Supporting Information

Schröder et al. 10.1073/pnas.1019074108

SI Results

The integration of translocated plasmid DNA in eight different stably transformed cell lines (A–H) was analyzed by Southern blot hybridization (see Fig. S1), PCR amplification (see Fig. 2*A* and Table S3), and sequencing (see Fig. 2*B* and Fig. S2), and the interpretation of the data is given in the following.

Cell Line A. The absence of hybridization signal for the yellow probe (Fig. S1*B*, *Left*) indicates the loss of the plasmid region encoding the Mob protein. This is confirmed by the absence of a PCR product using primer pairs 1 to 3 (Fig. 2*A* and Table S1). Two bands of 2.8 and 9 kb were detected with the blue probe (Fig. S1*B*, *Middle*). The 2.8-kb band could correspond to the *kan* gene and adjacent 3′ junction, whereas the 9-kb band could represent a fragment containing the *neo* gene and adjacent 5′ junction. The hybridization signal obtained with the black probe (Fig. S1*B*, *Right*) is consistent with the 9-kb band detected with the blue probe. The location of the 3′ junction was confirmed and mapped by TAIL-PCR (Fig. S2*A*).

Cell Line B. Detection of PCR amplification using primer pair 1 (Fig. 2*A*) indicates that the DNA region spanning the *oriT* has been integrated into the chromosome. This finding was confirmed by PCR amplification of a 1,401-bp fragment from the genomic DNA of cell line B using oligonucleotides prMQ1463 and prMQ1442 (Table S3). Sequencing of the amplified fragment showed 100% homology to the sequence of pRS130 over the 1,401 bp. This could be a result of the integration of a concatemeric fragment of pRS130 or could indicate the precise religation at the *oriT* before integration. The gap detected in the integrated pRS130 sequence (PCR primer pairs 4 and PCR primer pairs 9–10; Fig. 2*A*) could not be joined by over-spanning PCR, suggesting that at least two fragments of the plasmid are located at different chromosomal locations. These findings are consistent with the Southern blot analysis (Fig. S1), in which the size of the fragment hybridizing to both black and blue probes (larger than the expected 5.4 kb) suggests the loss of one of the HindIII sites surrounding the fragment. It could represent a chromosomal junction of the plasmid fragment containing the *neo* gene. The 1.1-kb fragment detected with the yellow probe most likely corresponds to the plasmid fragment flanked by the two left HindIII sites. The additional fragment (6.5 kb) detected with the yellow probe possibly corresponds to the 5′ junction and flanking chromosomal DNA. Finally, the additional 3-kb fragment detected with the blue probe could be a separate integration event.

Cell Line C. The detection of a 5.4-kb fragment by Southern blot hybridization with both black and blue probes indicates the integration of the DNA fragment flanked by the second and the third HindIII sites. The absence of a second fragment by hybridization with the blue probe suggests truncation of the 3′ region of the transferred DNA, which is confirmed by the absence of a PCR product for the primer pairs 10 to 13 (Fig. 2*A*). The yellow probe detects the expected 1.1-kb and a 4.8-kb fragment, representing the 5′ junction, in agreement with the results obtained for the mapping of the 5′ junction by TAIL-PCR (Fig. 2*B*).

Cell Lines D, E, G, and H. No signal was detected by Southern blot hybridization with the yellow probe in any of these four cell lines, indicating 5′ truncation of the integrated plasmid, as confirmed by the absence of a PCR product with the primer pairs 1 to 3 (cell lines D, E, and H) and 1 to 4 (cell line G; Fig. 2*A*). The absence of signal detection for hybridization with the black probe indicates 3′ truncation of the integrated plasmid, as confirmed by the absence of a PCR product with the primer pairs 10 to 13 (cell line D), 7 to 13 (cell line E), 6 to 13 (cell line G), and 8 to 13 (cell line H; Fig. 2*A*). The only indication of plasmid integration is provided by hybridization with the blue probe (fragment of 10, 3.2, 2.5, and 3 kb for cell lines D, E, G, and H, respectively), which should represent the integrated *neo* gene and both flanking borders. This interpretation is compatible with the 5′ junction mapping of the cell lines D and E (Fig. S2 *B* and *C*) with a predicted chromosomal HindIII site 4,423 bp and 724 bp upstream of the 5′ border, respectively.

Cell Line F. Strikingly, all primer pairs (pairs 1–13) produced a PCR fragment amplification when using the genomic DNA of cell line F, including the primer pair spanning the *oriT* (as in the case of cell line B). Confirmation for the presence of an intact *oriT* region was assessed by PCR using oligonucleotides prMQ1463 and prMQ1442 (Table S3) followed by the sequencing of the resulting 1,401-bp PCR product. The sequencing of the PCR product confirmed 100% homology of the amplified fragment to the sequence of pRS130. Similar to what was found for cell line B, this may reflect the integration of a concatemeric copy of pRS130 or the precise religation of the plasmid at the *oriT* before integration. The integration of a concatemer seems more likely, as all fragments predicted by a HindIII digest of the circular form of pRS130 have been detected (both blue and black probes detected the expected 5.4-kb fragment; the blue probe detected the predicted 4-kb fragment and the yellow probe detected both the 1.1-kb and 4-kb fragments). Only one additional band of 1.5 kb was detected with the yellow probe, which is likely to result from integration in close vicinity to the *oriT* of pRS130.

SI Materials and Methods

Southern Blot Analysis. Genomic DNA of cell lines was obtained by a standard procedure involving cell lysis, proteinase K treatment, and precipitation (1). Digests were performed overnight by using 9 μg of DNA and 50 U of restriction enzyme in a total volume of 200 μL. After addition of another 20 U of enzyme, digests were continued for 4 h. Next, DNA was precipitated, dried, and resuspended in 35 μL of Tris–EDTA buffer by incubation and gentle mixing for 4 h at 50 °C. After addition of loading buffer, samples were loaded onto 1% agarose gels, and gels were run for 4 h at 50 to 60 V or overnight at 20 V. For the subsequent steps of gel treatment (depurination, denaturation), transfer (capillary blotting) to nylon membranes (Hybond N+; Amersham), and hybridization (in tubes), the protocol given by the manufacturer (Amersham) was followed. After transfer, membranes were baked for 2 h at 80 °C in a vacuum oven. For generation of radioactive probes, plasmid fragments obtained by digestion and gel isolation or by PCR (as detailed later) were used as template with the Prime-it II labeling kit (Stratagene) and [α^{32}P]-dATP (10 μL of [α^{32}P]-dATP per reaction). Probes were purified by gel filtration by using Nick columns (Amersham), yielding 400 μL of probe with an activity of approximately 300,000 cpm/μL, all of which was used for hybridizations at 65 °C in 10 mL of hybridization buffer in hybridization tubes. Washings were performed at 65 °C with 0.1% SDS solutions containing, consecutively, 2× SSC, 1× SSC, 0.3× SSC, and 0.1× SSC. Probed membranes were exposed to X-ray films in enhancer screens for 1 to 3 wk at −80 °C or the membranes were exposed to PhosphorImager screens

(Molecular Dynamics) for 24 h. Three different probes (yellow/hatched, blue, and black) were used for mapping of the approximate plasmid integration sites. The yellow probe is a 2,305-bp KpnI fragment of pLRS130 encompassing the *mob* fusion with the *bepD* secretion signal (*mob-BID*) and part of the replication gene (*rep*) of the pBGR1 replicon. The blue probe consists of a 2,085-bp PCR fragment generated by using oligonucleotides prRS255 and prCHF08 (GATCGAATTCGAGCTCATTACCTCATATAGC and CAAATTGCTGTCCATCTACC) and pRS130 as template. This fragment encompasses the *neo* gene, the oriV (pBGR1), and part of the *rep* (pBGR1) gene. As the *kan* gene also present on pRS130 shares a high degree of homology with the *neo* gene, this probe also hybridized with plasmid or genome fragments harboring *kan*. Finally, the black probe is a 1,376-bp HindIII-XbaI fragment of pRS130 containing the e*gfp* gene.

1. Laird PW, et al. (1991) Simplified mammalian DNA isolation procedure. *Nucleic Acids Res* 19:4293.

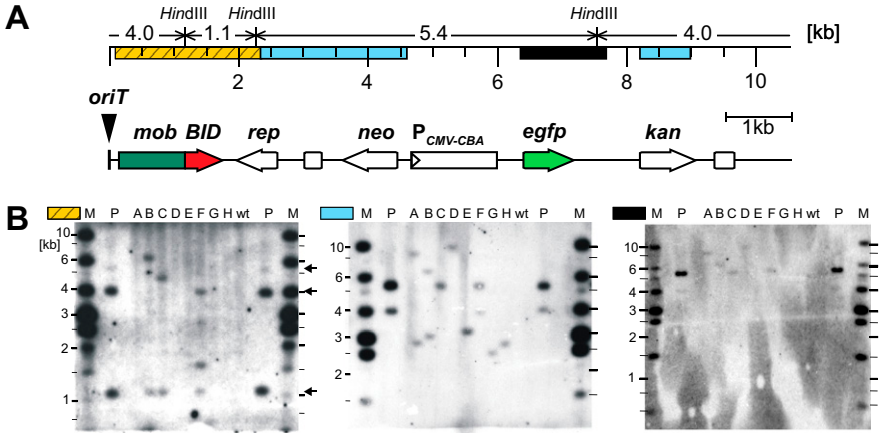

Fig. S1. Integration of translocated plasmid DNA into the host cell chromosome. Following infection of Ea.hy926 cells with *B. henselae* strain RSE581 carrying the reporter plasmid pRS130, eight individual cell lines (named A–H) were isolated and analyzed by Southern blot analysis by using three different plasmid-derived hybridization probes. (*A*) Schematic representation of pRS130 (linearized at the *oriT*), with internal HindIII sites. *Top*: Scale with the sizes of fragments expected from HindIII digest, and homology regions to three different probes (yellow/hatched, blue, black) are indicated. *Bottom*: Genetic organization of the plasmid is represented. (*B*) Southern blots of HindIII digested genomic DNA of cell lines A to H (as indicated above each lane), probed with ^{32}P-labeled probes. From left to right: probe 1 (yellow, hatched box), probe 2 (light blue box), and probe 3 (black box), which are accordingly color-coded in the scheme in *A*. Note that the probe 2 also hybridizes to the *kan* gene of pRS130, which shares 100% homology with the *neo* gene. M, molecular size marker; sizes of marker bands visible on the blot are indicated in kilobases; P, HindIII digested plasmid pRS130; wt, HindIII digested genomic DNA of untransformed Ea.hy926 cells.

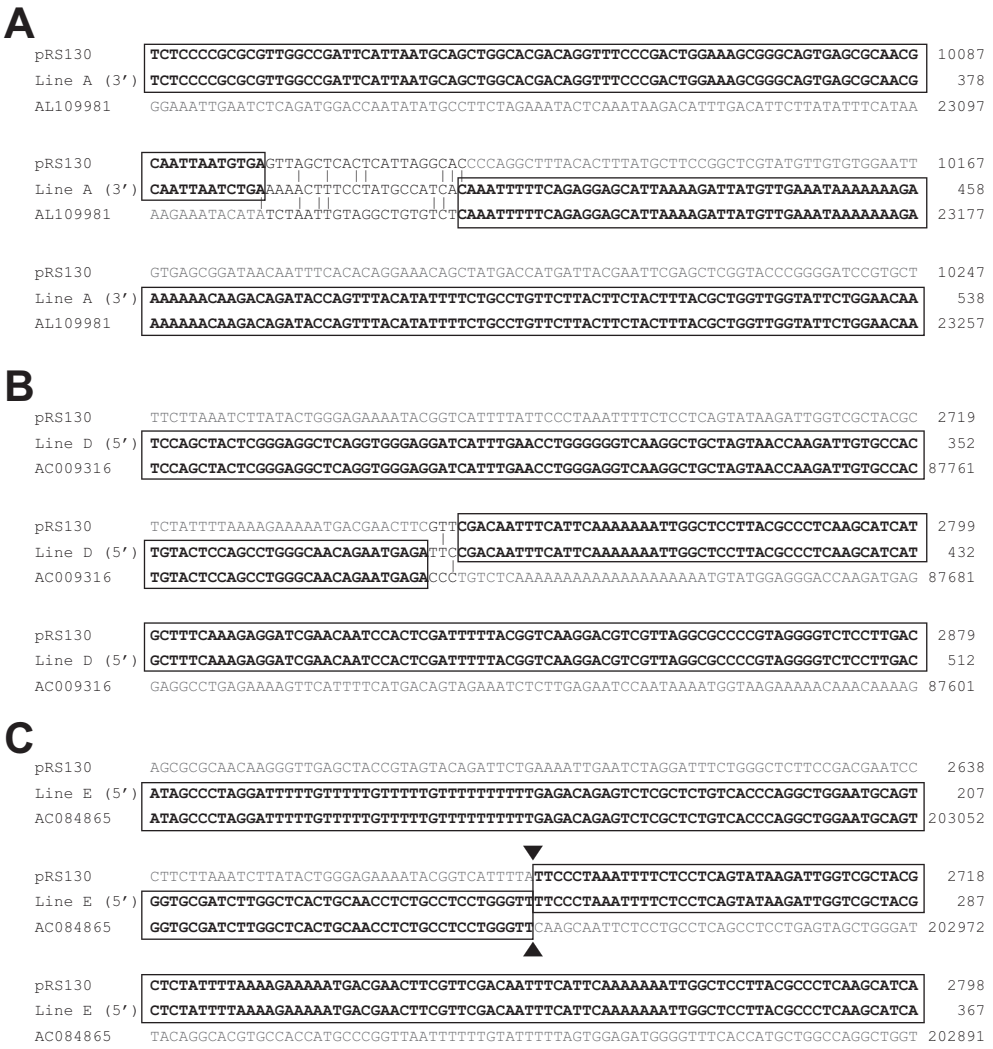

Fig. S2. Nucleotide sequence alignment of three integration junctions. Sequences of three integration junctions isolated by TAIL-PCR from genomic DNA of stable cell lines are aligned to the corresponding region in the human genome and in pRS130. Only 240 bp of the isolated fragment are displayed, centered around the integration side. (*A*) 3′ Junction between integrated DNA and the chromosome of cell line A, showing imprecise integration, with 20 bp filler DNA. (*B* and *C*) 5′ Junction between integrated DNA and the chromosomal DNA of cell line D and E, respectively, showing precise integration.

Table S1. Analysis of the transferred DNA integration of eight different Ea.hy926 cell lines by PCR amplification

Line	PCR 1	PCR 2	PCR 3	PCR 4	PCR 5	PCR 6	PCR 7	PCR 8	PCR 9	PCR 10	PCR 11	PCR 12	PCR 13
Start*	10,405	284	2,223	3,248	4,309	4,914	5,384	6,294	7,524	8,017	9,459	9,794	10,135
Stop*	85[†]	419	2,359	3,380	4,413	5,004	5,463	6,425	7,623	8,165	9,568	9,890	10,147
A	ND	ND	ND	ND	29.8	30.0	30.3	29.8	30.9	30.3	28.9	30.7	ND
B	28.6	28.7	27.8	ND	28.9	29.8	30.0	29.8	ND	ND	29.7	31.2	31.2
C	ND	28.5	27.5	30.0	28.6	29.5	29.5	28.9	30.1	ND	ND	ND	ND
D	ND	ND	ND	29.6	28.5	28.6	28.7	28.4	29.7	ND	ND	ND	ND
E	ND	ND	ND	29.7	28.7	29.4	ND	ND	ND	ND	ND	ND	ND
F	28.3	27.3	27.5	30.2	29.1	29.6	28.9	29.5	30.1	30.05	28.6	30.0	30.5
G	ND	ND	ND	ND	30.1	ND	ND	ND	ND	ND	ND	ND	ND
H	ND	ND	ND	31.1	29.3	30.1	30.4	ND	ND	ND	ND	ND	ND
Eahy	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Eahy/pRS130	28.5	27.5	25.3	30.6	29.4	29.9	29.2	30.1	30.9	29.9	29.2	30.4	30.9

Presence/absence analysis for the entire sequence of pRS130 in eight stably transformed Ea.hy926 cell lines (A–H) using 13 different primer pairs. Genomic DNA from untransformed Ea.hy926 cells was used as negative control (Eahy) and the same DNA spiked with purified pRS130 as positive control (Eahy/pRS130). Cycle threshold (Ct) values obtained from the amplification with a given primer pair on the genomic DNA isolated from cell lines are indicated. ND, not detected.

*The start and stop value refer to the position of the PCR product relative to the sequence of pRS130 linearized at the *oriT*.

[†]Amplicon of PCR 1 is spanning the *oriT* region of pRS130. Results are summarized graphically in Fig. 2*A*.

Table S2. Bacterial strains used in this study

Strain	Description	Source
E. coli NovaBlue	Strain used for cloning and preparation of plasmid DNA	Novagen
E. coli β2150	Strain used for performing matings with *B. henselae*; Δ*dap*A	1
B. henselae RSE242	Δ*virB4* mutant of *B. henselae* RSE247	2
B. henselae RSE247	Spontaneous streptomycin-resistant strain of *B. henselae* ATCC 49882′, serving as WT	2
B. henselae RSE308	RSE247 harboring pRS51	3
B. henselae RSE556	RSE247 harboring pRS122	Present study
B. henselae RSE557	RSE242 harboring pRS122	Present study
B. henselae RSE558	RSE247 harboring pRS117	Present study
B. henselae RSE559	RSE242 harboring pRS117	Present study
B. henselae GS759	RSE247 harboring pRGS06	Present study
B. henselae RSE581	RSE247 harboring pRS130	Present study

1. Dehio C, Meyer M (1997) Maintenance of broad-host-range incompatibility group P and group Q plasmids and transposition of Tn5 in *Bartonella henselae* following conjugal plasmid transfer from *Escherichia coli*. *J Bacteriol* 179:538–540.
2. Schmid MC, et al. (2004) The VirB type IV secretion system of *Bartonella henselae* mediates invasion, proinflammatory activation and antiapoptotic protection of endothelial cells. *Mol Microbiol* 52:81–92.
3. Schulein R, et al. (2005) A bipartite signal mediates the transfer of type IV secretion substrates of *Bartonella henselae* into human cells. *Proc Natl Acad Sci USA* 102:856–861.

Table S3. Oligonucleotide primers used in this study

Name	Sequence	Relevant information
Plasmid construction		
prRS350	ATGGTACCGGTTTACATACCAAAGGCCATTCC	pRS122, *Age*I site
prRS351	ATGGTACCGGTAAAAATCCCCTCTACGAAGG	pRS122, *Age*I site
PCR probes for the mapping of pRS130 insertion		
prMQ1465	AAAAATGGCTTTAGCCACGAACG	pRS130 PCR 1 forward
prMQ1466	TATCTGTTATTCGACTGTATTTAC	pRS130 PCR 1 reverse
prMQ1441	AAGCGACGGAAAGATGCTG	pRS130 PCR 2 forward
prMQ1442	GTCCGTACTTATCTGCCAACCAC	pRS130 PCR 2 reverse
prMQ1443	CCATAATAGAACGCCGAAAGC	pRS130 PCR 3 forward
prMQ1444	GAGAAATGCTTGGTACCCACG	pRS130 PCR 3 reverse
prMQ1447	GCATGCCTGCTATTGTCTTC	pRS130 PCR 4 forward
prMQ1448	CCACTCCCACTGTCCTTTC	pRS130 PCR 4 reverse
prMQ1451	GTGCAATCCATCTTGTTCAATC	pRS130 PCR 5 forward
prMQ1452	GTACGGTGGGAGGTCTATATAAGC	pRS130 PCR 5 reverse
prMQ1490	CATTATGCCCAGTACATGACC	pRS130 PCR 6 forward
prMQ1491	GCTCACCTCGACCCATGG	pRS130 PCR 6 reverse
prMQ1492	CCTCCGGGCTGTAATTAGC	pRS130 PCR 7 forward
prMQ1493	CGGAGCCCTTTAAGGCTTTC	pRS130 PCR 7 reverse
prMQ1453	CTGGTTGTTGTGCTGTCTCATC	pRS130 PCR 8 forward
prMQ1454	GCTGAACTTGTGGCCGTTTAC	pRS130 PCR 8 reverse
prMQ1455	CAGATTTTTCCTCCTCTCCTGACT	pRS130 PCR 9 forward
prMQ1456	ACGGCCAGTGCCAAGCT	pRS130 PCR 9 reverse
prMQ1457	GGCGCCCTCTGGTAAGGT	pRS130 PCR 10 forward
prMQ1458	CTGCGTGCAATCCATCTTGT	pRS130 PCR 10 reverse
prMQ1463	CAGTGGCGATAAGTCGTGTCTTA	pRS130 PCR 11 forward
prMQ1464	GCTCCAAGCTGGGCTGTGT	pRS130 PCR 11 reverse
prMQ1502	GCCTATGGAAAAACGCCAGC	pRS130 PCR 12 forward
prMQ1503	GAATCAGGGGATAACGCAGG	pRS130 PCR 12 reverse
prMQ1504	ATGCAGCTGGCACGACAGG	pRS130 PCR 13 forward
prMQ1505	CCGGAAGCATAAAGTGTAAAGC	pRS130 PCR 13 reverse
Primers used for TAIL-PCR amplification		
AD1	NGTCGASWGANAWGAA	AD primer 1
AD2	TGWGNAGSANCASAGA	AD primer 2
prMQ1484	ACCGCCTACATACCTCGCTCTGC	Cell line A (3′) GSP1
prMQ1485	CGATAAGTCGTGTCTTACCGGGTTGG	Cell line A (3′) GSP2
prMQ1486	ACGCCTGGTATCTTTATAGTCCTG	Cell line A (3′) GSP3
prMQ1481	GTACTTATCTGCCAACCACTGTTTTGATCG	Cell line C (5′) GSP1
prMQ1482	GCTGCTCTGGAGATGCTGTTGTCC	Cell line C (5′) GSP2
prMQ1483	GTCATAACATACTCGACGGCTAACACAGC	Cell line C (5′) GSP3
prMQ1478	GGCTACCCGTGATATTGCTGAAGAGC	Cell line D (5′), cell line E (5′) GSP1
prMQ1479	TGACCGCTTCCTCGTGCTTTACG	Cell line D (5′), cell line E (5′) GSP2
prMQ1480	GCCGCTCCCGATTCGCAGC	Cell line D (5′), cell line E (5′) GSP3
Primers used for validation of the TAIL-PCR amplification product		
prMQ1497	AGTAAGAACAGGCAGAAAATATGTAAACTGG	Cell line A (3′), AL109981
prMQ1507	GTGTAAAACTGTACTATAAAGCCAGC	Cell line C (5′), AC108035
prMQ1521	AACTGTGTTGCACAAGCCAAAGG	Cell line D (5′), AC009316
prMQ1515	CAGAGATGTGATCACAGTCC	Cell line E (5′), AC084865