
This copy is for your personal, non-commercial use only.

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by [clicking here](#).

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines [here](#).

The following resources related to this article are available online at www.sciencemag.org (this information is current as of January 10, 2011):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/content/330/6007/1102.full.html>

Supporting Online Material can be found at:

<http://www.sciencemag.org/content/suppl/2010/11/17/330.6007.1102.DC1.html>

This article **cites 16 articles**, 9 of which can be accessed free:

<http://www.sciencemag.org/content/330/6007/1102.full.html#ref-list-1>

This article appears in the following **subject collections**:

Microbiology

<http://www.sciencemag.org/cgi/collection/microbio>

is identical to the description of an electric circuit with two resistors (fig. S6), with Eqs. 1 and 4 being analogous to Ohm's law. Solving these equations simultaneously leads to the Michaelis-Menten relation known empirically for the dependence of cell growth on nutrient level (18)

$$\lambda(\kappa_t, \kappa_n) = \lambda_c(\kappa_t) \cdot \frac{\kappa_n}{\kappa_t + \kappa_n} \quad (5)$$

The value of the maximal growth rate $\lambda_c(\kappa_t) = \kappa_t \cdot (r_{\max} - r_0) \approx 2.85 \text{ hour}^{-1}$ (based on the average r_{\max}) corresponds well to the doubling time of ~ 20 min for typical *E. coli* strains in rich media. Moreover, Eq. 5 quantitatively accounts for the correlation observed between growth rate λ and nutritional capacity κ_n (fig. S3A).

This theory can be inverted to predict the effect of protein expression on cell growth. Unnecessary protein expression leads to diminished growth (19). Understanding the origin of this growth inhibition is of value in efforts to increase the yield of heterologous protein in bacteria (20) and to understand the fitness benefit of gene regulation (21, 22). Aside from protein-specific toxicity, several general causes of growth inhibition have been suggested, including diversion of metabolites (23), competition among sigma factors for RNA polymerases (24), and competition among mRNA for ribosomes (19, 25).

We modeled the expression of unnecessary protein (of mass fraction ϕ_U) as an additional (neutral) component of the proteome that effectively causes a reduction of r_{\max} to $r_{\max} - \phi_U/\rho$ (Fig. 4A). Equation 5 then predicts a linear reduction of the growth rate,

$$\lambda(\phi_U) = \lambda(\phi_U = 0) \cdot [1 - (\phi_U/\phi_c)] \quad (6)$$

extrapolating toward zero growth at $\phi_c = \rho \cdot (r_{\max} - r_0) \approx 0.48$. The prediction quantitatively described the observed growth defect caused by inducible expression of β -galactosidase (Fig. 4B), as well as previous results obtained for various proteins and expression vectors (Fig. 4C) (19, 26), without any adjustable parameters. These results suggest that growth reduction is a simple consequence of ribosome allocation subject to the constraints of Eqs. 1, 3, and 4.

Robust empirical correlations of the RNA/protein ratio with the growth rate (Figs. 1A and 2A and figs. S1 and S2) revealed underlying constraints of cellular resource allocation and led to the formulation of a simple growth theory that provided quantitative predictions and unifying descriptions of many important but seemingly unrelated aspects of bacterial physiology. Like Ohm's law, which greatly expedited the design of electrical circuits well before electricity was understood microscopically, the empirical correlations described here may be viewed as microbial "growth laws," the use of which may facilitate our understanding of the operation and design of complex biological systems well before all the underlying regulatory circuits are elucidated at the molecular level.

References and Notes

1. N. J. Guido *et al.*, *Nature* **439**, 856 (2006).
2. H. Youk, A. van Oudenaarden, *Nature* **462**, 875 (2009).
3. E. M. Airolidi *et al.*, *PLoS Comput. Biol.* **5**, e1000257 (2009).
4. S. Klumpp, Z. Zhang, T. Hwa, *Cell* **139**, 1366 (2009).
5. A. Zaslaver *et al.*, *PLoS Comput. Biol.* **5**, e1000545 (2009).
6. A. P. Arkin, D. A. Fletcher, *Genome Biol.* **7**, 114 (2006).
7. C. Tan, P. Marguet, L. You, *Nat. Chem. Biol.* **5**, 842 (2009).
8. R. Kwok, *Nature* **463**, 288 (2010).
9. M. Schaechter, O. Maaloe, N. O. Kjeldgaard, *J. Gen. Microbiol.* **19**, 592 (1958).
10. H. Bremer, P. P. Dennis, in *Escherichia coli and Salmonella*, F. C. Neidhardt, Ed. (ASM Press, Washington, DC, 1996), pp. 1553–1569.
11. O. Maaløe, in *Biological Regulation and Development*, R. F. Goldberg, Ed. (Plenum, New York, 1979), pp. 487–542.
12. J. Forchhammer, L. Lindahl, *J. Mol. Biol.* **55**, 563 (1971).
13. See supporting material on Science Online.
14. T. Ruusala, D. Andersson, M. Ehrenberg, C. G. Kurland, *EMBO J.* **3**, 2575 (1984).
15. B. J. Paul, W. Ross, T. Gaal, R. L. Gourse, *Annu. Rev. Genet.* **38**, 749 (2004).
16. A. L. Koch, *Can. J. Microbiol.* **34**, 421 (1988).
17. A particular protein species may belong to multiple classes; sometimes this is a result of expression from multiple promoters that are differently regulated.
18. J. Monod, *Annu. Rev. Microbiol.* **3**, 371 (1949).
19. H. Dong, L. Nilsson, C. G. Kurland, *J. Bacteriol.* **177**, 1497 (1995).
20. C. P. Chou, *Appl. Microbiol. Biotechnol.* **76**, 521 (2007).

21. E. Dekel, U. Alon, *Nature* **436**, 588 (2005).
22. D. M. Stoebel, A. M. Dean, D. E. Dykhuizen, *Genetics* **178**, 1653 (2008).
23. B. R. Glick, *Biotechnol. Adv.* **13**, 247 (1995).
24. A. Farewell, K. Kvint, T. Nyström, *Mol. Microbiol.* **29**, 1039 (1998).
25. J. Vind, M. A. Sørensen, M. D. Rasmussen, S. Pedersen, *J. Mol. Biol.* **231**, 678 (1993).
26. W. E. Bentley, N. Mirjalili, D. C. Andersen, R. H. Davis, D. S. Kompala, *Biotechnol. Bioeng.* **35**, 668 (1990).
27. R. Mikkola, C. G. Kurland, *Biochimie* **73**, 1551 (1991).
28. We thank H. Bremer, L. Csonka, P. Dennis, M. Ehrenberg, P. Geiduschek, M. Schaechter, A. Tadmor, and members of the Hwa lab for suggestions and discussions; D. Hughes for the Sm mutant strains; and P.-h. Lee and B. Willumsen for the use of unpublished data. Supported by NIH grant RO1GM77298, NSF grant MCB0746581, and the NSF-supported Center for Theoretical Biological Physics (grant PHY0822283). M.S. was supported by a Natural Sciences and Engineering Research Council of Canada fellowship.

Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6007/1099/DC1
 Materials and Methods
 SOM Text
 Figs. S1 to S6
 Tables S1 to S7
 References
 20 May 2010; accepted 6 October 2010
 10.1126/science.1192588

Symbiotic Bacterium Modifies Aphid Body Color

Tsutomu Tsuchida,^{1*†} Ryuichi Koga,^{2†} Mitsuyo Horikawa,³ Tetsuto Tsunoda,³ Takashi Maoka,⁴ Shogo Matsumoto,¹ Jean-Christophe Simon,⁵ Takema Fukatsu^{2*}

Color variation within populations of the pea aphid influences relative susceptibility to predators and parasites. We have discovered that infection with a facultative endosymbiont of the genus *Rickettsiella* changes the insects' body color from red to green in natural populations. Approximately 8% of pea aphids collected in Western Europe carried the *Rickettsiella* infection. The infection increased amounts of blue-green polycyclic quinones, whereas it had less of an effect on yellow-red carotenoid pigments. The effect of the endosymbiont on body color is expected to influence prey-predator interactions, as well as interactions with other endosymbionts.

The world is full of colors, and many animals have color vision, recognizing their environment, habitat, food, enemies, rivals, and mates by visual cues. Body color is thus an ecologically important trait, often involved in species recognition, sexual selection, mimicry, aposematism, and crypsis (1, 2). In the pea aphid *Acyrtosiphon pisum*, red and green color morphs are found in the same populations. Early work has shown that the aphid body color is genetically determined, with red being dominant over

green (3). Ecological studies show that ladybird beetles tend to consume red aphids on green plants (4), and parasitoid wasps preferentially attack green aphids (5). The predation and parasitism pressures appear to maintain the color variation in natural aphid populations (1, 4). An unexpected recent discovery showed that the aphid genome contains several genes for carotenoid synthesis not found in animal genomes. The genes are of fungal origin and appear to have been acquired in the evolutionary history of aphids via ancient lateral transfer. One of the genes is involved in synthesis of red color pigments, and the presence or absence of the gene is responsible for the red or green coloration of the aphids (6). Here, we report another factor affecting aphid color polymorphism: a previously unrecognized endosymbiont that modifies insect body color in natural populations.

While screening pea aphid strains from natural populations collected in France, we found several strains of green aphids producing red nymphs. As the nymphs grew, their body color changed from reddish to greenish, and the adults became

¹Molecular Entomology Laboratory, RIKEN Advanced Science Institute, Wako 351-0198, Japan. ²National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba 305-8566, Japan. ³Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima 770-8514, Japan. ⁴Research Institute for Production Development, Kyoto 606-0805, Japan. ⁵Institut National de la Recherche Agronomique, UMR 1099 BiO3P, Institut National de la Recherche Agronomique (INRA)/Agrocampus Ouest/Université Rennes 1, BP 35327, 35653 Le Rheu Cedex, France.

*To whom correspondence should be addressed. E-mail: t-tsuchida@riken.jp (T.T.); t-fukatsu@aist.go.jp (T.F.)
 †These authors contributed equally to this work.

green (Fig. 1A and table S1). A survey of endosymbiotic microbiota in these aphid strains from distinct geographic origins and bearing different genotypes (7) identified γ -proteobacterial facultative endosymbionts (one of which is a *Hamiltonella*- or *Serratia*-like organism) that confer protection to their host aphids against parasitoid wasps (8). In addition, we found a previously unrecognized aphid endosymbiont of the genus *Rickettsiella*, whose members are insect pathogens that are phylogenetically related to the human pathogens *Coxiella* and *Legionella* (Fig. 2A) (9).

By antibiotic treatments (7), we successfully eliminated the *Hamiltonella/Serratia* infection from the aphids without affecting *Rickettsiella* and *Buchnera* infections. Their body-coloring patterns did not change after the treatments (Fig. 1B). When we injected diverse aphid strains, which harbored only *Buchnera*, with hemolymph from the *Rickettsiella*-infected strains (7), the aphids produced both *Rickettsiella*-infected and -uninfected offspring. Notably, *Rickettsiella*-infected red aphids of distinct genotypes consistently changed body color to green as they developed, whereas neither uninfected

red aphids nor originally green aphids were affected (Fig. 1, fig. S1, and table S1). The body-coloring patterns in the experimentally infected aphid strains were similar to those in the naturally *Rickettsiella*-infected strains (Fig. 1). Quantitative polymerase chain reaction (PCR) analyses revealed that the intensity of green color was positively correlated with the infection density of *Rickettsiella* for different host and endosymbiont genotypes (fig. S1). These results indicate that the *Rickettsiella* infection is responsible for green body color in at least some green pea aphids in natural populations.

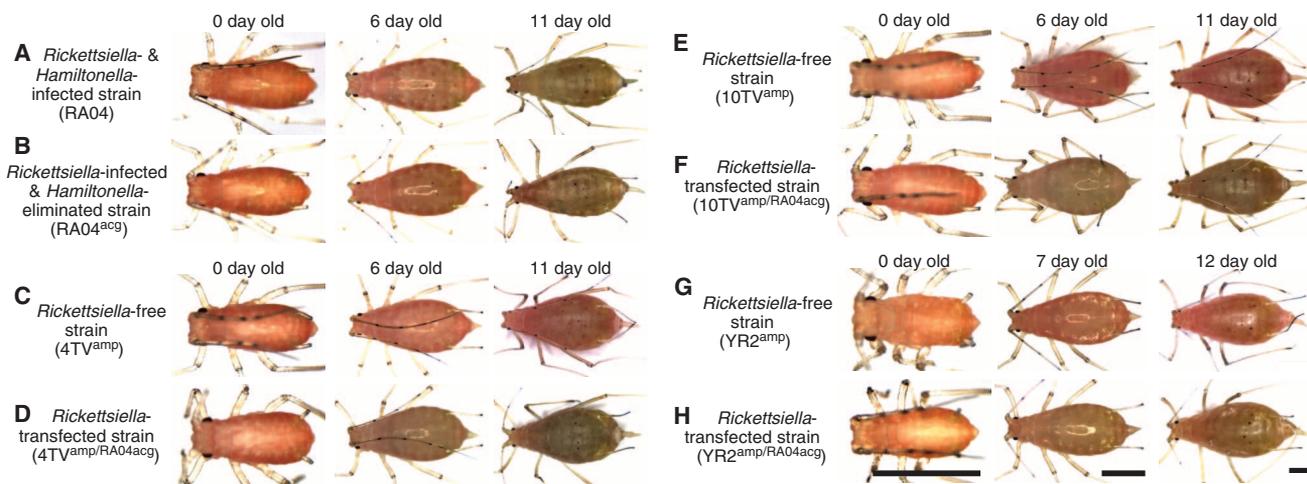


Fig. 1. (A to H) *Rickettsiella*-induced body-color change in pea aphids of different genetic backgrounds. Scale bars, 1 mm. For details of the aphid strains, see table S1.

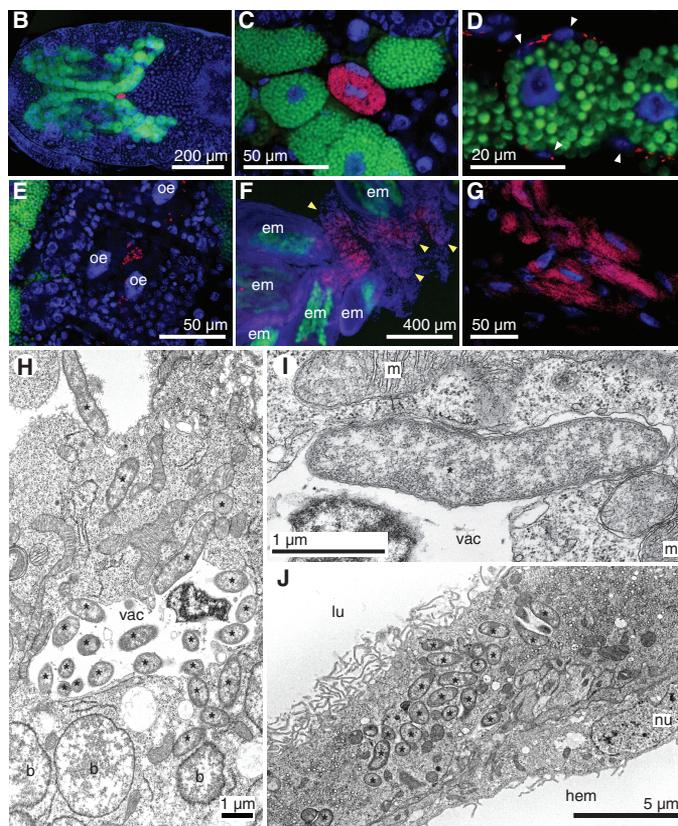
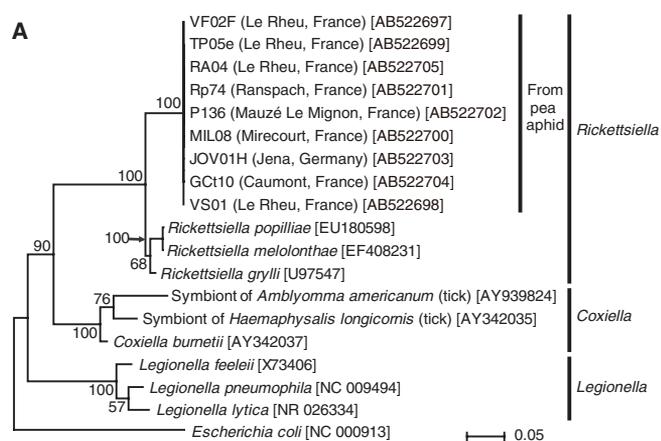


Fig. 2. (A) Phylogenetic analysis of *Rickettsiella* endosymbionts from European pea aphids on the basis of 16S ribosomal RNA gene sequences. A maximum likelihood phylogeny inferred from 1384 aligned nucleotide sites is shown with bootstrap values. (B to G) In situ hybridization of *Rickettsiella*. (B) A mature embryo (blue) containing many primary bacteriocytes harboring *Buchnera* (green) and a secondary bacteriocyte harboring *Rickettsiella* (red) that together constitute a huge bacteriome. (C) Enlarged image of the secondary bacteriocyte. (D) Sheath cells harboring *Rickettsiella* adhering to the periphery of primary bacteriocytes (white arrowheads). (E) Oenocytes (oe) infected with *Rickettsiella*. (F) Posterior part of an ovary, where ovariole pedicels are heavily infected with *Rickettsiella* (yellow arrowheads). em, embryo. (G) Enlarged image of an ovariole pedicel. (H to J) Electron microscopy images of *Rickettsiella*. (H) Image of a secondary bacteriocyte harboring *Rickettsiella* and a primary bacteriocyte harboring *Buchnera*. vac, vacuole; b, *Buchnera*; asterisks, *Rickettsiella*. (I) Enlarged image of a *Rickettsiella* cell. m, mitochondrion. (J) Image of the wall of an ovariole pedicel infected with *Rickettsiella*. hem, hemocoel; lu, lumen of ovariole pedicel; nu, nucleus.

Downloaded from www.sciencemag.org on January 10, 2011

Diagnostic PCR surveys detected 7.9% [28 of 353 insects (28/353)] *Rickettsiella* infection in Western European populations of *A. pisum* (fig. S2A). Fitness measurements revealed that infection status did not affect growth rate and body size for two aphid genotypes, although we observed significantly larger body size and faster growth with *Rickettsiella* infection for one aphid genotype (fig. S3). Similarly to *Hamiltonella* and *Serratia* (10, 11), *Rickettsiella* resided in secondary bacteriocytes and sheath cells in vivo and was also found intracellularly and extracellularly in various tissues and the hemolymph (Fig. 2, B to J). In natural populations, not all green aphids were infected with *Rickettsiella*, and some strains of red aphids were found with *Rickettsiella* infection (fig. S2, B and C). It appears that the combination of aphid genotype and the endosymbiont contribute to body color (3, 6): Some endosymbiont genotypes may fail to induce green coloration, whereas some host genotypes may attenuate or inhibit the activity of *Rickettsiella*. Similar interactions have been documented for other facultative endosymbionts in the pea aphid (12, 13).

Aphid body color mainly consists of two major groups of pigment molecules: (i) yellow-red colors from carotenoid pigments such as β -carotene, lycopene, and torulene (6, 14) and (ii) blue-green and other pigments from structurally complex polycyclic quinones and their glycosides, called aphins or aphinins (14, 15). When the naturally red aphid strain was infected with *Rickettsiella* and became green, we observed some changes in carotenoid compositions, but the differences were not sufficient to account for the degree of green pigmentation (fig. S4). Moran and Jarvik (6) have found that a fungus-derived carotenoid desaturase gene, *tor*, is present in red aphid clones but is absent in green aphid clones and is responsible for production of the red carotenoids. Our quantitative reverse transcription-PCR assay showed that expression levels of the *tor* gene were not significantly affected by the *Rickettsiella* infection (fig. S5).

We recovered almost all of the green pigments into the butanol fraction by water-butanol extraction of the aphids (7). Thin-layer chromatography of the extracts separated one major and several minor green bands, which presumably represented polycyclic quinone glycosides like aphinins (14), although their exact structures were not determined (7). The intensity of the green bands was greater in the *Rickettsiella*-infected green-aphid strains than in the original red-aphid strains (fig. S6A). Densitometric quantification of the major band revealed a 2.4- to 4.6-fold increase of the green pigment in the *Rickettsiella*-infected aphid strains compared with the uninfected strains (fig. S6B). We presume that *Rickettsiella* does not synthesize new green pigments for itself but stimulates aphid metabolism to increase green-pigment production (fig. S7). The murky hue of the *Rickettsiella*-induced green aphids (Fig. 1 and fig. S1) is probably a result of the combination of the green pigments and the reddish carotenoid pigments. Genome sequencing of the *Rickettsiella* endosymbiont and transcriptomic

analysis of the infected and uninfected aphid hosts should provide insights into the molecular and metabolic interactions between host and endosymbiont that lead to the body-color change.

Previous studies have identified a variety of biological roles for facultative endosymbionts in the pea aphid, including tolerance to high temperature, resistance against natural enemies, and plant adaptation (8, 16–19). We have added another endosymbiont relationship that potentially affects a host trait of ecological importance in ways that have yet to be verified. For example, the induced green color may reduce the predation risk by ladybird beetles. Notably, *Rickettsiella* is frequently found in co-infections with either *Hamiltonella* [55.6% (35/63)] or *Serratia* [20.6% (13/63)] endosymbionts (fig. S2D), both of which are protective against parasitoid wasps (8) and may act to offset the risk of green aphids attracting parasitoids.

References and Notes

- G. D. Ruxton, T. N. Sherratt, M. P. Speed, *Avoiding Attack: The Evolutionary Ecology of Crypsis, Warning Signals and Mimicry* (Oxford Univ. Press, Oxford, 2005).
- J. Leonard, A. Cordoba-Aquilar, *The Evolution of Primary Sexual Characters in Animals* (Oxford Univ. Press, Oxford, 2010).
- F. P. Müller, *Z. Pflanzenkr.* **69**, 129 (1962).
- J. E. Losey, J. Harmon, F. Ballantyne, C. Brown, *Nature* **388**, 269 (1997).
- R. Libbrecht, D. M. Gwynn, M. D. E. Fellowes, *J. Insect Behav.* **20**, 25 (2007).
- N. A. Moran, T. Jarvik, *Science* **328**, 624 (2010).
- Materials and methods are available as supporting material on *Science* Online.
- K. M. Oliver, J. A. Russell, N. A. Moran, M. S. Hunter, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 1803 (2003).

- R. Cordaux *et al.*, *Appl. Environ. Microbiol.* **73**, 5045 (2007).
- R. Koga, T. Tsuchida, T. Fukatsu, *Proc. Biol. Sci.* **270**, 2543 (2003).
- N. A. Moran, J. A. Russell, R. Koga, T. Fukatsu, *Appl. Environ. Microbiol.* **71**, 3302 (2005).
- K. M. Oliver, N. A. Moran, M. S. Hunter, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 12795 (2005).
- J. A. Russell, N. A. Moran, *Proc. Biol. Sci.* **273**, 603 (2006).
- K. S. Brown, *Chem. Soc. Rev.* **4**, 263 (1975).
- J. H. Bowie, D. W. Cameron, J. A. Findlay, J. A. K. Quartey, *Nature* **210**, 395 (1966).
- C. B. Montllor, A. Maxmen, A. H. Purcell, *Ecol. Entomol.* **27**, 189 (2002).
- T. Tsuchida, R. Koga, T. Fukatsu, *Science* **303**, 1989 (2004).
- C. L. Scarborough, J. Ferrari, H. C. J. Godfray, *Science* **310**, 1781 (2005).
- K. M. Oliver, P. H. Degnan, G. R. Burke, N. A. Moran, *Annu. Rev. Entomol.* **55**, 247 (2010).
- We thank J. Peccoud, Y. Outreman, S. Stoeckel, L. Mieuze, J. Jaquière, and W. Weisser for aphid sampling; J. Bonhomme, L. Mieuze, and J. Makino for aphid rearing and genotyping; and X.-Y. Meng and S. Hanada for help with electron microscopy. This work was supported by the Japan-France Integrated Action Program SAKURA of the Japan Society for the Promotion of Science (to T.F. and J.-C.S.); the Grant-in-Aid for Scientific Research on Innovative Areas (22128007) of the Ministry of Education, Culture, Sports, Science and Technology, Japan (to T.F.); and funds from INRA AIP Biosources (to J.-C.S.). T.T. was supported by a RIKEN special postdoctoral research fellowship.

Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6007/1102/DC1
Materials and Methods

Figs. S1 to S7

Table S1

References

22 July 2010; accepted 8 October 2010

10.1126/science.1195463

PiggyBac Transposon Mutagenesis: A Tool for Cancer Gene Discovery in Mice

Roland Rad,¹ Lena Rad,¹ Wei Wang,¹ Juan Cadinanos,^{1,2} George Vassiliou,¹ Stephen Rice,¹ Lia S. Campos,¹ Kosuke Yusa,¹ Ruby Banerjee,¹ Meng Amy Li,¹ Jorge de la Rosa,² Alexander Strong,¹ Dong Lu,¹ Peter Ellis,¹ Nathalie Conte,¹ Fang Tang Yang,¹ Pentao Liu,¹ Allan Bradley^{1*}

Transposons are mobile DNA segments that can disrupt gene function by inserting in or near genes. Here, we show that insertional mutagenesis by the *PiggyBac* transposon can be used for cancer gene discovery in mice. *PiggyBac* transposition in genetically engineered transposon-transposase mice induced cancers whose type (hematopoietic versus solid) and latency were dependent on the regulatory elements introduced into transposons. Analysis of 63 hematopoietic tumors revealed that *PiggyBac* is capable of genome-wide mutagenesis. The *PiggyBac* screen uncovered many cancer genes not identified in previous retroviral or *Sleeping Beauty* transposon screens, including *Spic*, which encodes a PU.1-related transcription factor, and *Hdac7*, a histone deacetylase gene. *PiggyBac* and *Sleeping Beauty* have different integration preferences. To maximize the utility of the tool, we engineered 21 mouse lines to be compatible with both transposon systems in constitutive, tissue- or temporal-specific mutagenesis. Mice with different transposon types, copy numbers, and chromosomal locations support wide applicability.

Genetic screening in higher organisms has been hampered for decades by the lack of efficient insertional mutagenesis tools. Retroviruses have been used for cancer gene discovery in mice, but their application has been

limited to the study of hematopoietic and mammary tumors because of viral tropism for these tissues (1). DNA transposons, which are the key insertional mutagens in lower organisms, were inactivated in vertebrate genomes millions of years