with the use of transcriptional profiling to identify appropriately expressed genes and transgenesis and RNA interference in adult females to identify those required for embryonic development, but it remains to be acquired. In addition, current models of the spread of *Medea* do not take into account important real-world variables, such as migration, nonrandom mating, and the fact that important disease vectors such as *Anopheles gambiae* consist of multiple partially reproductively isolated strains (*20*, *21*). Although an understanding of the above issues is critical for the success of any population-replacement strategy, the problems are not intractable, as evidenced by past successes in controlling pests by means of sterile-male release (*18*) and as implied by our growing understanding of mosquito population genetics, immunity, and ecology (*20*–*23*).

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Supporting Online Material

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Modeling the Initiation and Progression of Human Acute Leukemia in Mice

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Our understanding of leukemia development and progression has been hampered by the lack of in vivo models in which disease is initiated from primary human hematopoietic cells. We showed that upon transplantation into immunodeficient mice, primitive human hematopoietic cells expressing a *mixed-lineage leukemia* (*MLL*) fusion gene generated myeloid or lymphoid acute leukemias, with features that recapitulated human diseases. Analysis of serially transplanted mice revealed that the disease is sustained by leukemia-initiating cells (L-ICs) that have evolved over time from a primitive cell type with a germline immunoglobulin heavy chain (IgH) gene configuration to a cell type containing rearranged IgH genes. The L-ICs retained both myeloid and lymphoid lineage potential and remained responsive to microenvironmental cues. The properties of these cells provide a biological basis for several clinical hallmarks of *MLL* leukemias.

In human leukemia, only a subset of leukemic blast cells have the potential to initiate and recapitulate disease when transplanted into immunodeficient mice (*1*–*3*). To date, these approaches have not permitted identification of the cell type(s) from which leukemia-initiating cells (L-ICs) originate or assessment of how these L-ICs phenotypically evolve during disease progression. In order to investigate these questions, we have developed an in vivo model of leukemia initiated from primary human hematopoietic cells.

Over 50% of infant acute leukemias exhibit rearrangements of the *mixed-lineage leukemia* gene (*MLL*, also termed *ALL-1* and *HRX*) at human chromosome 11q23 (*4*). Translocations of *MLL* to >40 different partner genes have been identified, and the resulting fusion proteins are strong transcriptional activators that drive the aberrant expression of homeobox family genes (*5*). In view of the potent oncogenic properties of *MLL* fusion genes, we tested the leukemogenic potential of *MLL–eleven-nineteen*