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Migration Along Orthodromic Sun Compass Routes by Arctic Birds

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Flight directions of birds migrating at high geographic and magnetic latitudes can be used to test bird orientation by celestial or geomagnetic compass systems under polar conditions. Migration patterns of arctic shorebirds, revealed by tracking radar studies during an icebreaker expedition along the Northwest Passage in 1999, support predicted sun compass trajectories but cannot be reconciled with orientation along either geographic or magnetic loxodromes (rhumb lines). Sun compass routes are similar to orthodromes (great circle routes) at high latitudes, showing changing geographic courses as the birds traverse longitudes and their internal clock gets out of phase with local time. These routes bring the shorebirds from high arctic Canada to the east coast of North America, from which they make transoceanic flights to South America. The observations are also consistent with a migration link between Siberia and the Beaufort Sea region by way of sun compass routes across the Arctic Ocean.

How birds use different compass systems based on the sun, stars, and geomagnetic field to orient along their migration routes is not fully understood (*1–4*). The region at the Northwest Passage where magnetic declination varies markedly close to the Magnetic North Pole provides a natural cue-conflict situation, in the sense that predicted flight trajectories differ in a very distinct way if birds orient by time-independent celestial rotation cues (predicted trajectories lie along geographic loxodromes) (*2, 3, 5*), the magnetic compass (predicted trajectories lie along magnetic loxodromes) (*6*), or the sun compass (*7*). The sun compass is sensitive to the time shift associated with longitudinal displacement (*8–10*), as long as the internal clock is not reset to local time, which apparently requires some period of stopover (*11, 12*). Such a natural time shift becomes substantial during migratory flights at polar latitudes, where distances between longitudes are small. At high latitudes, sun compass courses are expected to change by approximately 1° for each degree of longitudinal displacement, with little daily and seasonal variation. This corresponds to the change in sun azimuth associated with the difference in local time between longitudes (*8*). The course change along a great circle equals sin θ degrees for each degree of longitude intersected, where θ is the latitude (*8*). This means

that at high latitudes, where sin θ approaches unity, sun compass routes constitute close approximations of orthodromes.

We have previously demonstrated regular and widespread east-northeast migration of shorebirds from northern Siberia toward North America across the Arctic Ocean (*13*). Evaluating predicted trajectories associated with different orientation principles showed orientation along sun compass routes to be most likely, although orientation along magnetic loxodromes could not be ruled out (*14*). In this study, we test this hypothesis of orientation along sun compass routes by investigating and evaluating the bird migration pattern in high arctic Canada, where there are extreme differences between predicted trajectories based on celestial and geomagnetic cues.

We used a tracking radar placed on board the Canadian Coast Guard icebreaker *Louis S. St-Laurent* to record the postbreeding bird migration pattern along the Northwest Passage during the period from 29 June to 3 September 1999 (*15, 16*). Radar observations were carried out when the ship was stationary (*17*), and more than 10 tracks of migrating birds were recorded at each of 11 sites between Baffin Island (66°W) in the east to Herschel Island (140°W) in the west. We focused on the dominating, high-altitude (mostly 400 to 3000 m above sea level), and broadfront migration in easterly and southeasterly directions as recorded at seven of these sites (*18*). Field observations from the ship and at tundra sites indicated that shorebirds (such as the American golden plover *Pluvialis dominica*, semipalmated sandpiper *Calidris pusilla*, white-rumped sandpiper *C. fuscicollis*, and pectoral sandpiper *C. melanotos*) made up most of the migrants in this

study, traveling in flocks up to about 100 individuals. The above-mentioned species of shorebirds have a wide breeding range in arctic North America, migrating mainly via the Atlantic region of North America to well-defined winter quarters in South America. Hence, the results from the different sites are expected to reflect, to a large extent, this major shorebird migration system in the New World (*19*).

The mean and scatter of flight directions are shown in Table 1. Track direction refers to the flight direction relative to the ground, as measured by radar, whereas heading direction was calculated by subtracting the wind vector from the birds' track vector. We primarily refer to observed track directions in our evaluation. The differences between track and heading directions are generally small or modest (Table 1), and our conclusions will be valid irrespective of whether partial wind drift has influenced the track directions on some occasions.

Eastbound migration was massive at all three Beaufort sites (combined in Table 1), with similar mean track directions 100 km north of the coast (105° at 70.5°N, 139.0°W), 27 km north of the coast (87° at 69.8°N, 133.3°W), and only a few kilometers from Herschel Island (104° at 69.6°N, 139.5°W). Combining all eastbound tracks (0° to 180°) from these three sites, 33, 42, and 16% fell in the sectors 60° to 90°, 90° to 120°, and 120° to 150°, respectively. Selected courses within these sectors have been used to illustrate predicted trajectories to and from the Beaufort sites for different orientation principles, whereas corresponding trajectories were calculated on the basis of mean directions for the Wollaston, King William, and Baffin sites (Fig. 1). When evaluating the alternative trajectories, we rejected those that do not take the birds through the Hudson Bay region or the interior and east coast of North America, where these shorebirds are known to pass during autumn migration (*19*).

Long-distance orientation along constant geographic or magnetic courses can be ruled out, because the geographic and magnetic loxodromes (*20*) in most cases extend too far north, with some of the latter spiraling toward the Magnetic North Pole (Fig. 1, A through D). Geographic loxodromes from the Beaufort sites toward 60 to 110° (65% of the recorded tracks), as well as the loxodrome along the mean direction at Wollaston Peninsula, can be rejected (Fig. 1, A and B). All magnetic loxodromes except one (Baffin Island) can also be rejected (Fig. 1, C and D). Magnetoclinic orientation (*21*) is also invalid in many cases (*14*). It has been recently suggested, on the basis of experiments testing the interactions between celestial and magnetic compasses in birds, that the problem of a changing magnetic declination is solved by recalibration of magnetic orientation by celestial rotation cues at stopover sites along

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the route (2). This is probably not a feasible solution for the shorebirds in this study, because it necessitates very frequent recalibration (eastbound movement between 120° and 90°W at the 70°N parallel is perpendicular to densely spaced declination isolines involving a change in magnetic declination by about 70° per 1000 km of distance, and inclination is very steep, 85° to 88°) and still leads the birds along unrealistic loxodromes (Fig. 1, A and B) in many cases.

Sun compass trajectories (Fig. 1, E and F)

Fig. 1. Predicted flight trajectories according to different orientation principles, extrapolated from observed directions of migrating birds at the Northwest Passage. Flight trajectories are extrapolated from the mean Beaufort position (**A**, **C**, and **E**) for five geographical compass courses (75°, 90°, 105°, 120°, and 135°) and from the Wollaston, King William, and Baffin positions (**B**, **D**, and **F**) for the mean geographic courses recorded at these sites (102°, 132°, and 168°, respectively). Trajectories are illustrated on a Mercator projection (26) for different orientation principles associated with migration along geographic loxodromes [(A) and (B)] magnetic loxodromes [(C) and (D)] and sun compass routes [(E) and (F)]. Isomagnetics for declination (20) are also indicated in (C) and (D). Magnetic loxodromes and sun compass routes were simulated by multiple stepwise loxodromic calculations (with step length ≤1° in longitude and 0.5° in latitude), recalculating the course for each successive step according to changes in magnetic declination (20) and to course changes of 1° per degree of longitude traversed, respectively.
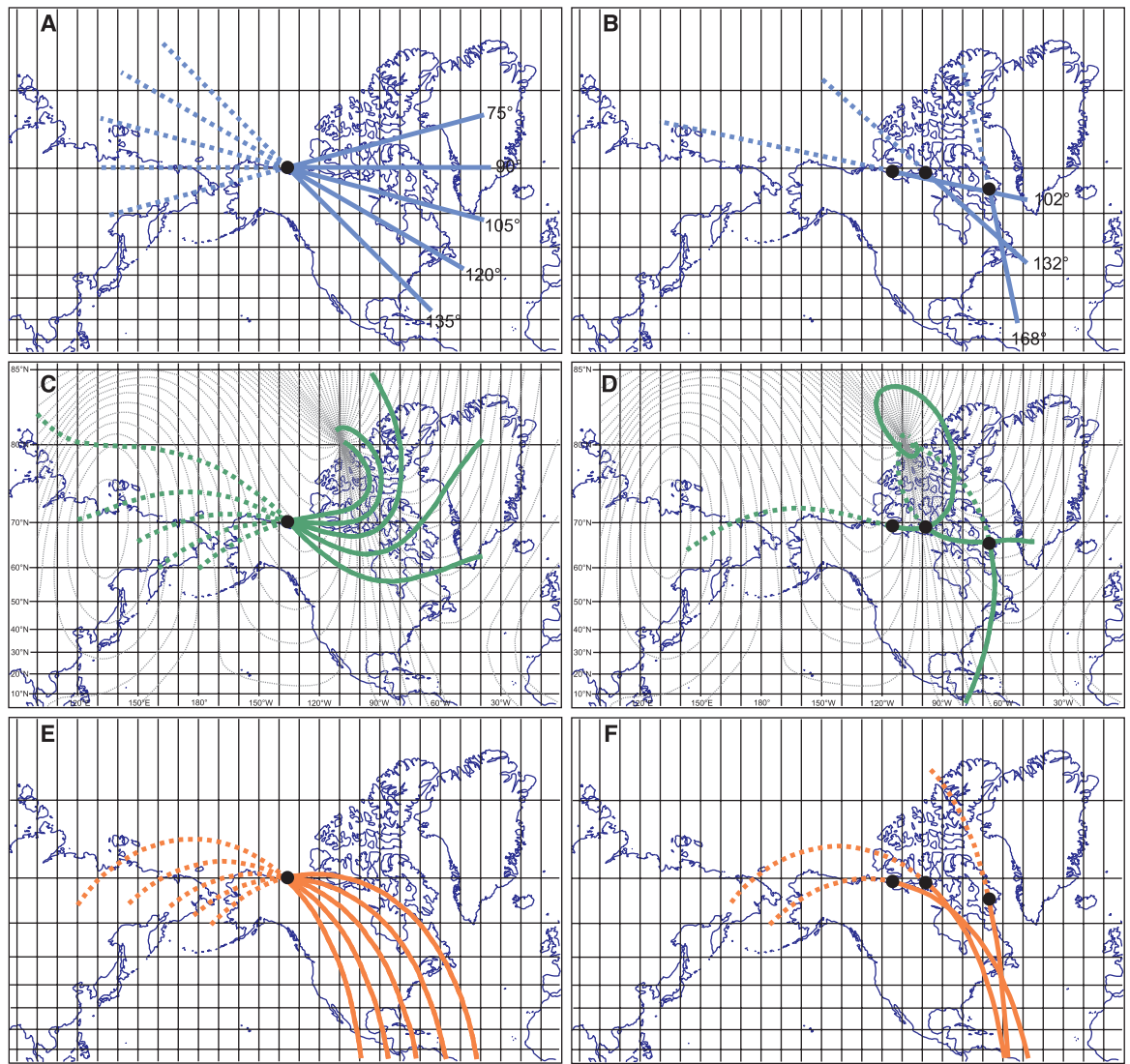

Table 1. Tracking radar data of bird migration in high arctic North America: Mean and scatter of flight directions of the main migratory movements toward the east and south at different sites along the Northwest Passage in 1999. The means and scatter of directional data were calculated as mean vectors and angular deviations. The mean vector length, r, is a measure (between 0 and 1) of directional concentration (29).

Sites	Position	Dates	Flight direction	Mean direction (degrees)	Mean vector length (r)	Angular deviation (degrees)	No. of tracks
King William Island*	69.4°N, 99.2°W	20–22 July	Track	132	0.90	26	18
			Heading	132	0.95	18	16
Wollaston Peninsula*	69.3°N, 115.0°W	23–25 July	Track	102	0.79	38	21
			Heading	100	0.52	56	20
Beaufort Sea*	70.0°N, 136.0°W†	31 July–7 Aug	Track	96	0.90	25	279
			Heading	115	0.91	24	265
Baffin Island‡	66.0°N, 67.0°W§	30 Aug–3 Sep	Track	168	0.82	35	50
			Heading	191	0.53	55	50

*Track directions in sector 0° to 180°.   †Data combined from three sites: Tuktoyaktuk (31 July to 1 August, 6 to 7 August; 69.8°N, 133.3°W), Beaufort Sea (2 to 3 August; 70.5°N, 139.0°W), and Herschel Island (3 to 5 August; 69.6°N, 139.5°W).   ‡Track directions in sector 90° to 270°.   §Data combined from two sites: East Baffin Island (30 to 31 August; 68.6°N, 66.3°W) and Iqaluit (3 September; 63.7°N, 68.5°W).

show an excellent agreement with known autumn concentration of shorebirds at, for example, James Bay and the east coast of North America, and with the transatlantic migration to South America (*19, 22–25*). These trajectories also agree with a migration system between Siberia and North America across the Arctic Ocean (*14*). Only for the migration at Baffin Island do all three orientation principles give similar trajectories, and no conclusion about the most likely alternative can be drawn for this site (Fig. 1, B, D, and F). The fact that sun compass routes are distance-efficient is illustrated in Fig. 2, showing trajectories on an equidistant azimuthal map projection (*26*). For this evaluation, it is the predicted trajectories at northerly latitudes that are critical. The birds may change to other orientation principles and cues for the transoceanic flights across the Atlantic Ocean (*23–25*) and at more southerly latitudes, where celestial and geomagnetic cues (and wind patterns) are quite different from those at high latitudes [sun compass trajectories in Figs. 1 and 2 are extended across the Atlantic Ocean and into more southerly latitudes (*8*) without implying any critical evaluation in relation to alternative possibilities of orientation in these latter regions].

Orientation along sun compass routes is facilitated by long nonstop flights (common among arctic shorebirds), when there is no time for the birds to reset their internal clock in phase with local time. To continue along the same sun compass route after a stopover period when the birds reset their internal clock to local time requires that the arrival course at the stopover locality be transferred from the sun compass to another cue system (such as magnetic compass or topography), to be transferred back to the sun compass as the departure course after resetting the internal clock (*8*).

Although the radar tracks showed that migration takes place on a broad front, apparently without responses to landmark features, large-scale topographical guidance could play some role in the birds' flight routes (such as along the south coast of the Beaufort Sea). However, there are long passages, across ocean, barrens, or low-level fog, where the migrants cannot rely on topographical orientation. Our analysis does not exclude the possibility that the birds use a more complex orientation program than assumed in the alternative cases above, perhaps involving changes of preferred compass course and/or switching between different celestial and magnetic orientation mechanisms during the passage across arctic North America. Genetically programmed changes in migratory direction have been demonstrated for passerines (*1, 5*), and it is also known that migratory birds may switch between magnetic and celestial orientation cues (*1–3*). Even if feasible in principle, it is difficult to propose a complex orientation program that accounts for the observed migration patterns under the special geomagnetic and environmental conditions at the Northwest Passage. Furthermore, the orientation from Siberia toward North America across the Arctic Ocean (*14*) would require the assumption of an analogous complex system programmed in relation to quite different geomagnetic and environmental conditions, which seems highly unlikely. Also, the long flights and rapid postbreeding migration from the tundra to temperate staging areas, which are common features among shorebirds (the passage across the Arctic Ocean from Siberia to North America is presumably completed in a single flight), make a division of the journey into a series of differently programmed orientation steps unlikely. Before considering such intricate solutions, we investigated whether fixed orientation on the basis of any of the birds' fundamental compass mechanisms may suffice to explain the long-distance orientation and migration routes at high magnetic and geographic latitudes.

This analysis supports the hypothesis of sun compass routes as an explanation for the long-distance orientation of many tundra shorebirds between Siberia, arctic North America, and the east coast of North America, whence the birds continue by transatlantic flights toward South American winter quarters. An important feature of this migration system is the fact that the resulting sun compass routes conform closely to distance-saving orthodromes.

The sun compass has probably provided the basis for the evolution of the above-mentioned extraordinary migration system in a region with an exceptional geomagnetic field. Along routes where magnetic declination is less variable, birds may use their interacting compass senses differently (*27, 28*). The sun compass has been demonstrated to be important to Adelie penguins (*Pygoscelis adeliae*) in Antarctica (*9, 10*)—another case of polar orientation. The fact that the arctic shorebirds in this study do not return in spring along the same routes as used in autumn [spring migration mostly occurs through the interior of North America (*19*)] testifies to the complexity of the global orientation performance of migrating birds.

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16. The icebreaker route extended from Iqaluit on Baffin Island (29 June 1999) through Hudson Strait, Foxe Basin, and northward to northern Bathurst Island, where the ship turned south- and westward, passing south of Victoria Island to Herschel Island at the Beaufort Sea as the westernmost position (3 to 5 August). The ship then sailed eastward by another route north of Banks Island via the Magnetic North Pole at Ellef Ringnes Island (79°N, 105°W), returning to Iqaluit (3 September) along the east coast of Baffin Island.
17. A tracking radar (3 cm wavelength, 200 kW peak power, 0.25/1.0 μs pulse duration, and 1.5° nominal pencil beam width) was placed on board the CCG icebreaker *Louis S. St-Laurent* (radar antenna 21 meters above sea level). The radar was operated when the ship was stationary in pack ice or open water, most often 2 to 8 km from the nearest tundra shores, except at two sites in the Beaufort Sea, 27 and 100 km north of the coast, respectively. The position of a target was recorded every 2 s by the radar operated in automatic tracking mode. Radar data were corrected for the heading, motion, and leveling of the ship recorded simultaneously with the radar tracking. Mean track direction, ground speed, vertical speed, and altitude were calculated for flocks or individual birds tracked for at least 30 s (mean tracking time was 160 s) within 10 to 12 km from the radar. Wind measurements at different altitudes were obtained within 2 hours of bird track records by radar tracking of helium-filled balloons. Mean heading direction and airspeed of the birds were calculated by subtraction of the wind vector from the birds' track vector. For some tracks, heading directions could not be calculated because of missing wind data.
18. A grand total of 692 radar tracks of birds was recorded during 494 hours of radar operation during the journey. In this analysis, we focused on a well-defined and intensive east- and southbound migratory passage recorded at seven sites, excluding sites with sparse (≤10 radar tracks) or widely scattered movements (three sites at Banks and Melville Islands) or mainly low-altitude (<200 m) movements over the sea (Amundsen Gulf). Included in our analyses are three sites at the Beaufort Sea with massive eastbound migration (279 tracks toward 0° to 180°; 23 tracks toward 180° to 360° excluded), one site at Wollaston Peninsula (21 tracks toward 0° to 180°; 4 tracks toward 180° to 360° excluded), one site at King William Island (18 tracks
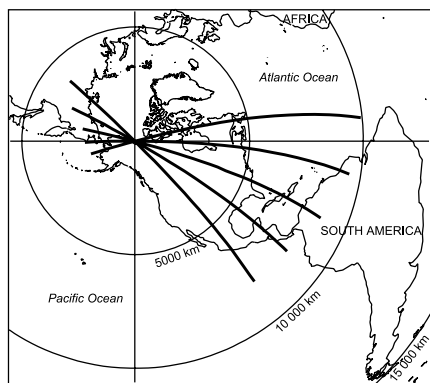
Fig. 2. Flight trajectories along sun compass routes extrapolated from the mean Beaufort position (for geographic courses 75°, 90°, 105°, 120°, and 135°) on an equidistant azimuthal projection with the Beaufort position as projection center. This map projection shows orthodromes through the projection center as straight lines (*26*).

toward 0° to 180°; at this site there was a distinctly bimodal distribution toward SE and W, and 32 tracks toward 210° to 350° were excluded), and two sites at Baffin Island (50 tracks towards 90° to 270°; 4 tracks toward 270° to 90° excluded).

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A Ribonucleotide Reductase Homolog of Cytomegalovirus and Endothelial Cell Tropism

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Human cytomegalovirus infects vascular tissues and has been associated with atherogenesis and coronary restenosis. Although established laboratory strains of human cytomegalovirus have lost the ability to grow on vascular endothelial cells, laboratory strains of murine cytomegalovirus retain this ability. With the use of a forward-genetic procedure involving random transposon mutagenesis and rapid phenotypic screening, we identified a murine cytomegalovirus gene governing endothelial cell tropism. This gene, M45, shares sequence homology to ribonucleotide reductase genes. Endothelial cells infected with M45-mutant viruses rapidly undergo apoptosis, suggesting that a viral strategy to evade destruction by cellular apoptosis is indispensable for viral growth in endothelial cells.

Human cytomegalovirus (HCMV) establishes a persistent lifelong infection. Infection of the immunocompetent individual is usually subclinical, but the virus can cause severe and life-threatening disease in transplant patients and people with acquired immunodeficiency syndrome (AIDS). HCMV infects a wide variety of cells and tissues (*1*). Several studies have implicated HCMV in the genesis of atherosclerosis, and particularly in rapidly progressing coronary artery disease and endothelialitis in cardiac transplant patients, and in the development of coronary restenosis after angioplasty (*2*). Migration of vascular smooth muscle cells stimulated by a CMV-encoded chemokine receptor has been proposed to promote vascular stenosis (*3*). In addition, increased endothelial and smooth muscle cell proliferation that is not counterbalanced by increased apoptosis may also result in thickening of the intima and media

of arteries (*4*). This suggests a mechanism by which a viral inhibitor of apoptosis contributes to vascular disease. Moreover, HCMV-infected endothelial cells circulate in the blood of patients with CMV disease and contribute to viral dissemination (*5*).

Studies on the interaction of HCMV with vascular tissues could clarify the contribution of HCMV to vascular disease. Unfortunately, the HCMV laboratory strains do not replicate in endothelial cell cultures. Clinical isolates, by contrast, can be propagated in endothelial cells, but this property is lost after virus propagation in fibroblasts (*6, 7*). Although there is evidence for a genetic basis of cell tropism (*6*), the gene(s) responsible are difficult to find, owing to the large genome of CMV, the lack of candidate genes, and the difficulty generating mutants of clinical HCMV isolates.

The HCMVs and murine CMVs (MCMVs) share a similar pathobiology and have collinear genomes. In the mouse, endothelial cells are known to play a role in viral dissemination, latency, and vascular disease (*8*). The genomes of the MCMV and HCMV laboratory strains were recently cloned as infectious bacterial artificial chromosomes (BACs) in *Escherichia*

coli (*9, 10*), where they can be mutated rapidly particularly with the use of random transposon (Tn) mutagenesis (*11*). MCMV derived from the BAC clone retains the capacity to propagate on cultured endothelial cells. In this work, we focused on the genetic basis of the tropism of MCMV for vascular endothelial cells.

In the absence of specific candidate genes, we constructed a library of virus mutants randomly mutated at a single position by combining a refined Tn mutagenesis procedure with a phenotypic screening approach. MCMV Tn mutants were generated by a single-step procedure using a Tn derivative, Tn*Max16*, bearing the enhanced green fluorescent protein (GFP) gene (*12*). To convert mutant genomes into a library of mutant viruses rapidly for phenotypic analysis, we directly transferred BAC DNA from *E. coli* to mammalian cells. The transfer of small multicopy plasmids can be done using naturally invasive bacteria or *E. coli* expressing the invasin gene of *Yersinia pseudotuberculosis* and the listeriolysin O gene of *Listeria monocytogenes* from the plasmid pGB2Ωinv-hly (*13*). We adapted this approach for the transfer of the 240-kb MCMV BAC into fibroblasts (*14, 15*). A library of 576 *E. coli* clones, each carrying the MCMV BAC, Tn*Max16*, and pGB2Ωinv-hly, was deposited in six 96-well microtiter plates. Two microliters of each bacterial culture was used to inoculate NIH-3T3 fibroblasts grown in 96-well tissue culture plates. Viable mutant viruses were easily detected because only MCMV with a Tn forms green fluorescent plaques, whereas wild-type MCMV or nonviable MCMV mutants do not. In this way, we retrieved 199 viable mutants.

Fibroblast and endothelial cells were infected in parallel with individual virus mutants to screen for loss of ability to grow on endothelial cells (*16*). Viral growth was assessed visually by observation of green fluorescent plaques and by titration. We identified six mutants that did not grow and spread in endothelial cells but did grow well in fibroblasts (Fig. 1). Using BAC DNA extracted from the corresponding *E. coli* clones we determined the Tn insertion sites by direct sequencing from within the Tn (*11*). Remarkably, the insertions in all six mutants mapped to two adjacent open reading frames

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