to *B*, which grows quickly on the selective plates. (For simplicity, assume that the rate of mutation from *A* to *B* is negligible.) We have investigated the properties of this two-step model, which we will present in more detail in a forthcoming paper. Under this model, many colonies are detected only after delays that appear inconsistent with the rate of growth of genotype *B* on the selective medium. Moreover, expected distributions of colonies obtained from sister cultures are similar to those presented by Cairns *et al.*[1]. Thus, the two-step model accounts for both types of observation used to support directed mutation.

Although two-step mutations are easily overlooked, they have been demonstrated previously. For example, it has been known for some time that it is quite difficult to isolate mutants of *Escherichia coli* B that are resistant to phage T2 (refs 7, 8). Using the standard protocol of plating for resistant mutants in the presence of excess phage, one of us[9] calculated the mutation rate to be of the order of 10^{-10} per cell generation. When bacteria and phage were put into continuous culture, T2-resistant bacteria appeared much sooner than could be anticipated from this rate. This occurred because mutants that were partially resistant to the virus increased in frequency, and these partially resistant intermediates mutated to the completely resistant genotype at a rate about 100-fold higher than did the original sensitive genotype[9]. What appeared superficially to be an improbable event was instead a sequence of two unremarkable events.

Shapiro[10], whose study of gene fusions generated by Mu excisions suggests directed mutation, observed that after cells were plated on selective medium, there eventually appeared ". . . numerous papillae on the background lawn. (Picking and testing of these papillae showed that some contained cells capable of full growth on minimal arabinose–lactose agar and some did not.)" Growth of cells other than those with the fully expressed mutant phenotype clearly indicates the potential for selective enrichment of an intermediate genotype and a secondary mutation. That the appearance of colonies was accelerated by exposure to arabinose[1] suggests to us that arabinose provides a substrate suitable for growth of some intermediate. Why might two mutations occur when apparently one suffices? It is known that excisions of Mu are usually imprecise, so that two steps are often required to reactivate fully a gene inactivated by an insertion[11].

If enrichment of an intermediate genotype is demonstrated, it could still be argued (as Hall[12] has done) that it occurs not by selection, but rather by directed mutation from *A* to *A'* on the selective medium. Hall studied an adaptation that required two mutations — excision of an insertion sequence from a structural gene, which preceded a second mutation in a regulatory gene. Hall could detect no growth of the intermediate genotype on the selective medium, which is consistent with its directed mutation. However, Hall's Table 2 shows that the frequency of excision mutants varied greatly among sister populations on the selective medium — after 12 days, for example, from less than about 1% to about 90% among six sister populations. This is not consistent with the random distribution expected under the hypothesis of directed mutation, unless one also invokes *ad hoc* variation among sister populations.

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● See the News and Views article by Neville Symonds on page 119 of this issue.

CO_2 from magma-chamber degassing

SIR—The high CO_2 concentration reported by Karl *et al.*[1] for hydrothermal vent fluids from Loihi Seamount underscores the need to place more emphasis on the role of magma-chamber degassing as a source of the CO_2 discharged at submarine hydrothermal vents. This source of CO_2 may be as important as, and possibly more important than, the customarily accepted source mechanism of hydrothermal stripping from sea-floor lavas and rocks of the oceanic crust. Although Karl *et al.* do not discuss the problem of source posed by the high CO_2 concentrations at Loihi, Cann and Strens do stress this problem in their News and Views article[2]. One suggestion by Cann and Strens that comes close to the magma-chamber-degassing source for the origin of the high CO_2 is volcanic degassing, that is, degassing of magmas as they ascend and erupt onto the sea floor.

Studies of the Kilaua volcano, the subaerial volcano 50 km north-north-east of Loihi Seamount and the next oldest product of the Hawaiian hotspot, have shown that CO_2 degassing occurs continuously from its summit magma chamber[3,4]. The CO_2 is derived from the basalt supplied to the summit chamber, and volatile budget models[3,4] require that 90–97% of the CO_2 initially present in the parental magmas is lost in this way. Consequently, eruptions such as the current east rift zone eruption, which involve magma that has degassed previously in the summit chamber, produce volcanic gases that are greatly depleted in CO_2 (typically less than 5 mol %). Currently, summit magma-chamber degassing of CO_2 at Kilauea is about 30 times more effective than volcanic degassing of CO_2, even though an eruption is in progress. It would not be suprising if magma-chamber degassing similarly dominates volcanic degassing, as well as hydrothermal stripping, of CO_2 at Loihi.

Magma-chamber degassing of CO_2 may also be an important source of the CO_2 discharged from the hydrothermal vents at mid-ocean ridges. The magmas supplied to ridges are probably poorer in CO_2 than those supplied to Loihi, but the extremely low solubility of CO_2 in mid-ocean-ridge basalt (MORB)[5] and the ubiquitous presence of CO_2-rich vesicles in MORB lavas make magma-chamber degassing of CO_2 a plausible hypothesis for these sites as well. Although it may be less intensive at mid-ocean ridges than at hotspot volcanic centres such as Kilauea and Loihi, it could nevertheless be significant on a global scale, as magmatism at mid-ocean ridges comprises 75% of the Earth's current magma supply and has probably been the largest single contributor of magma to the crust for more than 2×10^9 years. Thus, magma-chamber degassing of CO_2 from MORB may be important in the transfer of carbon from the mantle to the surface reservoirs of the carbon geochemical cycle. CO_2 degassing from sub-ridge magma chambers may also provide a carrier gas for transferring rare gases and other mantle-derived volatiles to the ocean–atmosphere system and for fractionating carbon isotopes in the magma supplied to mid-ocean ridges. The $\delta^{13}C$ values of sea-floor MORB lavas that have experienced magma-chamber degassing would be low relative to those of their parental magmas because of the large positive fractionation factor for $^{13}C/^{12}C$ between CO_2 fluid and carbon disolved in basaltic liquid[6].

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