# Gene Selection in Hemoglobin and in Antibody-Synthesizing Cells

A process of intrachromosomal crossing-over selects and activates genes in two different tissues.

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Close linkage of coordinately regulated genes occurs widely in microorganisms and has been the subject of much study. Analysis of such coordinate regulation in the *lac* operon of *Escherichia coli* has led to an extremely important advance in our understanding of gene control (*1*). Although data concerning gene linkage is relatively very difficult to obtain in higher organisms, it is now clear, as is discussed below, that gene clustering occurs also in the hemoglobin genes and in the genes for immunoglobulins in mammals. However, unlike the situation with bacteria in which the clustered genes are coordinately expressed, the situation in higher organisms appears to be the opposite. That is, the closely linked genes in mammals tend to be expressed in a mutually exclusive manner. The expression of one gene in the cluster precludes the expression of any other linked gene.

I describe here a testable theory which was designed to explain this mutual exclusivity of gene function in the hemoglobin system, but which is also in accord with numerous other previously unexplained observations concerning hemoglobin synthesis and proliferation of erythroid stem cells. The theory is also in surprising agreement with known data for immunoglobulin synthesis; there is strong and previously unrecognized similarity between gene selection in these two different tissues.

## Clustering of Mutually Exclusive Hemoglobin Genes in Man and Sheep

Hemoglobin is a tetrameric protein containing two $\alpha$ chains and two other (that is, non-$\alpha$) chains that are called $\beta$, $\gamma$, $\delta$, or $\varepsilon$. It has the structure $\alpha_2X_2$, where X is the non-$\alpha$ chain. The developmental changes in proportions of the non-$\alpha$ chains of man are shown in Fig. 1. The $\varepsilon$ chain is normally present only in very early embryonic fetuses, being soon replaced by the $\gamma$ or fetal chain and eventually by the simultaneous appearance of the adult $\beta$ and $\delta$ peptide chains (*2, 3*). The $\beta$ and $\delta$ chains occur together within single adult erythrocytes (*4*) in a ratio of approximately 97 to 3. Apart from this coordinate appearance of the $\beta$ and $\delta$ polypeptide chains, it is quite striking that the appearance of each non-$\alpha$ chain is closely correlated in time with the disappearance of the more primitive chain. Many other similar examples are known in hemoglobin development (*5, 6*) and imply that there may be a cause and effect relation between inactivation of one non-$\alpha$ gene and activation of another. These hemoglobin changes are not due simply to successive dilutions of more primitive erythroid cells with new clones of cells; in man (*7, 8*) and in chicken (*9*) the red cells of the transitional phases contain both types of non-$\alpha$ chains intermixed within single cells.

Evidence for linkage of mammalian genes is very difficult to obtain by classical genetic tests because the number of matings is severely limited. Therefore, it has been necessary to use chemical evidence in addition to limited genetic evidence to argue for close linkage. For example, the well-accepted conclusion that the $\beta$ and $\delta$ loci of humans are closely linked is based only partly on classical genetic analysis (*10*). Important evidence comes also from the existence of the Lepore type hemoglobins, which appear to have arisen from unequal or nonhomologous crossing-over between the $\delta$ and $\beta$ structural genes (*11*). The non-$\alpha$ chain in the Lepore hemoglobins is $\delta$-like at the amino terminal end and $\beta$-like at the carboxyl terminal end. Nonhomologous crossing-over between unlinked genes would very likely be lethal or lead to multiple anomalies, whereas the Lepore condition is relatively mild. It is therefore argued that the $\delta$ and $\beta$ genes of man must be very closely linked.

Evidence for a close linkage of the human $\gamma$ chain structural gene to the $\beta$ and $\delta$ loci has recently been obtained. In the human condition known as hereditary persistence of fetal hemoglobin (PHF), the $\gamma$ chains continue to be synthesized throughout adult life and the $\beta$ and $\delta$ polypeptides are never synthesized. The PHF gene has been shown to be closely linked to the $\beta$ locus (*12, 13*). Furthermore, individuals heterozygous for the PHF gene and for the $\beta^S$ gene synthesize normal amounts of sickle cell hemoglobin, Hb S ($\alpha_2\beta_2^S$), but there is a complete absence of normal adult hemoglobin, Hb A ($\alpha_2\beta_2$) (*12, 13*). Similar data have been obtained in PHF/$\beta^C$ heterozygotes and demonstrate that the PHF gene is active in suppressing $\beta$ chain synthesis only in the *cis* position. Recent evidence has shown that there are actually at least two $\gamma$ genes in man which have been designated $^G\gamma$ and $^A\gamma$ (*13*). The encoded polypeptides differ only in containing glycine or alanine at position 136 in the polypeptide, respectively. Analysis of the ratios $^G\gamma:{}^A\gamma$ in various types of PHF reveals that this locus is complex and very likely contains the $^G\gamma$ and $^A\gamma$ structural genes (*13*). Other explanations are difficult to reconcile with the heterogeneity of PHF mutations. Furthermore, $^G\gamma$ synthesis precedes $^A\gamma$ synthesis in normal development. The ratio $^G\gamma:{}^A\gamma$ is high in newborns and declines during subsequent development (*13*).

Indirect evidence consistent with a close linkage of mutually exclusive genes for the non-$\alpha$ chains also exists in sheep (and, similarly, in goats). Polymorphism at the $\beta$ locus occurs in sheep, which contain only one $\alpha$ gene but which may carry either the A or B $\beta$ chain alleles. The $\beta^A$ and $\beta^B$ genes are not isoalleles and represent a true stable polymorphism since the encoded

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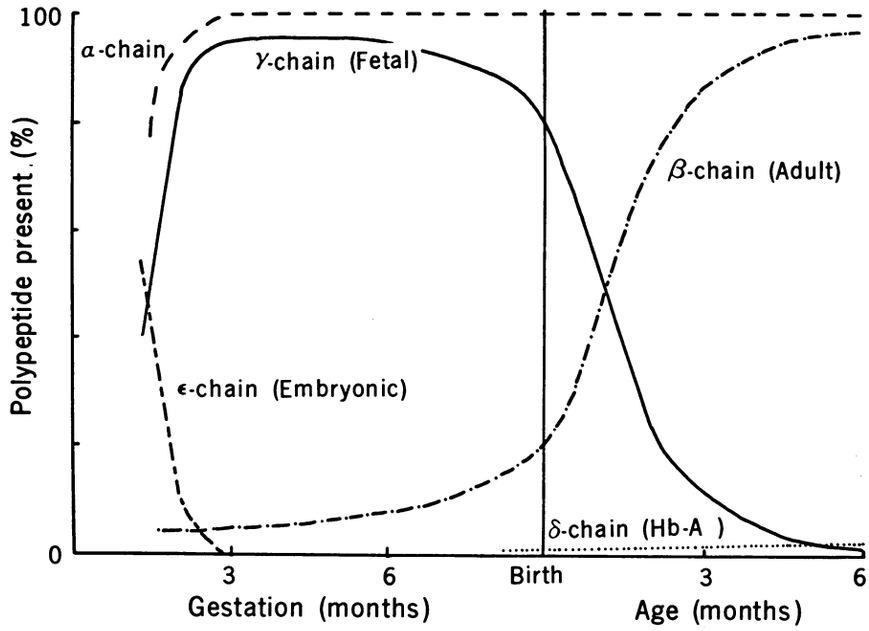


Fig. 1. The development of human hemoglobin chains. [From E. R. Huehns, N. Dance, S. Hecht, A. G. Motulsky, *Cold Spring Harbor Symp. Quant. Biol.* **29**, 327 (1964)]

polypeptides differ by seven amino acid replacements (*14*). Heterozygous sheep contain Hb A and Hb B intermixed within single erythrocytes (*15*). Furthermore, when adult sheep with the A allele are made anemic, they synthesize a different non-$\alpha$ chain, $\beta^C$, and cease producing any $\beta^A$ chains (*5*, *14–16*). An electrophoretic analysis of these sheep hemoglobins is shown in Fig. 2. The replacement of $\beta^A$ chains by $\beta^C$ chains is stoichiometric in anemic animals. The $\beta^C$ polypeptide chain differs from $\beta^A$ at 17 positions and from $\beta^B$ at 21 positions (*14*). The $\beta^C$ chain is normally present briefly during neonatal life (as synthesis of $\gamma$ chain declines) and it is later replaced with $\beta^A$ synthesis when the animal is approximately 80 days of age (*5*, *16*). Since $\beta^C$ synthesis is never observed in sheep homozygous for the B allele, it may be inferred either that the $\beta^A$ and $\beta^C$ structural genes are very closely linked and operate in a mutually exclusive manner or else that the $\beta^B$ allele somehow represses the unlinked $\beta^C$ gene. However, active expression of the $\beta^C$ gene occurs in anemic heterozygotes (Fig. 2) and is inconsistent with the latter interpretation. Similarly, the hypothesis that an activator for an unlinked $\beta^C$ gene is formed only upon repression of the A allele is complex and is difficult to reconcile with the stoichiometry of $\beta^A \rightarrow \beta^C$ replacement observed in heterozygotes (see Fig. 2). The data suggest that $\beta^A \leftrightarrow \beta^C$ conversion is a *cis*-dominant effect involving closely linked mutually exclusive structural genes.

## Looping-Out Excision Theory of Gene Selection

A chromosomal model that can explain the mutually exclusive functioning of linked hemoglobin genes is outlined in Fig. 3. Although derived without reference to earlier proposals, aspects of this model have been suggested (*17*). The terminology is borrowed from the models of gene control in microorganisms (*1*, *18*). Although the proposed functions of the promoter, operator, and terminator regions are considered analogous to their functions in bacteria, I do not mean to imply that the mechanisms of function are identical. The promoter is the site of RNA polymerase attachment to the chromosome, whereas the terminator is the detachment site. The operator loci occur on the promoter-proximal side of each non-$\alpha$ chain structural gene and are similar or identical in nucleotide sequence for all genes in the cluster. The operator loci are derepressed in erythroid cells, but are repressed in other tissues and in the multipotential stem cells that occur in mammalian bone marrow (see below). In this model, only the gene proximal to the promoter will be expressed because the more distal genes are separated from the promoter locus by a terminator.

The postulated genotype of the undifferentiated multipotential stem cells (MSC) of man is indicated in Fig. 3. It is well known that erythroid tissue contains stem cells capable of differentiating along several alternative pathways (*19*, *20*). Such stem cells from adult bone marrow can form colonies containing mixtures of erythrocytic, granulocytic, and megakaryocytic cells in spleens of lethally irradiated mice. These uncommitted MSC very likely

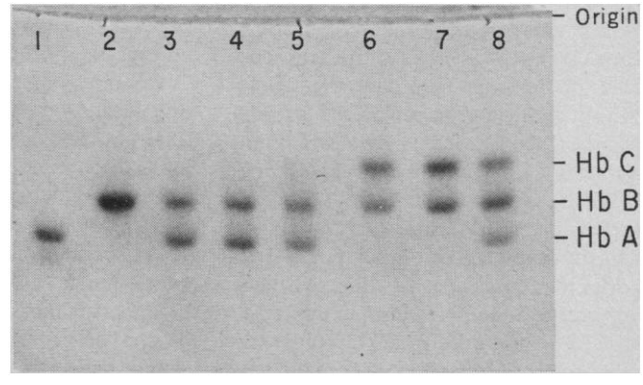


Fig. 2. Electrophoretic patterns of sheep hemoglobins on starch gel. The figure shows that hemoglobin A ($\alpha_2\beta_2^A$) is replaced with hemoglobin C ($\alpha_2\beta_2^C$) in anemic sheep. Hemoglobin B ($\alpha_2\beta_2^B$) is not affected by the anemia. An adult heterozygote containing Hb A and Hb B was made anemic by phenylhydrazine injections. Hemoglobin samples were obtained during the course of the experimental anemia. The samples are in the following order, from left to right. (position 1) Hemoglobin from an AA homozygote. (position 2) Hemoglobin from a BB homozygote. (position 3) Hemoglobin from the AB heterozygote before onset of anemia. (positions 4 to 7) Hemoglobin from the AB heterozygote at various times after beginning phenylhydrazine injections. Sample 8 contains a mixture of the hemoglobins analyzed in positions 3 and 7. The hematocrits were very low (approximately 12 percent) for the samples in positions 6 and 7 and the substitution of Hb C for Hb A appears to have been stoichiometric and complete. Stoichiometry was shown by the 1 : 1 ratio of Hb A + Hbb C) : Hb B throughout the production of anemia as measured by quantitative electrophoresis on polyacrylamide gels.

have unmodified hemoglobin genes, similar to those in the animal's germ line chromosomes. The MSC can replicate by mitosis or can undergo conversion into unipotential stem cells that are committed to only one pathway of differentiation. Such unipotential stem cells committed to erythroid differentiation are responsive to the hormone erythropoietin (*19, 20*). Like other authors, I will refer to these as erythropoietin-sensitive stem cells (ESC).

The ESC replicate rapidly by mitosis and are relatively numerous compared to the MSC in adult bone marrow or in spleen colonies (*20*). The ESC produce not only other ESC but they are also converted by erythropoietin into proerythroblasts, which are terminally differentiating precursors of the erythrocytes that enter the blood (*19, 20*). However, I propose here that ESC occasionally undergo a looping-out excision event in which the promoter-proximal gene for non-$\alpha$ chains is excised from the chromosome (Fig. 3). Looping-out excision is an intrachromosomal crossover between the two adjacent operator loci situated closest to the promoter, and is analogous to the well-known excision of lysogenic bacteriophage $\lambda$ DNA from the *E. coli* chromosome (*21*). The excised piece of DNA is presumably lost from the ESC as an acentric ring and cannot be transcribed since it lacks a promoter site. Thus, it is proposed that proliferation of ESC is accompanied by gradual conversion of these cells into a population that can generate erythrocytes containing adult hemoglobins. The latter class of ESC cannot engage in further looping-out excision because their genome contains only one operator locus (*22*).

Evidence in maize and in drosophila is consistent with this mechanism of generating variegated tissues by intrastrand crossing-over in somatic cells (*23–25*). For example, in maize the $P^r$ gene causes a red coloration of the pericarp. When $P^r$ is linked to a transposable or episome-like controller gene, Mp, a variegated irregular red striping occurs (*23*). It appears that Mp is unstable in somatic cells and that its loss from the chromosome permits $P^r$ gene expression. Furthermore, a variety of data in maize and in other plants supports the concept that directed gene loss occurs at complex or "paramutable" loci in somatic cells (*23*). Numerous other apparently analogous somatic variegations are known in higher organisms (*24*). A similar process may also be involved in the unstable integration and phenotypic variegation of transformed stocks of *Drosophila melanogaster* (*25*). Similarly, leucine synthesis in *Salmonella typhimurium* is activated by excision from the chromosome of an episome (*26*). Intrastrand crossing-over may also be involved at some stage in the production of circular DNA that contains ribosomal RNA genes in the amplified nucleoli of amphibian oocytes (*27*). Presumably, all such crossover events involving circular DNA are highly regulated and require the activity of specific protein factors (*21*). Although it is obviously difficult to prove that directed gene loss has occurred in somatic cells of higher organisms, there is at least good evidence for this in plants in which the germ line does not segregate early in development, and there is highly suggestive evidence that this may also occur in animals.

## Concordance of the Model with Hemoglobin Embryogenesis

The looping-out excision theory (Fig. 3) can adequately explain the developmental changes of non-$\alpha$ polypeptide chains, which I have discussed. In the early embryo, clones of MSC would arise and would generate unipotential subclones of ESC. These latter clones would initially produce erthrocytes containing only embryonic hemoglobin ($\alpha_2\varepsilon_2$). Looping-out excision would occasionally occur independently on the two homologous chromosomes of ESC, and the resultant ESC subclones would produce erythrocytes containing both $\varepsilon$ and $^G\gamma$ chains. As the ESC clones aged further, they would ultimately become capable only of synthesizing adult hemoglobins.

According to this model, the predominant type of non-$\alpha$ chain formed at any embryonic or neonatal stage would depend on the rate of looping-out excision in ESC and on the mean age of the ESC population. This would in turn depend on the relative sizes of the MSC and ESC populations and on the rate of conversion of MSC into ESC. Furthermore, the rate of looping-out excision might differ somewhat for different operator loci or for different erythropoietic tissue sites. Obviously, there exist enough unknown parameters for the observed hemoglobin changes during development to be consistent with this model. This conclusion is neither surprising nor supportive of the model. However, it is well known that the proportion of MSC in normal adult bone marrow or in spleen colonies is very low compared with the level of ESC (*18*). It is thus reasonable to conclude that only a small minority of adult ESC could be young (recently derived from MSC) and that the type of hemoglobin synthesized would therefore be almost exclusively adult hemoglobin. The proportion of fetal hemoglobin in the erythrocyte population of normal adult humans is approximately 0.7 percent (*6, 13*).

## Globin Synthesis Changes Accompanying Anemia

One of the most important corollaries of this theory is the idea that the adult bone marrow is constantly recapitulating its embryogenesis. The only change accompanying development is an increasing mean age of the ESC population in older animals. As I will now describe, this aspect of the theory is fully consistent with changes of hemoglobin synthesis which accompany anemia.

It is well known that anemia is accompanied by a greatly increased production (probably by the kidneys) of erythropoietin and with a premature release into the circulation of reticulocytes and of other immature bone marrow cells (*19, 20*). There is also much evidence that erythropoietin stimulates a conversion of undifferentiated unipotential ESC into proerythroblasts (*19, 20*), as is shown in Fig. 3. Although reticulocytes are generally depleted from bone marrow in anemia, the degree of ESC depletion is thought to depend on several species-specific factors (such as the size of the hematopoietic marrow) and on the severity of anemia. Excessive removal of ESC due to differentiation would be detrimental to an animal needing to compensate for a chronic blood loss, although it would be helpful as a means for recovery from a single bleeding. The depletion of ESC from anemic marrow may also be compensated by an enhanced rate of conversion from MSC to ESC by an unknown feedback mechanism (*19, 20*).

The consequences of anemia are predicted in any case to include generally a reduction in the mean age of the ESC population (due to a decrease in size of the ESC population causing a relative increase of the more immature

stages of ESC maturation and also due to compensated enhancement of conversion of MSC to ESC), with an associated shift of erythropoiesis toward earlier developmental forms of non-$\alpha$ hemoglobin chains. The shift from $\beta^A$ toward $\beta^C$ synthesis accompanying anemia in adult sheep was described earlier (in connection with the discussion on genes). The $\beta^C$ polypeptide is normally present in early neonatal life and is replaced with $\beta^A$ chains in adult animals (*5, 15, 16*). This activation of the $\beta^C$ "silent gene" can be induced by erythropoietin injections into nonanemic adult sheep (*5, 14–16*). Similar changes in hemoglobins of anemic animals occur in goats (*5, 16*) and to a lesser extent in mice and in ducks (*28*).

Similar changes in proportions of non-$\alpha$ hemoglobin chains are associated with anemias in humans. It is well known, for example, that many anemias in man (for example, thalassemia or Cooley's anemia, sickle cell anemia, and acquired aplastic anemia) are accompanied by elevated levels of fetal hemoglobin (*6, 8, 12, 29*). Furthermore, whereas the ratio of $^G\gamma$ to $^A\gamma$ in normal adults has a very low value, this ratio in anemic adults with $\beta$ thalassemia is often much higher and is closer to that occurring in normal newborns (*13*). These changes accompanying anemia are all fully consistent with the proposed theory of gene selection (Fig. 3). An additional observation which is consistent with this theory is the finding that adult human bone marrow cultures begin to shift their hematopoiesis toward predominant synthesis of fetal hemoglobin (*30*). It may be postulated that the ESC population becomes younger during the conditions of prolonged organ culture. These observations all provide strong support for the idea that adult erythroid tissue is constantly recapitulating its embryogenesis.

## Different Non-$\alpha$ Polypeptide Chains within Single Erythrocytes

Numerous hematological studies have been made of the distributions of fetal hemoglobin ($\alpha_2\gamma_2$) and of adult hemoglobin ($\alpha_2\beta_2$) among human erythrocytes during normal development and in various pathological conditions (*7, 8, 31, 32*). These studies are remarkably consistent with the looping-out excision theory (Fig. 3) in which gene selection occurs independently on the two chromosomes of the diploid set.

During normal human development, it can be predicted from Fig. 3 that $\gamma$-producer ESC would occasionally undergo looping-out excision on one of their chromosomes to yield mixed producers for $\gamma$ and $\beta$ polypeptide chains. Such mixed producers would subsequently undergo further looping-out excision to yield ESC capable only of adult hemoglobin synthesis. Thus, it would be expected that, during the developmental transition from fetal to adult hemoglobin, mainly erythrocytes containing mixtures of both hemoglobins would be found. This expectation is indeed what is observed during normal human development (*7, 8, 12, 31*).

Another situation in which Hb F occurs without anemia is in adult heterozygotes for PHF. The PHF chromosome is unable to mature beyond the $\gamma$-producer stage, in some cases perhaps due to a defective $\beta,\delta$ operator gene (see Fig. 3), and the result is persistent Hb F synthesis in erythrocytes derived from fully matured clones of ESC (*33, 34*). Since the vast majority of ESC in nonanemic adults are fully matured, we would expect the erythrocytes in adult PHF heterozygotes to all contain hemoglobin F (Hb F) as well as Hb A, as has been observed (*8, 12*) and is shown in Fig. 4.

A quite different result is observed in humans with various types of anemia (*7, 8, 12, 31*) who contain elevated levels of Hb F in their blood. As was described by Weatherall (*12*), there is a "clear distinction between hereditary persistence of foetal haemoglobin and the thalassemia syndromes and other inherited haemoglobinopathies, the foetal haemoglobin being quite homogeneously distributed in the former conditions and heterogeneously distributed in the latter states." Data illustrating this conclusion is shown in Fig. 4. More extensive substantiating data have been described (*8, 12*).

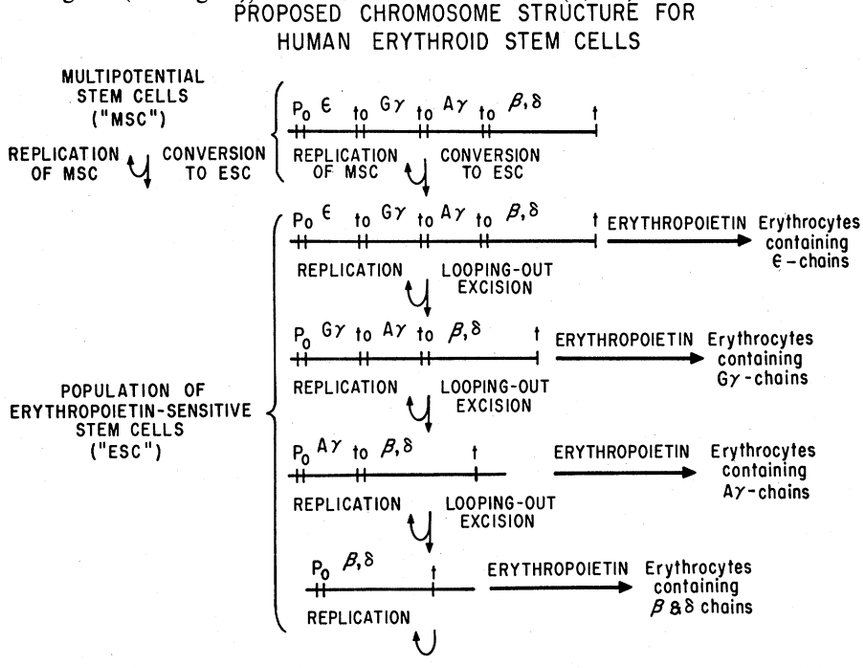


Fig. 3. Looping-out excision theory of erythropoiesis. Multipotential stem cells (MSC) exist in erythroid tissue and are converted occasionally into erythropoietin-sensitive stem cells (ESC) committed to erythroid differentiation (*19, 20*). The ESC replicate rapidly and are converted by erythropoietin into terminally differentiating proerythroblasts. The proerythroblasts are precursors of the blood erythrocytes. The proposed chromosome structure for the human stem cells is indicated. P is the promoter locus and is the site of RNA polymerase attachment to the chromosome, whereas the t loci are the terminator sites at which transcription is terminated. The O sites are the operator loci and are derepressed in erythroid tissue. Only the promoter-proximal gene can be expressed because the more distal genes are separated from the promoter by a terminator. It is proposed that ESC occasionally undergo an intrachromosomal crossover event called looping-out excision in which the promoter-proximal gene is excised from the chromosome as an acentric ring. The crossover occurs between the two homologous operator loci which are closest to the promoter locus and is analogous to the well-known excision of lysogenic bacteriophage $\lambda$ DNA from the *E. coli* chromosome (*21*). The looping-out excision occurs independently on the two chromosomes in the diploid ESC. By this mechanism a maturing population of ESC is obtained. Although no information is available about its linkage, the $\epsilon$ gene is tentatively included in this model.

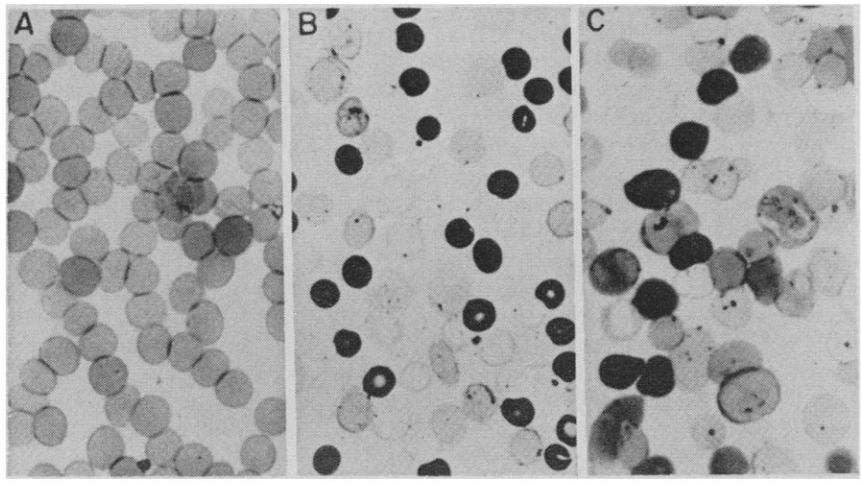


Fig. 4. Distribution of fetal hemoglobin in red cells as demonstrated by the technique of Kleihauer and Betke (*32*). The stained cells contain Hb F. (A) A person heterozygous for hemoglobin S and for hereditary persistence of fetal hemoglobin (S/PHF). Note the homogenous distribution of Hb F. (B) A mixture of red cells from a patient with sickle cell anemia (Hb F only approximately 5 percent) and from a homozygote of hereditary persistence of fetal hemoglobin (S/S + PHF/PHF). The darkly stained cells are from the PHF homozygote. (C) A patient with sickle cell anemia whose hemolyzate contained 21 percent Hb F. Note the heterogeneous distribution of Hb F in this anemic condition. [From Shepard *et al.* (*8*)]

The looping-out excision theory of gene selection (Fig. 3) is in full accord with this difference between the cellular distributions of Hb F in normal development (or in PHF heterozygotes) and in the anemias. In the absence of anemia, the ESC clones are relatively large, and the vast majority of ESC have completed the looping-out excision process; a rather homogeneous population of fully matured ESC is thereby obtained. However, in anemic animals the ESC population is relatively young and more heterogeneous in its maturational age. If the ESC population was only moderately reduced in mean age, as would be expected in a chronic mild anemia, then the youngest stages of ESC maturation would still be relatively rare, and one would expect to find progressively larger numbers of more mature ESC (that is, $\gamma/\gamma$ producers are fewer than $\gamma/\beta,\delta$ producers which are fewer than $\beta,\delta/\beta,\delta$ producers). And $\varepsilon$ producer ESC would presumably occur in undetectably low levels. Indeed, in rather mild anemias that are characterized by only slight increases of Hb F, all the Hb F is present in a small proportion of the cells and then apparently in moderate concentration. In more severe anemias there are generally larger numbers of cells containing Hb F and relatively fewer cells without any Hb F. Furthermore, the quantity of Hb F per cell becomes more variable as erythrocytes appear that may contain only Hb F (see Fig. 4). It would obviously be interesting to have quantitative estimates of the ratios of Hb F to adult hemoglobin for individual cells. Unfortunately, such quantitative data is not yet available. Nonetheless, it may be fairly concluded that the available information about cellular distribution of non-$\alpha$ hemoglobin chains is in close agreement with the expectations of the looping-out excision model of gene selection.

This theory of gene selection leads to the simple prediction that no more than two non-$\alpha$ genes should be expressed within a single erythrocyte. For example, we would expect that cord blood of humans heterozygous for the $\beta^A$ and $\beta^S$ alleles would consist of erythrocytes containing only two of the three possible hemoglobins, Hb A, Hb S, and Hb F. No cells should contain all three hemoglobins. I am currently testing this simple prediction of the theory.

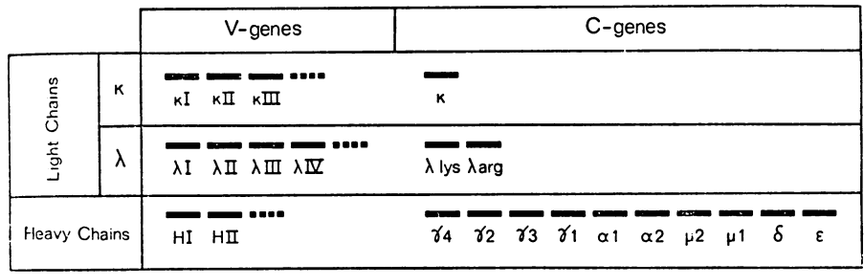


Fig. 5. The proposed minimum number of genes required for human immunoglobulins. The V genes encode the variable and the C genes the constant regions of the immunoglobulin polypeptide chains. The V genes are found expressed in association with products of C genes of the same row, and it is predicted by the authors (*35*) that in each case the set of V genes will be found linked to the corresponding C genes. Thus, it is proposed that there are $\kappa$, $\lambda$, and heavy chain gene clusters specifying each immunoglobulin polypeptide chain. The order of genes in this diagram is largely arbitrary. [From Milstein and Munro (*35*)]

## Gene Selection in Antibody-Synthesizing Cells

The looping-out excision theory of gene selection appears to be also in close agreement with the available data concerning immunoglobulin synthesis. In both tissues there is a close linkage of mutually exclusive genes and a selection mechanism for activating only one gene from among the set of linked genes. I will only briefly consider here the genetic aspects of immunoglobulin synthesis; the structure and genetics of these proteins has been reviewed (*35, 36*).

The minimum number of human genes that must be present in the germ line chromosomes to code for the immunoglobulin polypeptide chains is shown in Fig. 5 [see (*35*)]. These genes occur in three separate clusters of closely linked genes. The $\kappa$ and $\lambda$ gene clusters encode for light polypeptide chains, whereas a separate linkage group encodes for the various types of heavy polypeptide chains. Each gene cluster contains at least several mutually exclusive loci that specify the variable region and one or more mutually exclusive loci that encode for the constant region of the immunoglobulin polypeptide chain.

As compared with erythrocyte differentiation, the antibody-forming system is relatively complex; and it is clear that additional gene exclusion mechanisms are operative. For example, the immunoglobulin-forming cells make either $\kappa$ or $\lambda$ light chains, but not both (*37*). Furthermore, allelic exclusion oc-

curs in antibody-forming cells, only one of the two allelic gene clusters being expressed within a single cell (*37–39*). Much debate has also centered on whether all of the possible variable genes are present in the germ line chromosomes or whether additional variability arises due to hypermutability at these loci during somatic development of the embryo (*35, 36*). None of these complications are germane to my discussion; in any case there must occur a selection from among at least several linked variable genes and from among one or more linked constant region genes.

The problem of gene selection from among the mutually exclusive loci of an immunoglobulin gene cluster is closely analogous to the problem of non-$\alpha$ gene selection in the hemoglobin system (compare Figs. 3 and 5). The only difference is that two separate looping-out excision events are required for activation of synthesis of an immunoglobulin chain. One excision would place a particular variable gene proximal to the promoter locus, which lies on the left side of the gene clusters shown in Fig. 5; and a second looping-out excision would result in the splicing of this variable region gene with one of the linked constant region genes (*40*). Other workers have proposed that some type of crossover event could be used for splicing of variable and constant region genes at the DNA level (*35, 36, 41*). As was discussed above, such a mechanism could also account for gene selection in the hemoglobin system.

## Summary

Close linkage of mutually exclusive genes occurs in the non-$\alpha$ chain hemoglobin genes and in the immunoglobulin genes of man and other mammals. The expression of one gene in the cluster precludes the expression of any other linked gene. A simple, testable theory of gene selection called "looping-out excision" which was designed only to explain this mutual exclusivity in the hemoglobin system is described. The theory is closely concordant with a wide range of previously unexplained findings concerning hematopoiesis—including the developmental changes of hemoglobins, the increases in immature or fetal forms of hemoglobin that accompany anemia, and with the distribution of adult and fetal hemoglobins among erythrocytes during normal embryogenesis and in various pathological conditions. One corollary of this theory is that erythroid tissue in the normal adult bone marrow is constantly recapitulating the developmental stages of its embryogenesis. Another corollary is that the selection from among the linked globin genes occurs independently on the two chromosomes of the diploid organism. Both of these corollaries are supported by the available data.

The same theory of gene selection is also remarkably consistent with known data for immunoglobulin synthesis; it could explain not only the mutually exclusive activation of linked variable genes but also the splicing which occurs between genetically linked variable and constant region genes for the immunoglobulin polypeptide chains. The agreement between these two different tissues is considered to be strong evidence that the proposed mechanism is correct at least in broad outline. Evidence from the genetics of maize and of drosophila also supports this theory of somatic tissue variegation. On the basis of these comparisons, I suggest that looping-out excision probably occurs also in other tissues and may be one means of gene selection and activation in differentiating cells.

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P o $\gamma$ t o $\beta^C$ t o $\beta^A$ t,

whereas the predicted B genotype is

P o $\gamma$ t o $\beta^B$ t.

These genes are clearly not isoalleles since the amino acid sequences differ markedly

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33. There actually exist several types of PHF [see (*13*)] and all are consistent with the model in Fig. 3. For example, the type with only $^{G}\gamma$ chains could be an inactivating mutation of the $^{A}\gamma$ operator, whereas the type with both $^{G}\gamma$ and $^{A}\gamma$ chains might conceivably contain a defective $^{A}\gamma$ operator which participates only minimally in excision events.
34. Some other hemoglobinopathies associated with defective or altered synthesis of non-α chains can also be understood in terms of the looping-out excision theory and might be explained by deletions or mutations in various regulatory and structural genes plus secondary changes in ESC proliferation induced by anemia (see text). It would not be fruitful to discuss these hemoglobinopathies in detail. It is especially relevant, however, that the *cis* dominance of all of these conditions argues against any defective diffusible regulators involved in the etiology of these diseases, and is consistent with the proposed intrachromosomal mechanism of gene selection.
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40. There is evidence that the selection of constant region genes may occur sequentially, at least in the chick embryo [see (*38*)]. This is similar to the sequential activation of non-α hemoglobin genes. In the chick embryo, IgM synthesis precedes IgG synthesis. Furthermore, clones of cells capable of IgG synthesis all derive from cells synthesizing IgM immunoglobulins. This conversion occurs in the bursa fabricus and ceases when any cell leaves that organ.
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# A Comprehensive Ban on Nuclear Testing

Robert Neild and J. P. Ruina

The technical, strategic, and political aspects of a comprehensive test ban (CTB) treaty have changed since they were debated in the negotiations leading up to the limited test ban (LTB) treaty of 1963. Progress in the technical art of seismic monitoring may now permit nations to overcome the verification obstacle that blocked the CTB in the past. The gradual realization that new nuclear bomb technology is not critical to the strategic balance between the superpowers has also reduced concern about the risks of entering into a CTB.

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In this article, we review the range of issues involved in the consideration of a CTB. Often the negotiaions at Geneva and the internal debates in the United States have been so dominated by technical issues, notably those regarding verification, that the political and military significance of a CTB has been lost or ignored.

## Background

Any history of the LTB must consider the dimensions of the process of negotiations. The most obvious dimension is the record of official statements and the formal negotiating process itself—that process in which the parties to the negotiations make evident their policy decisions regarding negotiations. Much of the written history of this dimension is available (*1*). However, what is less evident is the nature of the intragovernmental negotiations that necessarily precede and accompany intergovernmental negotiations. We know a good deal about the United States' internal process. We know almost nothing about the Soviet Union's, but we must assume nevertheless that, in that country too, there was internal argument and debate on the test ban issue. The international negotiations consisted largely of sparring rounds on a few issues—the test ban's relation to more general disarmament and on-site verification. What we know about the internal debate in the United States and what we surmise about debate in the Soviet Union point up political and military issues that were never stated in the international negotiating process, but that were, nevertheless, motivating forces behind each country's position.

A test ban was proposed in 1954 after both the United States and the Soviet Union had tested thermonuclear devices and after the fallout from a particularly large U.S. test (15 megatons) in February 1954 had affected the crew of a Japanese fishing boat. From then until 1963, when the LTB treaty was signed, there were periods of serious negotiations, periods devoted to technical analyses, and a test ces-