RECENT ADVANCES IN HAEMATOLOGY IMMUNOLOGY AND BLOOD TRANSFUSION

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Proceedings of the Plenary Sessions of the Joint Meeting of the 19th Congress of the International Society of Haematology and the 17th Congress of the International Society of Blood Transfusion, Budapest, August 1–7, 1982

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PREFACE

The 19th Congress of the International Society of Haematology and the 17th Congress of the International Society of Blood Transfusion was held jointly in Budapest, August 1-7, 1982.

This volume contains the Plenary Session papers presented at the Congress and is intended to offer a summary of most recent advances in haematology, blood transfusion and those fields of immunology, which are intimately related to these two rapidly advancing fields of biomedicine.

We are deeply indebted to our colleagues for their help in organizing the Congress. Special thanks are extended to Mrs. Lenke Kevíczky and Ms. Krisztina Rozsnyai for their excellent secretarial work.

This volume appears some five months after the date of our Congress. Sincere thanks are due to the invited speakers and to the staff of Akadémiai Kiadó, Budapest for their cooperative efforts.

Susan R. Hollán

I. Bernát

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INTRODUCTION*

It is a great honour and pleasure for us to welcome the most distinguished experts, medical practicians, science graduates, gifted and skilled technicians attending the International Congress of Haematology and Blood Transfusion in Budapest.

It has become more and more difficult to organize an international congress not merely because the funds have been cut and the prices are soaring, but because in the field of haematology and blood transfusion an enormous amount of valuable experimental and clinical data has been produced over the past two years. Not only has the pace of discovery been quickened but observations deemed impossible only a few years ago have greatly contributed to these advances. Blood being a readily available part of the body, a great number of breakthroughs in medical science originate from discoveries in biochemistry, physiology, pathology and genetics of blood cells and plasma constituents, in other words in haematology, immunology and blood transfusion, since the substrates of research are essentially the same in these three disciplines.

The flood of new scientific information and the availability of more and more sophisticated technologies lead to an ever-increasing superspecialization. About twenty years ago we witnessed "The Death of the Professor of Medicine" being replaced by experts in cardiology, pulmonology, gastroenterology, endocrinology and haematology. Not much time has elapsed and the

*An abridged version of the Presidential Address of Susan R. Hollán, president of the Joint Congress of ISH-ISBT.

increasingly rapid advance in research, clinical and laboratory experience have broadened our knowledge to such an extent that the "Death of the Professor of Haematology and Blood Transfusion' is imminent. The floor has to be given to new emperors, these disciplines being already split up and ruled by molecular genetists, haemostaseologists, rheologists, haemato-oncologists, blood group serologists, immunohaematologists, membranologists, cryobiologists, fractionation, transplantation, plasma and cell-apheresis experts. We have also witnessed that brilliant directors of institutes and departments, founders of internationally well-known schools of haematology and blood transfusion cannot be replaced, just because research work in a very restricted field is so much more attractive. There is a dire need to prevent experts well versed in the whole spectrum of these fast-growing disciplines from dying out. It is of utmost importance to have leading experts who can cope with the most difficult task: the assignment of proper weights to new trends especially in times when funds for research and development in medicine are becoming more restricted and research and health service is becoming more costly. Careful stewardship is needed so that our disciplines shall not wind down irreversibly.

To cope with problems emerging from unavoidable 'super-specialization' necessitates a more intense cooperation between the International Society of Haematology and the International Society of Blood Transfusion. The time is ripe for coordinating the ever-increasing number of congresses, workshops, symposia and task forces, the proliferation of journals and periodicals, for obviating overlaps and costly duplications and multiplications of efforts and means, and for discovering how recent advances in these new specialities can be best applied in clinical practice and in the production of biologicals

Superspecialization and the new technological advances in automation and computerization also harbour the danger that physicians will be further removed from the patient. New sophisticated techniques are indispensable and highly specialized experts are invaluable in consulting colleagues about the management of patients. It is, however, of utmost importance that

physicians feel comfortable with recent advances and not rely solely on consultants and computer-integrated laboratory data.

The main importance of an international congress is to provide authoritative up-to-date information and concise summaries of the most significant recent advances in science and medical practice. Of course, no congress can cover all the important fields. Topics have to be selected. And this is the most difficult task in setting up the scientific programme.

The plenary session papers have been selected from those important topics where new breakthroughs provide exciting perspectives in our understanding of the basic phenomena underlying haematological and immunological diseases, in their diagnostics and therapy, and the most recent advances in haemotherapy.

The second main task of an international congress is to provide an opportunity for personal contacts, for the flow of ideas, for free discussions. Keeping this in mind in addition to symposia, workshops and poster discussions, we have organized an informal "Ask the Experts" evening session with a Wine and Cheese Party.

I wish to conclude my brief opening remarks by a statement which I believe you will all accept: the most important aim of our research, clinical and laboratory work should be to serve the interests of the patients to the best of our knowledge. The increasing sophistication of investigative and production methods have necessitated a steadily increasing number of science graduates in our haematological and blood transfusion services. One of the most fascinating experiences I constantly encounter is that irrespective of the university degree they also strive to improve the life and well-being of patients.

It is inspiring to know that the application of recent results in biomedicine have affected the life of millions, but one should never forget that the difference in life expectancy of patients suffering from these inborn or acquired population diseases is highly dependent on whether they live in developed or developing countries. We who fight day by day to save single

lives cannot remain neutral observers of how the arms race is killing people by diverting scarce resources from urgent health needs especially in developing countries.

We transfusiologists and haematologists have to be sincerely committed to helping our fellowmen, otherwise we cannot persuade people day by day to donate blood, to undergo plasma- and cell-apheresis, to become tissue or transplant donors. We who sometimes transfuse several hundred units of donated blood to a single patient have still another important duty. We have to explain to the public on a strictly scientific basis that although the individual genetic polymorphism is infinite, the main characteristics of human blood are the very same. There are differences in ABO and Rh groups which matter, but nationality, religion or political belief never preclude the transfusion of a donated blood. In the growing threat of thermonuclear war the future of mankind is at stake. I feel that it is our moral obligation to make our collective voices heard: Give blood and don's shed blood!

CONTENTS

Recent Advances in Haematology, Immunology and Blood Transfusion
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THE MOLECULAR GENETICS OF HUMAN GLOBIN GENES AND THALASSEMIAS

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The last five years have witnessed remarkable progress in our understanding of the structure, function, and abnormalities of human globin genes. At present we have available the structural features of each gene in exhaustive detail by gene cloning, mapping, and DNA sequencing and, the precise definition of specific lesions in many forms of thalassemia. Here the major aspects of the human globin gene system will be reviewed prior to discussion of some of the molecular defects identified in the thalassemias. Primarily illustrations of the pathology in the thalassemias will be taken from studies in my laboratory and those carried out in collaboration with the laboratories of Haig Kazazian, and Dean Hamer.

THE HUMAN GLOBIN GENES

Human globins are each encoded by a structural gene residing in the DNA. The α-like genes are clustered on the short arm of chromosome 16, whereas the β-like genes are located on the short arm of chromosome 11 (Orkin and Nathan, 1981). In the α-cluster there are two expressed α-globin structural genes (α1 and α2), located less than 3 kb apart at the 3'-end of the complex (Fig. 1) (Orkin, 1978). Upstream or 5' from these loci are two pseudogenes, ψα1 and ζ1, the latter an embryonic α-like gene with a single stop codon within the normal coding region. Further upstream is the embryonic, expressed ζ-gene, ζ2 (Lauer et al. 1980). In the β-complex, reading 5'-3' are the single embryonic gene, ε, two γ (or fetal) genes $^{G}\gamma$ and $^{A}\gamma$, a pseudo β-gene (ψβ1), the δ-gene, and the adult β-gene (Fig. 1) (Fritsch et al. 1980). Both complexes have genes arranged embryonic-fetal-adult in the DNA. All globin genes have two intervening sequences, separating the coding region into three portions, the exons. In the case of the α-genes these intervening sequences (IVS) are small (less than 150 bp), whereas in the β-like genes, the second IVS is generally about 850 bp in length. The intervening sequences are transcribed in erythroid cells into a single, colinear RNA that is a mosaic of coding and intervening sequences. Within the nucleus RNA processing enzymes excise the intervening sequences and ligate the coding blocks together to assemble the final mRNA molecule. The exact nature of these steps is unknown. Analysis of DNA sequences of many normal genes has indicated that there are preferred (or consensus) sequences at the boundaries of the exons and the IVS. IVS always begin with GT and end with AG (the so-called Chambon rule) (Breathnach et al. 1978).

The precise DNA sequences of all the human globin structural genes have been determined by work in many laboratories. For example, in Figure 2 is

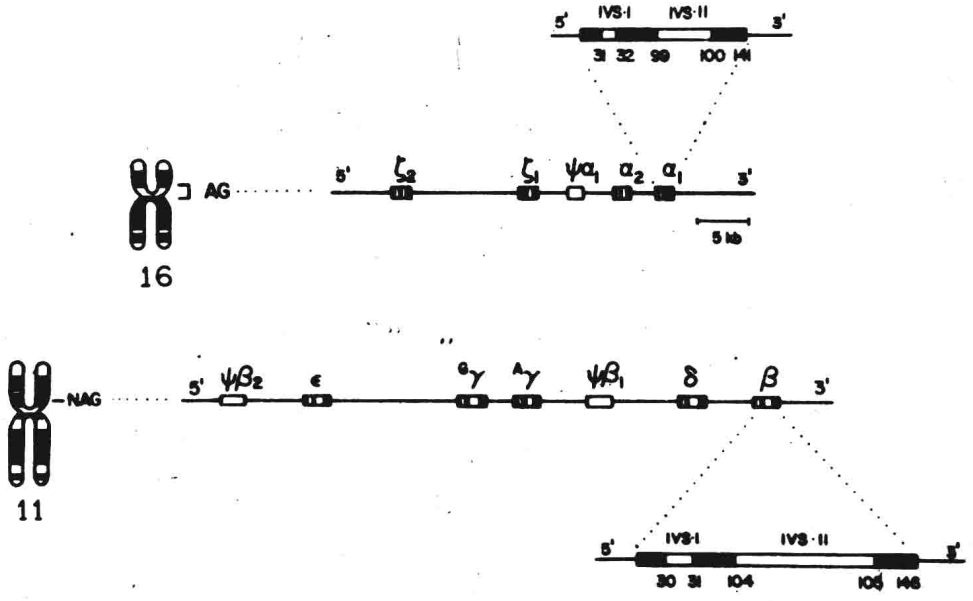

Figure 1. Globin Gene Organization.
The physical map of the globin gene clusters, their positions on the chromosomes, and their fine structures are illustrated above.

the structure of the two α-globin genes. What is particularly interesting about this pair is the sequence identity in the 5'-half and the divergence at the 3'-end, located in the 3'-untranslated region. Some of the implications of these sequence data are discussed below.

The normal sequences of the human globin genes provide background for work on the various forms of thalassemia, and for the examination of silent variations (polymorphisms) in populations.

THE ALPHA-THALASSEMIAS

The α-thalassemias are represented by several disorders, extending from the lethal form, homozygous α-thalassemia (hydrops fetalis with Hb Bart's), through a moderate hemolytic anemia, Hb H disease, to a minimal anemia (thalassemia trait), to a totally benign carrier state (the "silent carrier state"). For some time it was thought most likely that these various states mirrored the number of expressed α-globin genes. Normally four α-genes are present and expressed. If all genes are inactivated or missing in the DNA, the hydrops fetalis state would result in which no α-globin is synthesized in the fetus. Inactivation or deletion of three of the four normal genes would produce significant excess of β-chains and hemolytic anemia of the Hb H type. Loss of half the normal α-globin gene expression would produce a more mild state, and loss of a single α-gene would be quite insignificant. Initial molecular hybridization analyses strongly supported these conclusions and demonstrated deletion of α-genes in the hydrops fetalis state (Ottolenghi et al. 1974; Taylor et al. 1974). Restriction enzyme analysis using the method of Southern provided a more sensitive means of examination of the α-thalassemias and demonstrated that deletion

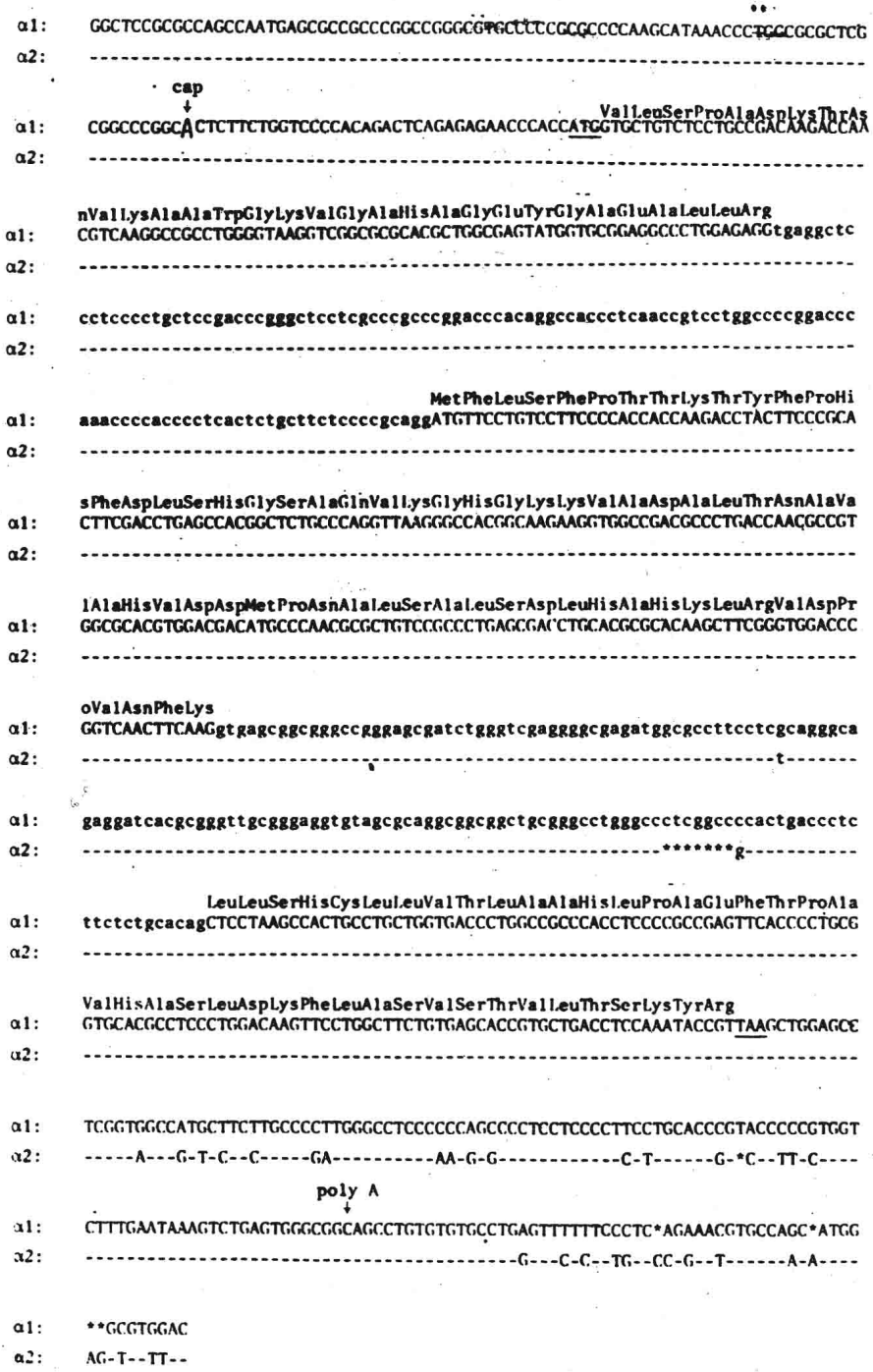

Figure 2. Nucleotide Sequences of the α1 and α2-Globin Genes.
The coding (mRNA) strand is depicted for the α1-gene with differences noted for α2.

of α-genes in α-thalassemias was most common, but that some individuals with α-thalassemias had α sequences in the DNA that did not effectively contribute to α-globin production (Orkin et al. 1979).

First, why is deletion of α-globin genes apparently so common? Part of the answer seems to lie in the physical make-up of the α-globin complex. The α-globin genes are highly homologous by their DNA sequence. What is most striking, however, is that the homology is not merely limited to the genes themselves, but extends into the 5'-flanking region for about a kb and then after some interruptions is maintained for short stretches (Lauer et al. 1980). Overall nearly 4 kb of DNA within the α-globin complex is highly homologous and duplicated. This is in marked contrast with the β-globin gene cluster in which homologous regions are limited to portions of the exons (excluding highly repeated Alu sequences in the flanking regions). The high degree of broad homology in the α-complex provides a large target size for recombination, or crossing-over, in the DNA. In fact, upon propagation of bacteriophage clones containing both α-genes in E. coli deletions of one gene, resembling those seen in humans, has been observed. If deletion of one α-gene from a chromosome is the result of this crossing-over process, we would expect to find the reciprocal three gene chromosome in the population. In fact, it has been observed in several groups, although at a somewhat reduced frequency relative to the single gene chromosome (Goossens et al. 1980; Higgs et al. 1980). The single-gene, or α-thal-2, allele can be generated in at least two ways, depending on which homology blocks alignment in the crossing-over (Embury et al. 1980). Strict homologous, but unequal, crossing-over within the α-genes leads to what appears to be a Lepore-like α-gene in which the 3'-untranslated region and the large IVS are derived from the α1-gene, but the 5'-flanking region is more like the α2-gene. The exact sequence features of a cloned α-thal-2 gene have led us to propose that unequal crossing-over in the α-complex preserves the sequence homology (by so-called gene conversion) and that the boundaries of identity are defined by small insertions or deletions of DNA (Michelson and Orkin, in preparation).

The deletions in the α-gene complex can be quite varied (Fig. 3). One that is particularly striking leaves a "2.6 kb Eco RI" fragment of the region, due to deletion of the entire complex except for the sequences 3'-to codon 57 of the α1-gene (Orkin and Michelson, 1980). This allele, which has been observed to date only in Mediterraneans removes ζ-gene sequences, and therefore would be lethal in the homozygous state. Other types of deletions of DNA in this cluster have also been described.

What about situations in which the structure of the α-globin gene region appears grossly normal but α-globin production is deficient? This is formally analogous to the situation in most β-thalassemias. In Mediterraneans we initially reported the identification of such an allele. Distinction of the α1 and α2-mRNAs on the basis of their 3'-untranslated regions allowed us to determine that the α2-gene was defective in one form of this disorder (Orkin and Goff, 1980). The gene was cloned and sequenced and thereby shown to have a pentanucleotide deletion at the first exon-IVS boundary, immediately after the invariant G nucleotide of the GT pair (Fig. 4) (Orkin et al. 1981). We proposed that this mutation alters RNA processing and prevents formation of the α2-mRNA species. This gene was linked to an SV40 vector and introduced into monkey kidney cells by transfection. The nature of the RNAs produced from this gene could then be compared with those generated by a normal α2-gene. Surprisingly the mutant α2-gene directed the production of a stable, but abnormally processed mRNA species. The normal 5' or donor splicing site of the IVS-1 was not used due to the tiny deletion. However, a donor-like sequence in the first exon was instead used

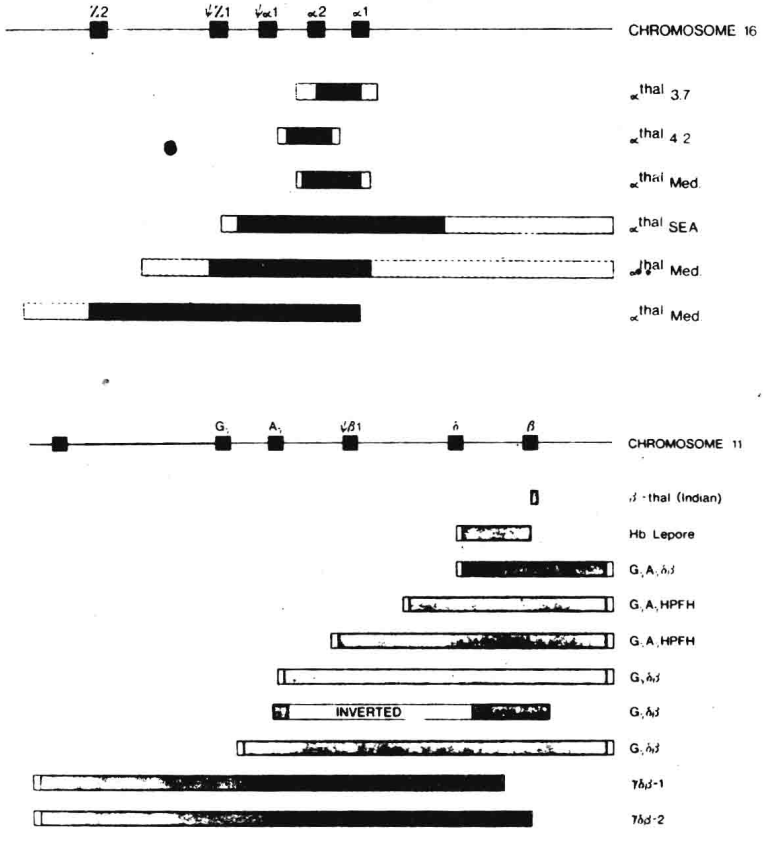

Figure 3. Deletions of DNA in Various Thalassemias.
Areas known to be deleted are shown in black. Uncertainties at the boundaries of some deletions are shown by the cross-hatching.
Top, α-complex - bottom, β-complex.

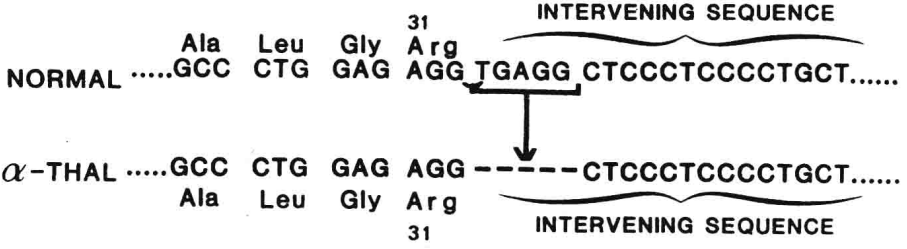

Figure 4. Pentanucleotide Deletion in a "Nondeletion" α-Thalassemia Gene.
The ends of exon-1 of the normal and α-thalassemia genes are shown along with the initial segment of IVS-1. Following the GT of the invariant GT, the pentanucleotide TGAGG is deleted in the mutant α2-gene.

for splicing to the normal end of IVS-1 (Fig. 5) (Felber, Orkin and Hamer, 1982). Therefore, a segment of the first exon was missing in the assembled mRNA. Such alternative splicing appears to occur at an exceedingly low rate from the normal gene as well. In the bone marrow of the affected individual we could demonstrate the existence of the abnormally processed mRNA. However, its level was lower than would be predicted by the cultured cell experiments and it was totally absent from reticulocytes. Taken together, these data suggest that the abnormally processed mRNA is unstable in vivo but not in the cultured cells. Similar conclusions have been reached in the study of several β-thalassemia mutations (Busslinger et al. 1981; Treisman et al. 1982). This one form of so-called "nondeletion" α-thalassemia is in fact due to a small deletion in the gene that leads to aberrant RNA processing. More recently another variety of nondeletion α-thalassemia, first described in a Chinese family, has been reported (Goossens et al. 1982). In this instance, the disorder is actually a hemoglobinopathy, in that the mutation is within the second exon and leads to production of a very unstable α-globin chain. The abnormal α-globin is removed so rapidly by precipitation or proteolysis that it is comparable to no production of α-globin from the affected gene. This, too, is a mutation of the α2-gene.

Some abnormal α-globin products are regularly associated with the phenotype of α-thalassemia. These are elongated α-globins produced by mutation of the normal termination signal for translation and subsequent read-through into the 3'-untranslated region of the mRNA (Clegg et al. 1971). These globins, of which Hb Constant Spring is the best described, are all mutations of the α2-globin gene. This is the case, since the elongated peptide of these globins cannot be encoded by the 3'-untranslated region of the α1-gene (Michelson and Orkin, 1980).

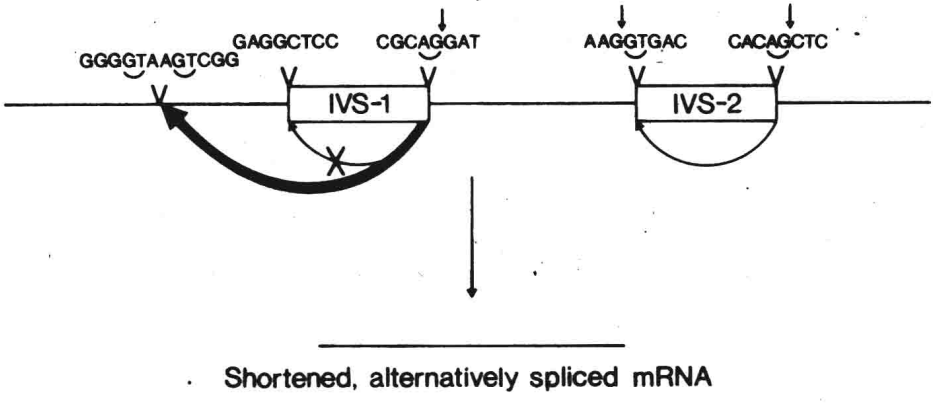

Figure 5. Alternative Processing of the "Nondeletion" α-Thalassemia Gene. Normal processing of the extended RNA at the top occurs from the ends of the IVSs. The pentanucleotide deletion (see Fig. 5) destroys the normal 5'-IVS-1 splicing site (shown by the x). Alternative splicing, however, occurs from the ends of IVS-1 to a donor-(5') like splicing signal in exon-1. This generates a shortened, alternatively spliced RNA.