The Peopling of the Pacific from a Bacterial Perspective
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G and H, and table S5). Because mil/SIP2 is expressed in the mesoderm just lateral to the midline (4), mil/SIP2 is proposed to function in mesoderm over the YSL. Further, cardia bifida in the spns2Δ15 mutant was restored by the injection of spns2 mRNA but not spns2(R153S) mRNA into the YSL at shield stage (Fig. 4, I to L, and table S6). The rescue frequency by injection of spns2 mRNA into the YSL was slightly lower than for injection into the blastomere (tables S2 and S6). One explanation is that spns2 mRNA injected into the blastomere at the one-cell stage is widely distributed in the YSL because the YSL is constituted by marginal blastomeres collapsing onto the yolk around the 1000-cell stage. Another explanation is that the function of Spns2 in embryonic tissues as well as in the YSL may be partly required for the migration of myocardial precursors. Furthermore, transplantation analysis showed that Spns2 at least functions in a cell-nonautonomous manner, because ko157-derived donor cells were incorporated into single beating hearts of wild-type recipients, and wild-type-derived donor cells were incorporated into one of two beating hearts of ko157 recipients (movies S1 to S3). One attractive interpretation is that Spns2 in the YSL regulates the SIP export from the yolk to the embryonic body, leading to the activation of Mil/SIP2 in mesoderm just lateral to the midline (fig. S1). Recent reports have pointed out the importance of ferroportin1 (fpn1) as a transporter of iron from the yolk to the embryonic body (21) and the clinical relevance to hypochromic anemia and hemochromatosis in humans (22, 23).

By investigating characteristic features of the zebrafish spns2ko157 mutant and analyzing the biological activity of Spns2, we have demonstrated that Spns2 functions as a SIP transporter and that Spns2 in the extraembryonic YSL is a prerequisite for the migration of myocardial precursors, presumably mediated by the SIP-Mil/SIP2 pathway. The identification of Spns2 not only contributes to our understanding of the molecular mechanism of biological SIP delivery, but may also elucidate the physiological importance of Spns2 in autoimmune disease (24), cardiovascular diseases, and cancer (25) in which SIP plays a central role.

**References and Notes**

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**Supporting Online Material**

www.sciencemag.org/cgi/content/full/316/7449/DC1

**Materials and Methods**

Figs. S1 to S6

Tables S1 to S6

Movies S1 to S3

References

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**The Peopling of the Pacific from a Bacterial Perspective**

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Two prehistoric migrations peopled the Pacific. One reached New Guinea and Australia, and a second, more recent, migration extended through Melanesia and from there to the Polynesian islands. These migrations were accompanied by two distinct populations of the specific human pathogen *Helicobacter pylori*, called hsPahul and hsPamori, respectively. hsPahul split from Asian populations of *H. pylori* 31,000 to 37,000 years ago, in concordance with archaeological history. The hsPahul populations in New Guinea and Australia have diverged sufficiently to indicate that they have remained isolated for the past 23,000 to 32,000 years. The second human expansion from Taiwan 5000 years ago dispersed one of several subgroups of the Austronesian language family along with one of several hsPamori clades into Melanesia and Polynesia, where both language and parasite have continued to diverge.

After modern humans dispersed “out of Africa” about 60,000 years ago (60 ka) (1), they reached Asia via a southern coastal route (2). That route extended along the Pleistocene landmass, known as Sundaland (i.e., the Malay peninsula, Sumatra, Java, Borneo, and Bali), that was joined to the Asian mainland as a result of low sea levels during the last ice age (12 to 43 ka) (3). Low sea levels also meant that Australia, New Guinea, and Tasmania were connected in a continent called Sahul, separated from Sundaland by a few narrow deep-sea channels. It seems Sahul was colonized only once, ~40 to 50 ka (3, 4), although backed-blade stone tool technology and the dingo appear to have been introduced from India at a later date (3, 6).

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Human genetic data are compatible with these interpretations, but have not provided the details. Redd and Stoneking identified multiple mitochondrial DNA (mtDNA) lineages among New Guinea peoples with coalescence times of 80,000 to 122,000 years (80 to 122 ky), predating the out-of-Africa migrations (5). In subsequent analyses, Australian aboriginals and Melanesians fell into multiple, distinct mtDNA haplogroups interdispersed among lineages from East Asia and India (4), with one exception: haplogroup Q, which had a coalescent estimate of 32 ka and contained both Australian and Melanesian lineages. Y-chromosome markers yielded one lineage for Australians and a second one for Melanesians (4). Australia and New Guinea remained connected by a land bridge until sea levels rose ~8 to 12 ka, and it is surprising that the native inhabitants of Sahul are not genetically associated except for haplogroup Q.

Subsequent prehistoric migrations to island East Asia and the Pacific have been designated differently depending on whether they were traced by language, archaeological remains, or genetic studies. Most of the native Pacific languages from near the African coast (Madagascar) through to Polynesia are Malayo-Polynesian, a subgroup of the Austronesian language family (7). The nine other subgroups of Austronesian are only spoken in Taiwan, suggesting that Taiwan is the origin of Austronesian (7). In support of this interpretation, agriculturists spread from Taiwan via insular and coastal Melanesia into the Pacific, as marked by the Lapita cultural complex, including red-slipped pottery, Neolithic tools, chickens, pigs, and farming (8). A human genetic marker of this route of spread is the “Polynesian” mtDNA HV1 motif of lineage B4a1a, which is found at high frequency among native Taiwanese (9), Melanesians, and Polynesians (10, 11).

We attempted to trace human prehistory in the Pacific by analyzing the distribution of a bacterial parasite of humans, Helicobacter pylori. H. pylori accompanied modern humans during their migrations out of Africa (12). Subsequent founder effects, plus geographic separation, have resulted in populations of bacterial strains specific for large continental areas. Thus, Africans are infected by the H. pylori populations hpAfrica1 and hpAfrica2, Asians are infected by hpAsia2 and hpEastAsia, and Europeans are infected by hpEurope (12, 13). It seemed possible that the distribution of H. pylori genotypes among native inhabitants might provide insights into migrations throughout the Pacific. We cultivated 212 bacterial isolates from gastric biopsies or mucus obtained from aboriginals in Taiwan and Australia, highlanders in New Guinea, as well as Melanesians and Polynesians in New Caledonia (table S1). Concatenated sequences of seven gene fragments (3406 base pairs, of which half are polymorphic) from these isolates yielded 196 unique haplotypes. These were compared with 99 unique haplotypes from 100 Europeans in Australia and 222 other unique haplotypes from Asia and the Pacific, including 15 haplotypes from Chinese inhabitants of Taiwan, as well as ~1700 haplotypes from other sources.

According to Bayesian assignment analysis, our samples from native inhabitants yielded 50 unique haplotypes that formed a distinct biogeographic group called hpSahul (14). Twenty-eight percent (26 of 92) of the haplotypes from aboriginals in Australia and 89% (24 of 27) of the haplotypes from highlanders in New Guinea were hpSahul (Fig. 1A). One hpSahul haplotype was found among 99 haplotypes from Europeans in Australia and none among the other haplotypes from elsewhere.

hpMaori is a subpopulation of hpEastAsia, isolated from Polynesians (Maoris, Tongans, and Samoans) in New Zealand (13) and three individuals in the Philippines and Japan. hpMaori isolates have not previously been isolated from other individuals, including the 15 Chinese inhabitants of Taiwan (12). Fifty-four of the 196 unique haplotypes from native inhabitants were hpMaori (14), and all came from Austronesian sources. These included native Taiwanese (43 of 59, 73%), Melanesians (6 of 13, 46%), and Polynesians (3 of 5, 60%) in New Caledonia, and two inhabitants of the Torres Strait islands that lie between Australia and New Guinea and which have been visited extensively by Polynesians (Fig. 1A and table S1). These observations suggest that hpMaori is a marker for the entire Austronesian expansions rather than only for Polynesians. The remaining unique haplotypes from native inhabitants were hpEurope, hpEAsia, and hpAfrica1, which can be attributed to very recent human travels.

If Taiwan were the source of the Austronesian expansions, hpMaori haplotypes would be expected to be widespread among aboriginal Taiwanese tribes. Indeed, hpMaori was isolated...
frequently (44 to 100%) from five of the six tribes sampled (Fig. 1A). Taiwan should also harbor the greatest diversity, and the branching order within a phylogenetic tree should reflect the direction of subsequent migrations. The phylogenetic analyses showed that genetic diversity was significantly higher in Taiwanese hsPMaori (π = 1.79 to 1.82%) than in non-Taiwanese hsPMaori (π = 1.58 to 1.62%). All non-Taiwanese hsPMaori haplotypes form a single clade, the Pacific clade, which originates from one of several clades among indigenous Taiwanese haplotypes (Fig. 1B). The sequence of branching events within the Pacific clade is consistent with sequential migrations from Taiwan via the Philippines and island Melanesia to Polynesia (Fig. 1B). These results also support an association between language and haplotype group. The indigenous Taiwanese haplotypes were isolated from tribes that speak 5 of the 10 subgroups of the Austronesian family of languages, whereas the Pacific clade was isolated from individuals that speak variants of Malayo-Polynesian. The sole exception to these generalizations was one haplotype from the Yami of Lanyu, a small island off the coast of Taiwan, where the language is a variant of Malayo-Polynesian but the haplotype clustered with the indigenous Taiwanese haplotypes. Together, these observations provide support for a Taiwanese source of the Austronesian expansions.

Using the isolation with migration model (IMa), we calculated the magnitude of migrations in both directions after the initial split between the Taiwan and Pacific clades of hsPMaori (15). IMa uses sequence data within a probabilistic framework to simulate a model of initial geographic separation between two populations followed by occasional migration in both directions. Because homologous recombination is frequent within H. pylori (13, 16), we excluded blocks of sequences that had a high likelihood of recombination (14). The calculations indicated that migrations subsequent to the initial split were unidirectional, from Taiwan to the Pacific (Fig. 2A).

Other splits between pairs of H. pylori populations were also unidirectional: for example, the Amerind colonization over the Bering Strait and the subsequent colonization of South

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**Fig. 2.** Global patterns of migration between eight pairs of H. pylori populations as calculated by the isolation with migration model (IMa). (A) Map. The magnitudes of migration are denoted by numbers and arrow thickness and their direction is indicated in blue or red. (B) Graph showing a linear relation between the calibration time (table S2) of six events (filled blue circles) that are dated by archaeological estimates and the estimated time (t). (C) Population tree reconstructed from a consensus of 1000 bootstrap samples from the range of calculated t values to determine the ages of nodes (thousands of years, kyr) associated with the peopling of the Sahul (unfilled circles). Ages (in light blue) are the 95% confidence limits of estimated coalescence times obtained by applying global rate minimum deformation (GRMD) ratesmoothing, as implemented in Treefinder, to the range of t values within the limits of calibration dates.

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**Fig. 3.** Global phylogeny of H. pylori as calculated by a haplotype approach based on the 80% consensus of 100 ClonalFrame analyses. (A) Phylogenetic tree of divergence time, as indicated by node height versus geographic sources (bottom line) and population assignments (second line). Detailed sources of clades within populations are indicated in the third line from the bottom. Node heights were used to date the two hpSahul nodes (unfilled circles) based on six calibration times (filled blue circles, table S2). Age ranges (right blue numbers) are the 95% confidence limits of estimated coalescence times obtained with GRMD ratesmoothing over the range of node height values and calibration time limits. hpAFR2, hpAfrica2; hpAFR1, hpAfrica1; AM, America. (B) Graph showing a linear relation of calibration time with the range of heights for each node.
Rapid Membrane Disruption by a Perforin-Like Protein Facilitates Parasite Exit from Host Cells

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Perforin-like proteins are expressed by many bacterial and protozoan pathogens, yet little is known about their function or mode of action. Here, we describe Toxoplasma perforin-like protein 1 (TgPLP1), a secreted perforin-like protein of the intracellular protozoan pathogen Toxoplasma gondii that displays structural features necessary for pore formation. After intracellular growth, TgPLP1-deficient parasites failed to exit normally, resulting in entrapment within host cells. We show that this defect is due to an inability to rapidly permeabilize the parasitophorous vacuole membrane and host plasma membrane during exit. TgPLP1 ablation had little effect on growth in culture but resulted in a reduction greater than five orders of magnitude of acute virulence in mice. Perforin-like proteins from other intracellular pathogens may play a similar role in microbial egress and virulence.

Perforin (PF) and members of the membrane attack complex (MAC) (complement proteins C6 to C9) are pore-forming proteins of the innate and adaptive immune response that constitute the founding members of the MACPF domain family (1). Recent studies (2, 3) have suggested a shared mechanism of pore formation between the MACPF domain and...