Myllykallio et al. (1) narrowed the replication origin of the archaeon *Pyrococcus abyssi* down to an 80-kb region (bar on left axis of Fig. 1A), an observation consistent with our prediction (2) of the replication origin of a closely related species, *Pyrococcus* OT3 (triangle on upper axis of Fig. 1A). Here, we report that a comparison of the genomic sequences of these two *Pyrococcus* species suggests that the orientation of a 350-kb region was reversed relative to the rest of the genome at some point in their evolution. Moreover, the center of this inversion is close to the replication origin (I in Fig. 1, A and B) discussed by the two research groups (1, 2). In the light of this new information, pieces of evidence can be assembled into a general model of genomic reorganization.

We compared the two *Pyrococcus* genomes using FASTA (3) and plotted the sequences against each other to gauge similarity (Fig. 1A). The genomes can be compared by relating similar sections in the original sequences (red dots in Fig. 1A) or by replacing one of the two sequences with its complementary sequence (blue dots in Fig. 1A). When the *P*. OT3 genome is scanned from left to right, the position related by blue dots trends generally down and to the right, an indication of the sequence's broad similarity to that of *P. abyssi*. Inside the 350-kb region (I in Fig. 1A), however, red dots are dominant and trace out a trend orthogonal to that of the blue dots, an indication of the inversion (4).

Evidence of similar shuffling can be found in other contexts. In two strains of the eubacterium *Helicobacter pylori*, for example, a pair of DNA fragments, 75 to 83 kb in length, are found replacing each other's position (5)—and, moreover, the two fragments are equally spaced from the putative replication origin (II in Fig. 1B). The replacement thus can be explained by an accumulation of two inversions of the same type as observed in *Pyrococcus*. Additionally, there is evidence that the genome of a third *Pyrococcus* species has differentiated from those of the two discussed above through the accumulation of similar exchanges (6). Such replacement thus seems to be fairly common among eubacterial and archael species in the same genera (6–9), and it seems possible to relate evolutionary distances of species to such shufflings of genomes.

We propose a model (Fig. 1C) to explain the observed patterns in the *Pyrococcus* genome. The long distance between the two inversion points in the genome can be explained if two protein assemblies replicating the same circular DNA molecule in opposite directions from the replication origin actually form a single complex, so that the two inversion points approach each other during replication. The symmetric positioning of the inversion points may simply reflect the fact that the two replication forms are working at similar speeds. The inversion points would thus move into the single complex that remains stationary in the cell (9) and would thereby approach each other. Exchange of two sections of the DNA attached to the two protein assemblies would result in the inversion observed in the *Pyrococcus* genome; accumulation of two such incidents would cause the exchange of fragments observed in *Helicobacter*.

Reconnection of one fork to another can take place if each protein assembly has two subunits that bind to DNA on different sides of each inversion point and if two assemblies reassemble by exchanging their subunits with the ends of DNA still attached (Fig. 1C). Indeed, such a mechanism has been proposed to explain recombination induced by DNA gyrase (10). Two forks replicating a circular DNA in vivo will necessarily increase positive superhelicity of the unreplicated part of the DNA (11); it is very likely that enzymes similar to DNA gyrase are placed at a replication fork to relax this superhelicity.

The number of genomic shufflings that could have taken place within these closely related species is remarkably small. In contrast, genomes in different genera do not show clear correlations over sizable lengths. This suggests that shuffling might have occurred numerous times, thereby accelerating evolution of these organisms.

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**References and Notes**

4. Coincidental matching between base pairs takes place with a frequency of 50%, because only two types of base pairs exist. Noise is filtered out (3, 6) in Fig. 1A by widening the size of the sliding window to 350 bp in one genome and 700 bp in the other, and by requiring FASTA percent identity to be larger than $940/N_{\text{bp}} + 59$, where $N_{\text{bp}}$ is the number of base pairs in the match. This threshold is chosen because it excludes more than 99% of coincident matches found between random sequences when their G-C contents are between 25% and 75%.
Intra-replichore rearrangements.

Intramolecular segment shuffling. Solid arrows represent inter-replichore rearrangements; shaded arrows represent intra-replichore rearrangements.

**Response:** Makino and Suzuki correctly note that the major rearrangement that has occurred between the genomes of two closely related species, *P. abyssi* and *P. horikoshii*, is an inversion of a large fragment carrying the single replication origin of these chromosomes. Furthermore, because this origin is located roughly at the midpoint of the inverted fragment and because DNA replication is bidirectional in *Pyrococcus* (1), they have concluded that these chromosomal rearrangements have occurred at two physically linked replication forks moving in the opposite directions. Such a model implies the existence of a single replication factory coupling two replication forks in archaea, as in bacteria (2). Tillier and Collins (3), relying on pairwise comparison of two *Heliocobacter* strains as well as two *Chlamydiae* and *My- cokbacterium* species, have likewise proposed a major role for replication-directed translocations in genome evolution, leading to local and genome-wide rearrangements.

Makino and Suzuki further propose that the observed rearrangements have been caused by DNA topoisomerases that are associated with the two replication forks. This proposal is consistent with our earlier finding of a type II DNA topoisomerase in both archaea and bacteria. Interestingly, the eukaryotic homolog of the archaeal Topo II is the endonuclease Spo11, which triggers meiotic recombination by making double-strand breaks in the chromosome (4). Because Spo11-induced recombination appears to function also in DNA replication (5), these observations together suggest an evolutionary link between prokaryotic genome rearrangements and eukaryotic crossing-over.

Finally, Makino and Suzuki suggest that a third *Pyrococcus* species, presumably *P. furiosus*, has differentiated from the two others by similar rearrangements. Whereas all major rearrangements that have occurred in *P. abyssi* and *P. horikoshii* (solid arrows in Fig. 1) are indeed symmetrical to the replication axis, however, we found that rearrangements that have taken place between *P. furiosus* and the other two *Pyrococcus* species (shaded arrows in Fig. 1) have relocated within the same replicohore—the portion of the chromosome separated by the origin and the terminus of replication. These rearrangements correspond to four inversions and four transpositions (Fig. 1). Therefore, at least two different types of rearrangements are driving plasticity of *Pyrococcus* genomes, one occurring between the two replicohores and symmetrical to the replication axis, and another one decoupled from chromosome replication. The confinement of this second type of rearrangement to one replicohore could correspond to some physical or topological barrier that separates the chromosome into two distinct domains. Alternatively, such rearrangements may only operate between elements separated by short distances, which thereby reduces the probability that they will occur between two replicohores. Future comparisons of closely related genomes should help to determine which explanation is correct.

The two types of genomic rearrangements detected among the three *Pyrococcus* genomes also probably correspond to different mechanisms of recombination. Whereas the first one can indeed be related to specific mechanisms operating at the replication forks, the second one could be due to transposition events. Indeed, the genome of *P. furiosus* contains 23 genes encoding a putative transposase that have no homologs in *P. abyssi* and *P. horikoshii* (6).

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**References**

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**Fig. 1.** Two different types of genomic rearrangements in *Pyrococcus*. OriC, the replication origin, and the symmetrically located putative terC terminus are connected by a dashed line that divides the chromosome in two replicohores. Outer-circle arrows (A, C, E, F) show *P. abyssi* versus *P. horikoshii* segment shuffling; inner-circle arrows (a, d, g) depict *P. furiosus* versus *P. horikoshii* and *P. abyssi* segment shuffling. Solid arrows represent inter-replichore rearrangements; shaded arrows represent intra-replichore rearrangements.