observation has been made (*18*) for the LF phytochrome responses of *cab* RNA and *rbcS* RNA in *Lemna*.

There are approximately 6 to 12 copies each of *cab* and *rbcS* in *P. sativum* var. Alaska (*19*, *20*). In petunia, these gene families comprise several subfamilies distinguishable by the sequence immediately 3′ to the coding region (*21*). If such subfamilies exist in pea, it is possible that different light responses or responses in different tissues (or both) are the result of transcription by different copies of the gene. Hence, for the *cab* genes one copy or subfamily may be responsible for the VLF accumulation while another may be responsible for the LF accumulation. The cDNA clones used in our study could not differentiate among transcripts originating from the different copies of the genes (*20*). Studies of the individual genes must await the isolation and characterization of clones capable of discriminating among the different copies.

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Hemoglobin I Mutation Encoded at Both α-Globin Loci on the Same Chromosome: Concerted Evolution in the Human Genome

Abstract. *Genetic analysis of an individual expressing an unexpectedly high level of hemoglobin I, an α-globin structural mutant, reveals that the mutation is present at both the α1- and the α2-globin gene loci. Kindred analysis confirms that the two affected genes are located in cis. The most likely explanation for this finding is that a recent conversion event occurred within the human α-globin gene cluster.*

The structures of the two adjacent α-globin genes within individual mammalian species are highly homologous despite the significant divergence of α-globin gene structure among the mammalian species during 400 million years of evolution (*1–9*). This parallel development of gene structure during evolution, referred to most commonly as concerted evolution (*5*), appears to be a general pattern shared by a number of linked (*4–7*, *10*) as well as unlinked gene families (*11*). The gene conversion events that result in concerted evolution occur at a high frequency within the α-globin clusters of primates (*5–9*). Comparison of the human and chimpanzee α-globin gene sequences suggests that at least one gene conversion event has occurred in the two human α-globin genes since the divergence of humans and chimpanzees 4 million years ago (*9*). We report here a more recent gene conversion in the human α-globin gene cluster. This conversion, unlike the one previously described, results in a parallel change in the structure of the α-globin protein encoded at both α-globin loci. We have identified an individual whose two adjacent α-globin genes, α1 and α2, encode the same α-globin protein mutation—the lysine at position 16 of the amino acid sequence is replaced with glutamine (α^{16Glu}). Delineation of this genotype supports the concept of concerted evolution in the human genome and clarifies the genetic basis of the α^{16Glu} structural hemoglobinopathy.

The two human α-globin genes, α1 and α2, are expressed equally at the protein level. This conclusion is based on studies of individuals heterozygous for α-globin structural mutations. In such individuals, one of the four α-globin genes encodes the mutant, and this structural variant is usually expressed at 25 percent of total α (*12*). If the function of one, two, or three of the normal α-globin genes in such individuals is lost because of α-thalassemia (*13*), the amount of the variant can approach 33, 50, or 100 percent of total α-globin, respectively (*14–16*). We now describe an individual in whom the level of the α-globin mutant α^{16Glu} cannot be explained by interaction of α-thalassemia with a single mutant locus. The concentration of hemoglobin I (HbI) ($\alpha_2{}^{16Glu}\beta_2$) in this otherwise normal black woman constitutes 65 percent of total hemoglobin (*17*, *18*). Southern blot analysis (*19*) (data not shown) revealed that her DNA contains three α-globin genes. One chromosome contains the normally arranged α1- and α2-globin genes (*4*, *20–22*). The homologous chromosome contains only a single α-globin gene, as a result of a rightward type α-thal-2 deletion (*4*, *23*, *24*). Her genotype is thus αα/−α. This woman's daughter, who has mild α-thalassemia (hemoglobin, 11.1; mean corpuscular volume, 74.2), is homozygous for the α-thal-2 chromosome (−α/−α). Since she has no HbI and has inherited her mother's α-thal-2 chromosome, the α gene on the α-thal-2 chromosome of the mother must encode normal α-globin. Therefore, the α^{16Glu} mutation must reside on the mother's chromosome that contains two α genes.

The high (65 percent) level of globin variant expression in this case could arise by several mechanisms. If there is only one α^{16Glu} locus in the mother, then a mutation that increases some combination of α^{16Glu} transcriptional output, translational efficiency, and messenger RNA (mRNA) stability could account for her 65 percent level of variant. Alternatively, if all three genes are expressed equally at the protein level, the 65 percent HbI would suggest the presence of two α^{16Glu} genes. Although generation of such an arrangement by two identical point mutations is extremely unlikely, the extensive regions of homology surrounding the α genes (*4*, *8*, *25*) could lead to a gene conversion event (*5*, *7*, *8*, *25*) that could produce two adjacent α^{16Glu} genes.

We used the technique of hybrid-selected translation (*26–28*) to identify the locus of the α^{16Glu} mutation on the structurally intact chromosome. The α1- and α2-globin mRNA's were separately isolated by hybridizing total reticulocyte RNA to nitrocellulose paper containing plasmid DNA specific for α1- or α2-globin mRNA. Since the structural divergence between these two mRNA's is limited to the 3′ nontranslated region (*7*, *29*), we used recombinant complementary DNA (cDNA) plasmids containing

only these regions of the mRNA (pH3α1B and pH3α2A, respectively) (*30*) to select α1- or α2-globin mRNA. Total α- and β-globin mRNA were also selected by hybridizing reticulocyte RNA to nitrocellulose paper containing full-length α-specific (pMC18) (*9*) and β-specific (pSAR6) (*31*) recombinant plasmids. The relative concentration of α1- and α2-globin mRNA's in each of the hybrid-selected samples was determined by S_1 nuclease analysis with ^{32}P-end-labeled probes. These probes are protected to different degrees by α1- and α2-globin mRNA's (*32*) (Fig. 1). The S_1 nuclease analysis of the α1 and α2 samples demonstrates that both are relatively pure. The hybrid-selected mRNA in the α1 sample contains only α1 mRNA, whereas the α2 sample is highly enriched in α2 mRNA (Fig. 1, lanes 1 to 4). The hybrid-selected total α-globin mRNA contains approximately equal amounts of α1 and α2 mRNA (Fig. 1, lanes 5 and 6). This ratio, which is identical to that measured in a large number of individuals with the αα/−α genotype (*33*), decreases the likelihood that a single α^{16Glu} gene is overexpressed.

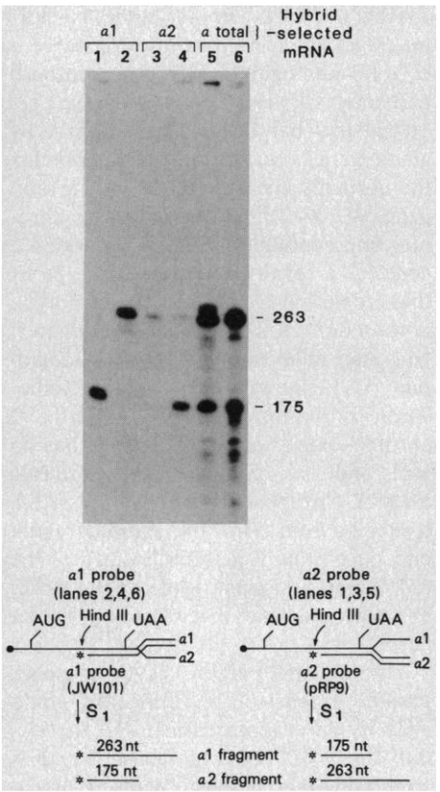

Fig. 1. S_1 mapping of hybrid-selected α1- and α2-globin mRNA. (Lanes 1 and 2) α1-, (lanes 3 and 4) α2-, and (lanes 5 and 6) total α-globin samples were analyzed for α1 and α2 mRNA content by a modification of the S_1 mapping analysis (*32*). Single-strand α1 and α2 probes were isolated from plasmid JW101 (*35*) and pRP9 (*36*) as described (*32*) end-labeled with ^{32}P at the Hind III site (codon 90 of an α-globin mRNA) and hybridized to reticulocyte RNA at an estimated tenfold molar excess relative to α-globin mRNA. Hybridization and S_1 digestion were performed as described (*30*), and S_1 nuclease–resistant fragments were analyzed on a 5 percent acrylamide, 8*M* urea gel. A 263-nucleotide fragment was generated when the probes hybridized to their homologous mRNA's (that is, the α1 probe to α1 mRNA), and a 175-nucleotide fragment was generated when the probe hybridized to the nonhomologous mRNA (that is, α1 to α2). This shorter fragment results from the sequence divergence between the two α-globin RNA's, which begins 175 nucleotides 3′ from the Hind III site (*7, 8*). This assay is shown schematically below the gel. End-labeled probes isolated from both α1- and α2-globin cDNA's are used for mutual confirmation of results. The sizes of the fragments shown were determined from a sequence ladder of the end-labeled probe run in a parallel lane (not shown). Positions of the initiation codon (AUG), termination codon (UAA), and the mRNA cap site (●) are shown.

In order to define the locus encoding the mutant protein, each hybrid-selected sample was translated in vitro in the presence of [^{35}S]methionine, and the labeled proteins were separated on a Triton-urea acrylamide gel (Fig. 2A). Translation of unfractionated reticulocyte RNA or hybrid-selected total α-globin mRNA produces α^{16Glu} in the expected excess relative to normal α (*17*). The purified α2-globin mRNA encodes only α^{16Glu}, whereas the α1-globin mRNA sample encodes both α and α^{16Glu}. Since the α-globin encoded on the chromosome containing the single α-globin gene is known from family studies to be normal, the α1- and α2-globin genes on the intact chromosome must both contain the α^{16Glu} mutation. Therefore, as schematicized in Fig. 2B, the genotype of the proband is $\alpha^{16Glu}\alpha^{16Glu}/-\alpha$.

Structural analysis of the two α-globin genes shows large regions of homology (*4, 7, 8*). This homology appears to result from correction events between the two genes. Boundaries have been proposed for the gene conversions in the human α-globin clusters (*4, 6, 7, 25*). Although the exact boundaries of the α^{16Glu} correction cannot be determined from our data, the 3′ border must be on the 5′ side of the termination codon since the divergent nature of the 3′ nontranslated region of the α1 and α2 mRNA's is maintained. The 5′ border of a recombination product will be difficult to define with accuracy because of the high degree of sequence homology of the α1 and α2 genes in this region (*8, 25*). Sequence analysis of both the α1 and α2 genes that encode α^{16Glu} will therefore be necessary to determine whether the boundaries of the α^{16Glu} conversion coincide with segmental conversion units previously defined for the α-globin gene.

The presence of the same structural mutation at both adjacent α-globin loci is a novel arrangement. Although individuals doubly heterozygous for two α-chain structural mutations have been described (*34*), this is, to our knowledge, the first reported instance of the same mutation occurring at both α-globin loci in cis. The presence of the α^{16Glu} mutation in two of three genes explains the high level (65 percent) of HbI.

The α^{16Glu} mutation we have described was assigned to both the α1- and α2-globin loci by the method of in vitro translation of hybrid-selected α1- and

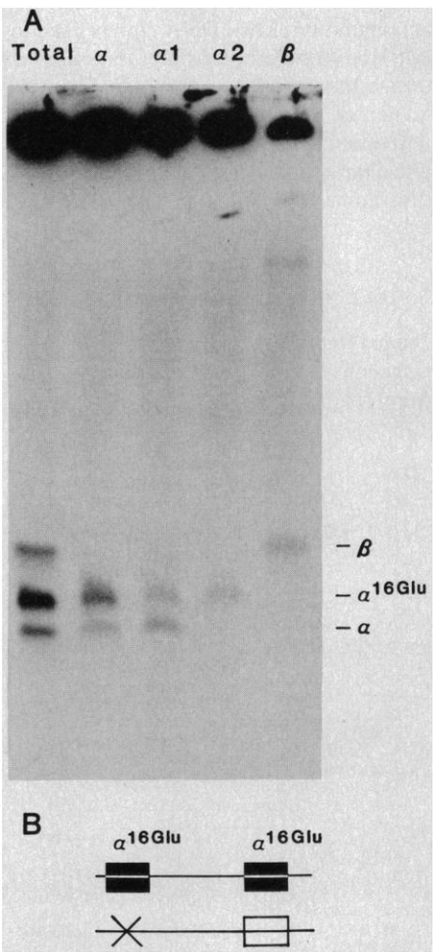

Fig. 2. Identification of the α-globin locus encoding the α^{16Glu} mutation by in vitro translation of the hybrid-selected mRNA's. RNA's from the indicated sources were translated in a micrococcal nuclease–treated rabbit reticulocyte translation system prepared from phenylhydrazine–treated New Zealand white rabbits (*37*). (A) [^{35}S]Methionine-labeled translation products were separated on a 12 percent acrylamide, Triton-urea gel (*38, 39*) and autoradiographed. Translation products of reticulocyte RNA (total), hybrid-selected α, α1, α2, and β mRNA's are shown, with positions of β-, α^{16Glu}-, and α-globin indicated. (B) The α-globin genotype of the proband as determined from the above data demonstrates the α^{16Glu} mutation at both α-globin genes in cis. The position of the α2 and α1 genes are 5′ and 3′, respectively.

α2-globin mRNA. Before the development of this method of analysis, direct sequence analysis of cloned α1- and α2-globin genes or their cDNA's would have been required. The hybrid-selection approach should allow a more rapid analysis of other structural mutations and may result in additional examples of sequence conversion between the human α-globin genes or between duplicated loci in other gene clusters. Such information may further define the frequency of other DNA recombination events in the human genome.

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Larval Development and Dispersal at Deep-Sea Hydrothermal Vents

Abstract. *Deep-sea hydrothermal vent communities exhibit an array of reproductive strategies. Although a few vent species undergo planktotrophic, high-dispersal modes of development, most exhibit relatively low dispersal, but probably free-swimming nonplanktotrophic development. This predominance of nonplanktotrophy may be largely a reflection of phylogenetic constraints on the vent colonizing taxa; intervent dispersal among these forms may be facilitated by reduced developmental rates in the cold abyssal waters away from the vents. It is proposed that for those vent species with nonplanktotrophic development, larval dispersal is a stepwise process with oceanic ridge axes serving as discrete dispersal corridors.*

The biological communities associated with deep-sea hydrothermal vents have been the subject of intense study since their discovery in May 1976 (*1*). Active vent systems accompanied by dense benthic assemblages are now known at a number of widely separated sites along midocean rift zones in the eastern Pacific, extending from 48°N along the Juan de Fuca Ridge to 22°S along the East Pacific Rise. One basic question concerning the ecology and evolution of the vent biota is how the species and associations are established and maintained in a habitat that is markedly patchy in time and space. Most of the species are sedentary, and it has been suggested (*2, 3*) that free-swimming larval stages are the principal agents of recruitment and gene flow among hydrothermal sites (*4*).

We surveyed the modes of larval development in 18 molluscan species collected from two vent sites, the Galápagos spreading center near the equator and the East Pacific Rise at 21°N. Mollusks are the only vent taxa that lend themselves to such a survey because their early ontogenetic history is recorded in the shell of juveniles and well-preserved adults (*5, 6*). Although a few vent species undergo planktotrophic, high-dispersal modes of development, most of these mollusks exhibit relatively low dispersal, but probably free-swimming nonplanktotrophic development. The sparse data accumulated to date on the reproduction of other vent taxa [for example, decapod crustaceans (*6*) and ampharetid polychaetes (*7, 8*)] suggest a similar diversity of larval development modes in deep-sea hydrothermal systems. Our results indicate that not all vent species require a high-dispersal larval stage to persist in these ephemeral environments and suggest that the reproductive strategies in the hydrothermal vent community are more complex than previously believed.

Hundreds of minute mollusks with at least portions of the larval shell intact were isolated from the washings of biological materials collected by the deep-sea research vessel *Alvin* from the Galápagos and 21°N sites (*9*). The specimens were immediately fixed in 10 percent buffered seawater formalin for 48 hours and subsequently preserved in 80 to 95 percent ethanol. Cleaned shells were mounted on copper tape, coated (under vacuum) with approximately 400 Å of gold-palladium or a combination of gold and carbon, and examined under an ETEC Autoscan or AMR 1000 scanning electron microscope.

The diversity of larval shell (protoconch) morphologies in the vent gastropods is illustrated by the representative species in Fig. 1. Modes of larval development can be inferred on the basis of protoconch size and form (*5, 10*); although there may be pitfalls to this technique, such a comparative approach has