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Genetic Control of Hemoglobin Synthesis

Thalassemia and related disorders may be explained by known properties of regions of genetic duplication.

Walter E. Nance

The widespread application of modern techniques of protein chemistry has led to the recognition of a growing number of genetically determined conditions in which there is either a quantitative or a qualitative alteration in hemoglobin synthesis, or both. In this article I indicate the limitations of current concepts of the genetic control of human hemoglobin synthesis and also describe a new hypothesis which resolves many previous difficulties. The suggested mechanisms are based on known properties of the hemoglobin loci; they account for many known genetic abnormalities as well as for certain apparently adventitious phenomena relating to hemoglobin synthesis, and they may be of general significance to other important problems in human genetics.

Normal Hemoglobins and Abnormal Conditions

Hemoglobin A, the major component of adult hemoglobin, is composed of two α and two β polypeptide chains. In hemoglobin A_2 and fetal hemoglobin, respectively, the β chains are replaced by δ or γ chains, yielding $\alpha_2\delta_2$ and $\alpha_2\gamma_2$. Hemoglobin A_2 is a minor component which normally accounts for less than 3 percent of the total adult hemoglobin. Fetal hemoglobin is normally present in significant amounts only during prenatal and neonatal life. Although adult hemoglobin has been detected as early as the 9th week of fetal life (*1*), fetal hemoglobin usually remains the major component throughout gestation. The exact proportion of fetal hemoglobin at birth is correlated with gestational age (*2*) and may vary from 50 to 90 percent. The normal adult level of 0 to 1 percent is usually attained by the end of the first year, although occasionally levels of fetal hemoglobin of 2 to 3 percent may persist throughout the first decade of life (*3*). Studies of amino acid sequences (*4*) have revealed a remarkable degree of structural homology among the four types of hemoglobin polypeptides; this is particularly striking in the case of the β and δ chains. These two chains are thought to differ by possibly fewer than 12 substitutions out of a total of nearly 150 amino acid residues. The β chain differs from the γ chain, on the other hand, by more than 30 amino acid substitutions, and from the α chain by more than 70 substitutions.

Genetically determined qualitative variants of each of the four human hemoglobin chains have now been recognized. Thus, approximately a dozen each of β- and α-chain mutations are known, and, in spite of the technical difficulties involved in their detection, at least two γ- and two δ-chain mutants have also been described. All of these mutant types are comparatively rare, with the exception of the β-chain mutants S, C, and E and, possibly, the δ-chain mutant B_2 (*5*). In addition, conditions are known in which there is either a quantitative change in the types of hemoglobin that are found or both a qualitative and a quantitative change. Thalassemia is the most important condition of this type. In the syndrome of persistent fetal hemoglobin, although the adult hemoglobin of affected individuals may be partially or totally replaced by fetal hemoglobin, there may be no significant clinical abnormalities (*6*). Similarly, pedigrees have been described in which a twofold to threefold elevation of hemoglobin A_2 has been transmitted as a genetic trait without other abnormalities (*7*). More recently a case has been described in which there was no demonstrable A_2 fraction (*8*). Hemoglobins "Bart's" and H are two variants which result not from point mutations but from the abnormal association of γ and β chains to form γ_4 and β_4 tetramers, respectively (*5*). These two conditions frequently occur in association with thalassemia. A final group of disorders that is of particular significance is the "Lepore trait" syndrome (*9*). In this syndrome a qualitatively abnormal hemoglobin is invariably associated with thalassemia, a disease we will now consider in some detail.

Thalassemia

Classic thalassemia is a hereditary, microcytic, hypochromic anemia which is frequently lethal during childhood in homozygotes but is of extremely variable severity in heterozygotes. Once thought to be limited to populations of Mediterranean origin, where the frequency of heterozygotes may exceed 20 percent in some areas, variants of the syndrome are now known to be prevalent in many parts of the world.

The author is a Markle scholar in academic medicine and a postdoctoral fellow in the department of medical genetics of the University of Wisconsin Medical School, Madison. He is on leave of absence from the department of medicine at Vanderbilt University Medical School, Nashville, Tenn.

Clinical and hematologic observations have made it clear that the thalassemic homozygote is unable to maintain an adequate supply of functional erythrocytes, despite an intense stimulation of erythropoiesis resulting in marked bone-marrow hyperplasia. The red blood cells that are formed have an abnormally short life span, which is in part a consequence of their rapid destruction in the spleen. The anemia results, therefore, from both inadequate production and excessive destruction of the erythrocytes. These conclusions have been confirmed and extended in studies with radioactive iron or glycine —studies which have consistently indicated that, although the incorporation and turnover of hemoglobin precursors are markedly elevated in thalassemia, the effective utilization of these precursors for the production of functional erythrocytes is, in comparison, quite low.

It has long been recognized that thalassemia may be associated with a marked elevation of fetal hemoglobin in the homozygote and, less consistently, with minor elevations of fetal hemoglobin in the heterozygote. More recently it has been observed that the level of hemoglobin A_2 is frequently, but not invariably, elevated in individuals with the thalassemia trait (*7, 10*). Although once thought to be virtually pathognomonic for the thalassemia trait, a high level of the A_2 fraction, as noted earlier, has been observed in families without any other hematologic signs of thalassemia.

Important observations relating to thalassemia have been made in persons who were presumably heterozygous both for thalassemia and for a gene determining an abnormal hemoglobin, such as hemoglobin S (sickle). These individuals fall into two classes: those who have marked elevation of hemoglobin S and fetal hemoglobin, with no detectable hemoglobin A, and those in whom fetal hemoglobin, hemoglobin S, and normal adult hemoglobin occur in variable proportions. In the first type, called the "interacting type" (*11*), it seems clear that the production of normal β chains has been completely "suppressed" by a genetic event which is operationally indistinguishable from a deletion. In the second or "noninteracting type," the suppression is incomplete and both genes are active.

As indicated previously, hemoglobins H and Bart's are frequently associated with thalassemia. Furthermore,

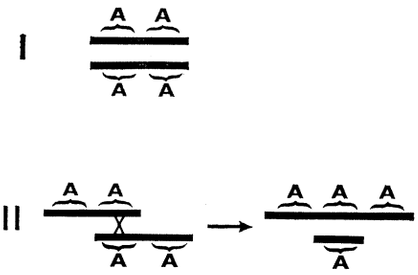

Fig. 1. Synapsis between homologous chromosomes each containing a region of duplication (A) of the order 2 may be either equal (I) or unequal (II). Each "synaptype" involves the apposition of homologous regions of DNA, so there should be no impediment to crossing over within synapsed homologous regions. In the case of synaptype II, crossing over leads to the generation of higher and lower orders of duplication. However, since the total length of homologous synapsis is twice as great with synaptype I, the total frequency of crossing over within synaptype II would be half the total frequency within synaptype I. Similar considerations apply to higher orders of duplication.

both of these hemoglobins may be present in the same individual (*12*). The type of thalassemia in these families is usually the "normal A_2" type, but examples of the "high A_2" type have been observed in association with both hemoglobins Bart's and H (*13*). More than a dozen pedigrees have been described in which an electrophoretically abnormal hemoglobin (the Lepore trait) was segregating in association with thalassemia (*14*). In heterozygotes, about 20 percent of the hemoglobin was of the abnormal type. In homozygotes there was no detectable hemoglobin A, and in some, no detectable hemoglobin A_2, either. Recent biochemical studies suggest that the abnormal Lepore polypeptide has an amino acid sequence which begins like that of the δ chain and ends like that of the β chain (*15*). Thalassemia has also been reported in association with other rare hemoglobin variants which were presumed to be point mutants. It is possible that some of these cases actually belong in the Lepore trait category.

Previous Hypotheses

Bannerman (*7*) has suggested that the primary defect in thalassemia may be a block in heme synthesis. However, as we have seen, in interacting sickle-cell–thalassemia there is good evidence of an interference in globin synthesis in that the activity of one of the β loci is completely suppressed. It is difficult to conceive of a mechanism by which allele-specific suppression such as this could arise as a secondary effect of a block in heme synthesis.

Ingram and Stretton (*16*) have suggested two alternative hypotheses to account for known genetic abnormalities of hemoglobin synthesis. In their first hypothesis they proposed that the thalassemia syndromes are the consequence of "hidden" point mutations of the structural hemoglobin genes. They assumed that these mutant genes act with reduced efficiency to produce a polypeptide chain that is indistinguishable from the normal chain by electrophoretic screening procedures. This hypothesis requires that the amino acid sequence of at least one of the hemoglobin polypeptide chains of *every* thalassemic homozygote be abnormal. A sufficient number of hemoglobin samples from such individuals have been examined by appropriate techniques and found normal, requiring a virtual abandonment of this hypothesis (*17*).

Ingram and Stretton, in their second hypothesis, proposed that thalassemia resulted from a mutation of a "tap" gene, which controlled the rate of action of the structural hemoglobin genes. This idea was subsequently elaborated by Neel, Motulsky, and others (*18, 19*) who used terminology and reasoning based to a large extent on the operator-gene model of Jacob and Monod (*20*). According to this model, as it has been applied to the hemoglobin locus, the switch from fetal to adult hemoglobin at birth is determined by an operator gene, closely linked to the structural genes which it controls. This operator gene in effect turns off the γ-chain locus and turns on the closely linked β- and δ-chain loci. Beta-chain thalassemia is thought to result from an operator-gene mutation in which there is a partial failure of the activation of the β locus. Whereas the normal operator gene controls both the δ and the β loci uniformly, this mutation is assumed to affect only the β locus, and δ-chain production may actually be increased in a compensatory manner. The syndrome of persistent fetal hemoglobin is viewed as a *different* operator-gene mutation, in which either the γ locus is not properly suppressed or the β and δ loci are not activated at all, or in which

both of these failures occur. Presumably, the high A_2 syndrome and the absence of A_2 might be examples of additional types of operator-gene mutation, in which only the δ locus is affected. Hemoglobin H, hemoglobin Bart's, noninteracting sickle-cell–thalassemia, and thalassemia with normal hemoglobin A_2 are thought to be caused by another operator-gene mutation, in which there is suppression of α-chain production. Why hemoglobins H and Bart's may occur independently or together is not specified by this theory, and pedigrees in which a high-A_2 variety of thalassemia is associated with these hemoglobins must be regarded as unexplained exceptions. The essential elegance of the operator-gene hypothesis of Jacob and Monod is its economy: the activity of several structural genes is controlled by the interaction of exogenous effector substances with the product of a regulator gene and a single operator locus. In attempting to interpret genetic events at the human-hemoglobin locus in terms of an operator-gene hypothesis, one is forced, at the very least, to postulate an operator gene for every structural-gene locus. The mechanism of integrated control would then remain a mystery unless one postulated a higher order of operator genes. Such complexities seem hardly justified by the evidence, although it must be admitted that in man the opportunity for excluding such a system by observation of critical matings is remote. Be this as it may, there is at least one type of thalassemia for which no number or combination of operator genes can account; this is the Lepore trait, in which qualitative hemoglobin abnormalities are uniquely associated with typical thalassemia.

An Alternative Hypothesis: The General Model

Two of the most striking biochemical and genetic facts that have recently been established about human hemoglobin are the linkage relationships of the four hemoglobin loci and the chemical homology of their products, as stated earlier. Neel has recently given a careful summary of the available data relating to linkage (*18*). There is considerable evidence that the β and δ loci are closely linked, but less information is available concerning the presumed linkage of the γ locus to the $\beta\delta$ complex. The α locus, on the other hand, is sufficiently far removed from the $\beta\gamma\delta$–complex locus—perhaps even on another chromosome—to show independent segregation. These facts led Ingram (*21*) to propose that the α and γ genes evolved by duplication from a primitive myoglobin gene and were subsequently separated, possibly by inversion or translocation. The γ gene gave rise, by further duplication, to the β gene, which at a later time reduplicated, to generate the δ locus. Thus, the origin of these loci accounts for their residual homology, and subsequent differentiation accounts for their present differences.

Smithies, Connell, and Dixon (*22*) have recently shown that serum haptoglobin, another polymorphic human protein, may also be determined by an allele which arose by a process of duplication, in this case involving only part of a gene. Reasoning from known properties of regions of duplication in other genetic systems, they were able to predict successfully the existence of genetic triplication at the haptoglobin locus, as well as certain other allelic variants (*23*), and to outline some of the general consequences that might be expected in any region of duplication. Since the hemoglobin locus is known to involve duplication, it seems reasonable to attempt to explain the peculiarities of the locus in terms of genetic properties of regions of duplication. Once a duplication occurs and becomes established in a population by reason of its selective advantage, the opportunity arises, as discussed by Smithies *et al.*, for the generation of higher orders of duplication (that is, triplication, quadruplication, and so on) by means of displaced synapsis, with unequal but homologous crossing over. These relationships are illustrated in Fig. 1. Crossing over during displaced synapsis may occur with appreciable frequency—once per thousand meiotic divisions at the Bar locus in *Drosophila*, for example. In the absence of any selective forces whatsoever, this process would lead to the production and accumulation of higher and higher orders of duplication at an accelerating pace. A system of this kind would not lead to a stable polymorphism. It is highly improbable, however, that all orders of duplication would have the same selective advantage. It would seem more reasonable to assume that there is an order of duplication which has optimum fitness and that higher or lower orders have a lesser fitness which is a function of the degree to which they depart from the optimum order. Such a system will reach equilibrium when the total number of chromosomes produced each generation and containing duplications of higher or lower order than optimum exactly equals the number lost by reason of their reduced fitness. It is apparent that the mean fitness of such a population at equilibrium would be less than that of a theoretical population in which all individuals had the optimum order of duplication. Using the terminology of Morton, Crow, and Muller (*24*), we may identify this abstract decrease in mean population fitness as the duplication load, a previously unrecognized and possibly important component of the total genetic load. The greater the optimum order of duplication is, the greater the opportunity for deviation from the optimum order, and consequently the greater the duplication load will be.

It is apparent that any genetic event which reduces the frequency of unequal crossing over in a region of duplication will stabilize the region, and that it could therefore have a selective advantage. Thus, translocation might effectively break up the duplication complex; a genetic inversion might "suppress" crossing over; and any mutation, such as a small deletion, which reduces homology between the duplicated regions could have a positive selective advantage if it is compatible with normal physiologic activity. Conversely, any event which increases the frequency of unequal crossing over in a duplication complex might cause a previously stable locus to become unstable and show "directed mutation." It is of particular significance that heterozygous advantage is not required to maintain a duplication equilibrium. *Any* particular gamete or genotype may have optimum fitness; as long as it contains a duplication of order 2 or more the maintenance of a polymorphic system is assured, regardless of whether selection is assumed to act at the gametic or the zygotic level. The frequency of the alleles at equilibrium will depend upon the instability of the complex and the relative fitness of the alleles in question. The nature of the alleles may be quite variable and to some extent unpredictable. As Smithies and his associates have emphasized, in the case of partial gene duplication, at least, the members of a duplication series

may determine proteins which have strikingly different biochemical properties. Lacking knowledge concerning the genetic fine structure, one might erroneously interpret unequal crossing over within a duplication complex as an example of "directed mutation" at a "highly mutable locus."

Relevance of the Model to Thalassemia

We may now proceed to apply the foregoing model to the hemoglobin locus. We postulate that Ingram's concept of the origin of the hemoglobin genes by duplication is correct, and we further assume that the γ locus is closely linked to the $\beta\delta$ complex. While the latter assumption is not critical to the general validity of the argument, it would, if found to be valid, suggest that the optimum order of duplication at the complex locus is at least 3 and possibly more. If 3 is considered to be the optimum, orders of duplication ranging from 1 to 5 would be generated with appreciable frequency by the following events:

$$\frac{AAA}{\times\ AAA} \rightarrow \frac{AAAA}{+\ AA}$$

$$\frac{AAA}{\times\ AAA} \rightarrow \frac{AAAAA}{+\ A}$$

In the case of the hemoglobin locus, the nature of the crossover products can be predicted by replacing the A's with $\gamma\beta$ and δ. In this way, the alleles listed in Table 1 have been generated by events comparable to those represented by the following example:

$$\frac{\gamma\,\beta\,\delta}{\times\ \gamma\,\beta\,\delta} \rightarrow \underline{\gamma\,\beta(\delta-\beta)\delta} + \underline{\gamma(\beta-\delta)}$$

The symbols $(\delta-\beta)$ and $(\beta-\delta)$ indicate the reciprocal fusion genes (*22*) that are formed by crossing over within the β–δ region during displaced synapsis. A particular order—namely, $\gamma\beta\delta$—has been assumed. Similar results may be obtained if the order $\gamma\delta\beta$ is assumed, but it is interesting to note that the order $\beta\gamma\delta$ gives results that are not so readily interpretable. The characteristics of the fusion genes $(\delta-\beta)$ and $(\beta-\delta)$ would depend upon where the crossover event took place. Thus, as illustrated in Fig. 2, if the β and δ genes differ only at points D, H, J, K, P, and T, crossover within regions I or V would yield two recombinant chromosomes, one of which contains two β or δ loci and the other of which lacks either a β or a δ locus. Crossing over within regions II or IV would give two new recombinant genes each containing a "point mutation." Crossing over at any other site (region III) would lead to genetic regions coding for polypeptides which differ by more than one amino acid substitution from the parental gene products. If the fusion product is a new gene which determines a physiologically active protein, it will manifest itself by the production of a qualitatively altered hemoglobin. If the fusion gene does not determine a physiologically active protein, its presence may be suspected only by its effect on the activity of other genes within the complex. There are at least three ways in which a fusion gene of this kind might disturb normal hemoglobin synthesis: (i) by interference at the chromosomal level with the normal transcription of messenger RNA; (ii) by competition of normal and fusion-gene messenger RNA for ribosomal attachment sites; or (iii) by production of a physiologically inactive protein or smaller polypeptide fragment which participates in the normal feedback control mechanisms of hemoglobin synthesis, whatever they may be. This general concept of gene expression has been developed in an essay by Woolf (*25*) and provides an attractive explanation of the apparent paradox that syndromes involving total gene deletion may, in the heterozygote, be clinically less severe than other syndromes in which an abnormal product causes "feedback" inhibition of normal genes. In Table 1, fusion genes which lead to a physiologically active product, such as $(\beta-\delta)$, are distinguished by an asterisk [for example, $(\beta-\delta)^*$] from those which are detectable only as a consequence of feedback inhibition.

Thus we see that we must distinguish between two types of "thalassemia genes": those which involve deletion of the β locus and others in which a fusion gene is present at the $\beta\gamma\delta$ complex which competitively inhibits expression of the normal β gene. We have also noted that unequal crossing over can result in duplication of one or more of the members of the $\beta\gamma\delta$ complex. Using these alleles and the concept of "compensatory stimulation," we may proceed to suggest possible genotypes (Table 2) for the various syndromes that have been reported, through appropriate combination of the alleles

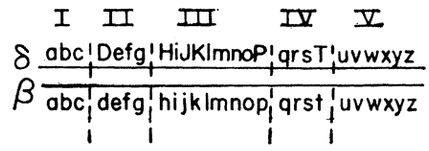

Fig. 2. Possible regions of crossing over within β and δ genes during displaced synapsis (see text).

listed in Table 1. The suggested genotypes are not to be construed dogmatically. It is evident that alternative interpretations and combinations of the given alleles are possible. Furthermore, it would indeed be surprising if the hemoglobin locus is as simple as our model assumes; other alleles undoubtedly occur. What I do propose, however, is that many of the genetic abnormalities of hemoglobin synthesis can be accounted for, and do in fact arise, through some combination of the abnormal alleles which are produced by unequal crossing over at the hemoglobin locus.

Several general features of the proposed genotypes are noteworthy. Syndromes which are clinically most severe, such as classic thalassemia and the Lepore trait, are assumed to include a fusion gene which interferes with the activity of the normal β loci by "competitive" or "feedback" inhibition. Other conditions, which generally are clinically less severe or totally asymptomatic, such as the high-fetal-hemoglobin syndrome and the condition in which hemoglobin A_2 is lacking, are assumed to be the consequence of complete deletion of a locus. Similarly, the disorders I assume to be associated with gene duplication—high levels of hemoglobins A_2, H, and Bart's—are frequent-

Table 1. Alleles resulting from unequal crossing over at the $\gamma\beta\delta$ locus.

Event	Possible products	
	Duplication	Deficiency
$\frac{\gamma\,\beta\,\delta}{\times\ \gamma\,\beta\,\delta}$	$\underline{\gamma\beta\beta\delta}$	$\underline{\gamma\delta}$
	$\underline{\gamma\beta\delta\delta}$	$\underline{\gamma\beta}$
	$\underline{\gamma\beta(\delta-\beta)\delta}$	$\underline{\gamma(\beta-\delta)}$
	$\underline{\gamma\beta(\delta-\beta)^*\delta}$	$\underline{\gamma(\beta-\delta)^*}$
$\frac{\gamma\,\beta\,\delta}{\times\ \gamma\,\beta\,\delta}$	$\underline{\gamma\beta\beta\delta}$	$\underline{\gamma\delta}$
	$\underline{\gamma\gamma\beta\delta}$	$\underline{\beta\delta}$
	$\underline{\gamma(\beta-\gamma)\beta\delta}$	$\underline{(\gamma-\beta)\delta}$
	$\underline{\gamma(\beta-\gamma)^*\beta\delta}$	$\underline{(\gamma-\beta)^*\delta}$
$\frac{\gamma\,\beta\,\delta}{\times\ \gamma\,\beta\,\delta}$	$\underline{\gamma\beta\gamma\beta\delta}$	$\underline{\delta}$
	$\underline{\gamma\beta\delta\beta\delta}$	$\underline{\gamma}$
	$\underline{\gamma\beta(\delta-\gamma)\beta\delta}$	$\underline{(\gamma-\delta)}$
	$\underline{\gamma\beta(\delta-\gamma)^*\beta\delta}$	$\underline{(\gamma-\delta)^*}$

ly not as severe as classic thalassemia. As noted earlier, convincing biochemical evidence has recently been presented that the Lepore trait does in fact arise from a fusion gene of the type postulated. According to the proposed model, thalassemia with compensatory elevation of the A_2 fraction results from a $\underline{\gamma(\gamma-\beta)^*\beta\delta}$ complex. A $\underline{\gamma\beta(\beta-\delta)^*\delta}$ complex, on the other hand, would lead to relative inhibition of the β and δ loci, and thalassemia with elevated fetal but normal or low A_2 hemoglobin would result. Although considerable variation might be expected, depending upon the composition of the fusion gene, it is apparent that this interpretation would imply a reciprocal relationship between the elevations of fetal and of A_2 hemoglobin in thalassemia. A correlation of this kind has in fact been proposed (*26*). Current evidence suggests, however, that there may be at least two different mechanisms involved in the persistence of fetal hemoglobin, either of which could obscure the reciprocal relationship if it does exist. Histochemical studies (*27*) and isotopic labeling experiments (*28*) suggest that in thalassemia fetal hemoglobin is at least partially limited to a discrete clone of cells. In the high-fetal-hemoglobin syndrome, on the other hand, fetal hemoglobin is uniformly distributed among all the cells. These findings are compatible with the idea that, in thalassemia, elevation of fetal hemoglobin may be, at least in part, a consequence of the persistence of a clone of fetal cells, and they emphasize the fundamental difference between the alleles which give rise to thalassemia and those which give rise to the high-fetal-hemoglobin syndrome.

Although I do not deny that duplication at the α-chain locus is a possibility, there is as yet no evidence of it. Consequently, the present hypothesis postulates no duplication, deficiency, or fusion alleles at the α locus. As we have seen, the vagaries of A_2 hemoglobin in the thalassemia syndromes can be reasonably accounted for without postulating a series of α-chain thalassemias, and thus the inconsistencies such postulates entail can be avoided. The alleles and genotypes I have proposed cannot account for all of the conditions described in the literature as thalassemia. At least four entirely unrelated mutations are known (*29*) which may cause striking alterations in red-cell morphology and variable anemia. It seems quite likely, therefore, that other mutations may exist which may mimic some of the subtle morphologic characteristics of thalassemia minor. Although confusion of these disorders with thalassemia will undoubtedly persist until specific diagnostic tests are available, in general one would expect these other disorders to show independent segregation with respect to the $\gamma\beta\delta$ locus, and one would not expect them to be associated with changes in the proportion of fetal or A_2 hemoglobin. I believe that some of the genetically transmissible disorders that have been interpreted as "α-chain thalassemia" (*30*) may fall into this category.

If I am correct in saying that thalassemia and the other conditions I have discussed arise from combinations of the abnormal alleles which are produced by unequal crossing over at the hemoglobin locus, then it is clear that these syndromes may collectively be regarded as the duplication load at the hemoglobin locus—that is to say, the total extent to which population fitness is reduced as a consequence of the genetic instability of the hemoglobin locus. It is of particular significance that this model offers a possible alternative to the assumption that thalassemia is maintained solely by heterozygote advantage, for, as we have seen, the maintenance of duplication equilibria may be independent of heterosis. Thus, the high frequency of thalassemia in certain populations may not invariably result from peculiarities of the environment but could be caused either by the existence of other genes in these populations, which increase the frequency of crossing over of the hemoglobin locus, or by the loss of a genetic mechanism (such as an inversion polymorphism or, conceivably, a heterochromatin effect) which, in other populations, stabilizes the locus. Selection could not operate directly against a mutation whose sole effect was to render another locus unstable. Hence, inbreeding or a small effective population size could facilitate the fixation of such an allele, or the loss, for example, of a "protective" inversion polymorphism. There is a clear precedent for such mechanisms in other genetic systems (*31*).

These conjectures are strikingly illustrated by reports of the persistence of hemoglobin polymorphisms in mouse lines, despite many generations of sib mating (*32*). It is difficult to see how selection for heterozygosis could be sufficiently intense to counteract the inbreeding effect. On the other hand, if the locus is complex, as is suspected (*33*), and the observed hemoglobin variants result from higher or lower orders of duplication, then, on the basis of the present hypothesis, the frequent recurrence of the variant types in previously homozygous strains would be predicted.

Table 2. Genotypes of certain heritable disorders of hemoglobin synthesis.

Condition	Possible genotypes	
	Heterozygous	Homozygous
Thalassemia		
With high A_2	$\frac{\gamma(\beta-\gamma)^*\beta\delta}{\gamma\beta\delta}$	$\frac{\gamma(\beta-\gamma)^*\beta\delta}{\gamma(\beta-\gamma)^*\beta\delta}$
With high fetal hemoglobin	$\frac{\gamma\beta(\delta-\beta)^*\delta}{\gamma\beta\delta}$	$\frac{\gamma\beta(\delta-\beta)^*\delta}{\gamma\beta(\delta-\beta)^*\delta}$
Interacting	$\frac{\gamma\delta}{\gamma\beta^S\delta}$	
Noninteracting	$\frac{\gamma\beta(\delta-\beta)^*\delta}{\gamma\beta^S\delta}$	$\frac{\gamma\beta^S(\delta-\beta)^*\delta}{\gamma\beta^S(\delta-\beta)^*\delta}$
High fetal syndrome		
With A and A_2	$\frac{\gamma\delta}{\gamma\beta\delta}$	
Without A		$\frac{\gamma\delta}{\gamma\delta}$
Without A or A_2		$\frac{\gamma}{\gamma}$
With anemia	$\frac{\gamma(\beta-\delta)^*}{\gamma\beta\delta}$	
High A_2 syndrome	$\frac{\gamma\beta\delta\delta}{\gamma\beta\delta}$	$\frac{\gamma\beta\delta\delta}{\gamma\beta\delta\delta}$
A_2 lacking		$\frac{\gamma\beta}{\gamma\beta}$
Lepore traits		
With high A_2	$\frac{\gamma(\beta-\gamma)\beta\delta}{\gamma\beta\delta}$	$\frac{\gamma(\beta-\gamma)\beta\delta}{\gamma(\beta-\gamma)\beta\delta}$
With normal A_2	$\frac{\gamma\beta(\delta-\beta)\delta}{\gamma\beta\delta}$	$\frac{\gamma\beta(\delta-\beta)\delta}{\gamma\beta(\delta-\beta)\delta}$
With no A or A_2		$\frac{\gamma(\beta-\delta)}{\gamma(\beta-\delta)}$
Bart's	$\frac{\gamma\gamma\beta\delta}{\gamma\beta\delta}$	$\frac{\gamma\gamma\beta\delta}{\gamma\gamma\beta\delta}$
H, dominant inheritance	$\frac{\gamma\beta(\delta-\gamma)^*\beta\delta}{\gamma\beta\delta}$	
H, recessive inheritance		$\frac{\gamma\beta\beta\delta}{\gamma\beta\beta\delta}$
Bart's + H	$\frac{\gamma\beta\gamma\beta\delta}{\gamma\beta\delta}$	

Relevance of the Model to Cellular Differentiation

In order to understand the normal change that occurs during development from the production of fetal to adult hemoglobin, we must consider two remaining types of unequal crossing over. As indicated by Smithies and his associates in their discussion of partial gene duplication, there is a precedent in

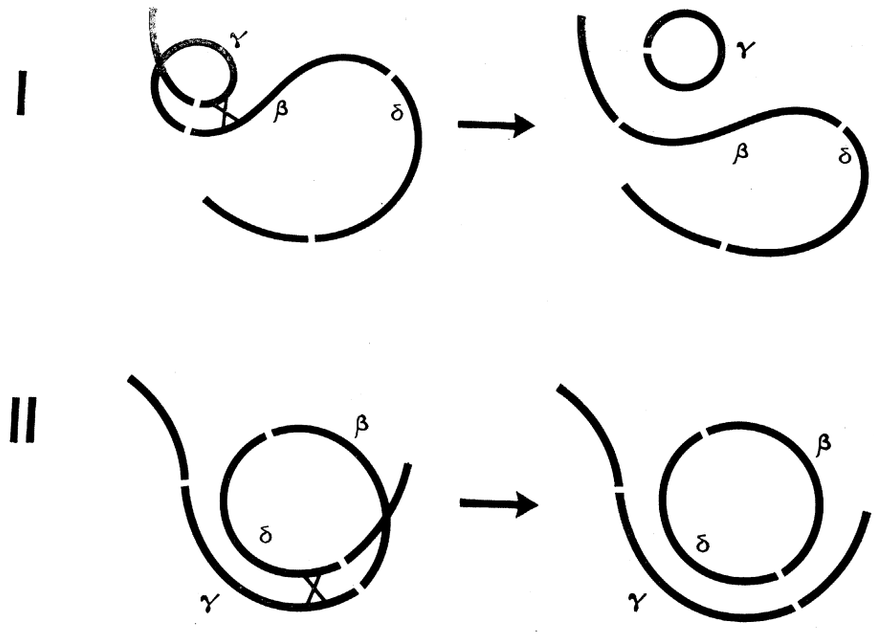

Fig. 3. Schematic diagram of intrastrand crossing over in a region of genetic triplication, leading to the deletion of one locus (I) or two adjacent loci (II) and the production of an acentric ring.

maize genetics for interchromatid and intrastrand crossing over. The significance of these events is that presumably they may occur in somatic cells during mitosis even in the absence of somatic pairing. The consequences of interchromatid exchange differ in no essential way from the consequences of interchromosomal exchange, but, as shown in Fig. 3, intrachromosomal crossing over in a region of duplication leads to a novel result. An event of this kind can lead to the loss of one or two adjacent loci and the production of an acentric ring. If embryonic erythrocyte precursors are assumed to possess all three hemoglobin loci, then clonal selection of lineages with the appropriate somatic deletion would provide a ready explanation for the production initially of fetal and subsequently of adult hemoglobin. The existence of small numbers of cells containing a normal complement of adult hemoglobin during early fetal life seems more compatible with a change in cell population by clonal selection than with environmental induction as the mechanism of differentiation from fetal to adult hemoglobin. The detection of fetal hemoglobin in certain cases of leukemia and pernicious anemia (*3; 34*, p. 38) suggests that primordial cells containing a functional fetal-hemoglobin gene may be selected in later life by an appropriate environmental stimulus. It is apparent that the property of the hemoglobin complex to which I attribute the differentiation from fetal to adult hemoglobin—namely, the property of unequal crossing over in a region of duplication—is the same property that results, during meiosis, in the duplication load. If genetic instability is a critical mechanism in cellular differentiation, then the ability to differentiate may be the selective force which accounts for the initial persistence of a duplication complex, and for its failure to become completely stabilized.

The fate of the acentric ring resulting from intrachromosomal crossing over is conjectural. It appears, however, that by reason of its DNA homology it could re-enter the chromosome by crossing over, or it could perhaps interact with distant loci (such as the α-chain and myoglobin loci) which possessed sufficiently homologous base-pair sequences. A similar mechanism has been proposed to explain the entry and exit of phage genes into the bacterial chromosome. If such events do occur, it is apparent that regions of duplication must be added to the growing list of factors known as episomes, or genetic elements, which may exist within or outside of the chromosome. Although the significance of episomes in higher organisms is not known, McClintock (*35*) has suggested that the observable effects of certain episomal elements in maize are prototypes of an important class of genetic interactions which may be of importance in normal cellular differentiation.

Relevance of the Model to Sickle-Cell Polymorphism

We have seen how duplication of the β locus can occur. It seems inevitable that in populations where the hemoglobin S gene is common, individuals with a β^A and a β^S gene on the same chromosome should eventually appear. An individual homozygous for such a chromosome might have all of the presumed advantages of sickle-cell heterozygosity with none of the liabilities. The "segregation load" would be entirely circumvented, since, regardless of the genotype of his mate, such an individual could have only carrier offspring (the most favorable genotype) and no normal (AA) or sickle-cell anemic offspring. What evidence exists that there may be variation of the number of β loci that are normally present on a single chromosome? The most convincing proof would be the demonstration of the simultaneous transmission of two β-chain abnormalities from one parent to the offspring. It is possible that such pedigrees have been observed but misinterpreted. A second consequence that we might anticipate is that the expression of a sickle-cell gene in a heterozygote might well depend upon the number of β^A and β^S genes and their distribution between the two chromosomes. A rather convincing trimodality of the relative concentrations of hemoglobin S and normal hemoglobin in sickle-cell heterozygotes has, in fact, long been recognized (*36*). These observations have been variously attributed to "isoalleles" or to the action of modifying genes (*37*). I believe that variation in the relative number and position of β^A and β^S loci in heterozygous individuals is a more straightforward interpretation of these findings. According to this interpretation, decreasing proportions of hemoglobin S might be expected in heterozygotes of the following genotypes: $\underline{\beta^S\beta^A}/\beta^S > \beta^S/\beta^A > \beta^S/\underline{\beta^A\beta^A}$.

Segregation analysis would provide a final test of this hypothesis. Among matings between heterozygous parents, those in which at least one parent was of the genotype $\beta^A/\underline{\beta^S\beta^A}$ could not produce any offspring with homozygous sickle-cell anemia. Therefore, if some heterozygotes actually have the genotype $\beta^A/\underline{\beta^A\beta^S}$, then fewer offspring with homozygous sickle-cell anemia would be found in matings between heterozygotes than would be expected if all heterozygotes have the genotype β^A/β^S. The method of ascertainment, or iden-

tification, of the family would be of critical importance. Families ascertained through an offspring with sickle-cell anemia would provide little information concerning the possible existence of $\underline{\beta^A\beta^S}$ chromosomes, since each parent in families of this kind must necessarily have at least one chromosome with only β^S loci, or with no β locus at all. In view of all that has been written about sickle-cell anemia, it is indeed remarkable that no adequate body of family data exists with which to test this hypothesis. Nevertheless, despite the fact that families which include offspring with sickle-cell anemia are more likely to come to the attention of physicians than families which do not include such offspring, the feeling persists, particularly among clinicians in Africa (*38*), that there are far fewer sickle-cell anemic individuals, in comparison with carriers, than would be expected with "single gene" inheritance, even if allowance is made for the reduced fitness of such individuals. Thus, in a population with a β^S gene frequency of 0.10, the expected ratio of carriers to homozygotes, if one assumes "single gene" inheritance, would be 18 to 1. It is estimated that the actual ratio in Africa may be closer to 1000 to 1 (*39*). If the ideas here developed have any validity, it is clear that the magnitude of the selective advantage that sickle-cell heterozygosity has been presumed to confer with respect to resistance to hyperendemic malaria (*40*) will have to be critically reappraised.

Relevance of the Model to Other Genetic Systems

The genetic model which has been developed here has general relevance to at least two major problems in human genetics. I have suggested that duplication equilibria may provide a previously unrecognized mechanism for the maintenance of genetic polymorphisms, which is independent of heterosis. Certain of the blood group polymorphisms—the Rh and MNS systems, for example—are determined by complex loci in which alleles that have been interpreted as partial deletions exist. Furthermore, somatic segregation has been suggested as a possible explanation for an important group of puzzling observations involving the existence, appearance, and disappearance of antigenically dissimilar clones of cells in certain individuals (*34*, pp. 40, 88, 173). These observations and ideas take on new significance when viewed in the context of a genetic model which predicts deletions and permits mitotic segregation, even in the absence of somatic pairing. Polymorphisms that are maintained by duplication equilibria would be expected to show the relatively frequent occurrence of "directed mutation." In the human blood group systems these events might not be recognized, or they might be attributed to illegitimacy. If, in an appropriately analyzed human population, the frequency of single-factor parental exclusions in certain systems significantly exceeds expectation, then I believe that the existence of unstable alleles in these systems may reasonably be inferred.

The possibility that certain human disease states, including sickle-cell anemia and thalassemia, might be caused by highly mutable loci has been previously suggested by Goodman and Reed (*41*). The model I have developed of a complex locus involving duplication which can become unstabilized as a result of other gene mutations or genetic events provides a specific mechanism by which a locus can show directed mutation with high frequency. Unstabilized loci of this type may provide an explanation for apparent racial differences in specific mutation rates, and for the high frequency of certain deleterious mutations in different population isolates. For example, the model provides an attractive supplement to selection, genetic drift, and inbreeding as possible explanations for the extraordinary frequency of the genes for kuru among the Fore natives of New Guinea, for amyotrophic lateral sclerosis among the Chamorros of Guam, for albinism among the Cuna Indians of Central America, and perhaps even for the prevalence of diabetes and cystic fibrosis among Caucasians (*42*).

Summary

There is compelling evidence that human hemoglobin is determined in part by a complex genetic locus which involves duplication. I have described a hypothesis concerning the control of hemoglobin synthesis which is based on known properties of regions of genetic duplication. I suggest that many abnormalities such as the variants of thalassemia, the high-fetal-hemoglobin and high-A_2-hemoglobin syndromes, and hemoglobins H and Bart's result from combinations of the abnormal alleles which can result from unequal but homologous crossing over at the hemoglobin locus. One clear example of this phenomenon has already been described; this is the Lepore trait, where the resulting fusion gene determines a physiologically active hemoglobin variant. The significance of the model we have developed in providing a mechanism for the maintenance of genetic polymorphisms has been stressed, and its possible relevance to cellular differentiation and other important problems in human genetics discussed (see *43*).

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The Magnetopause: A New Frontier in Space

The interface of the sun's atmosphere and the earth's is the site of many phenomena of geophysical import.

C. O. Hines

Up until a decade ago it was widely believed by atmospheric scientists that the earth's atmosphere terminated, for all practical purposes, several hundred kilometers above ground level. Any atmospheric molecules that still remained at that height would be moving freely in ballistic orbits, without significant mutual interaction, and some of them would be spraying off from the earth's domain at speeds exceeding the escape velocity. This "exospheric" region provided the upper fringe of the atmosphere as it was then conceived; beyond lay interplanetary space, and whatever minor quantities of dust or particles it might contain.

In the same period few astronomers pictured the sun's atmosphere as extending far above the visible disk. Photographs taken during periods of eclipse had long since shown the existence of a hot "corona," rising on occasion to heights of a few solar radii above the photosphere, but there all trace of the gaseous envelope was lost (unless it made some contribution to the dust-dominated zodiacal light).

True, the sun was believed to eject at times, strong jets of highly ionized gas (or "plasma," in modern parlance) and these jets were taken by many to be the cause of geomagnetic and auroral storms. But their life was transitory, and their influence on low-lying layers of the earth's atmosphere was thought to be exerted only after deflection in the distant regions of the geomagnetic field and subsequent passage through the intervening void.

These views have undergone drastic change in recent years, to such an extent that a whole new picture must now be adopted. In this picture the neutral atoms of the terrestrial atmosphere still form an exosphere, as before, but their ionized counterparts extend to heights of several earth radii (R_E) and fully occupy the region of geomagnetic domination. The solar corona streams ever outward, in what has come to be called a "solar wind." It streams past the earth, confines the earth's magnetic field, and makes contact with the outermost plasma of the terrestrial atmosphere.

The region occupied by the terrestrial plasma, and dominated by the geomagnetic field, is now commonly called the "magnetosphere." Its boundary with the solar wind—the newly recognized frontier between the terrestrial and the solar atmospheres—is called by some the "magnetopause," in a somewhat inelegant analogy to the "tropopause" of lower levels. This article is concerned with the nature of the magnetopause, and with the processes it influences.

The Developing Picture

The decisive change of attitude came, insofar as the terrestrial atmosphere is concerned, with the work of Storey (*1*). He studied the characteristics of "whistlers"—radio signals that are generated by lightning flashes and that yield, on detection, a characteristic whistling or swishing tone which descends in pitch from high to low audio frequencies. It had been known that this characteristic behavior could be imposed on the initial noiselike emission by a dispersion of the various frequency components it contained, if the radio wave passed through the ionized gas of the upper atmosphere, but the path to be followed by the signal in its return to earth was difficult to chart. Storey concluded, on the basis of mathematical analysis, that the energy would tend to travel along the lines of the geomagnetic field; it would then rise from the region of its generation in one hemisphere, pass over the equator at some great height, and be guided back down toward the earth in the opposite hemisphere near the geomagnetically "conjugate point" (see Fig. 1, A–A′). In order for this explanation to hold, however, there must be appreciable amounts of ionization even at the highest point of the geomagnetic arch—some hundreds of electron-ion pairs per cubic centimeter at heights of 3 R_E; this is much more than had been proposed previously. With this conclusion, a new era in the exploration of the upper atmosphere began.

The author is professor of aeronomy in the department of the geophysical sciences, University of Chicago, Chicago, Ill.