Gene therapy: **Repairing haemoglobin disorders with ribozymes**

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A ribozyme-mediated approach has made it possible to replace the region in β globin mRNA containing the sickle-cell-anaemia mutation with a γ-globin-encoding sequence. This is an interesting new way of correcting monogenic disease, but there are major problems to overcome before it could be applied in the clinic.

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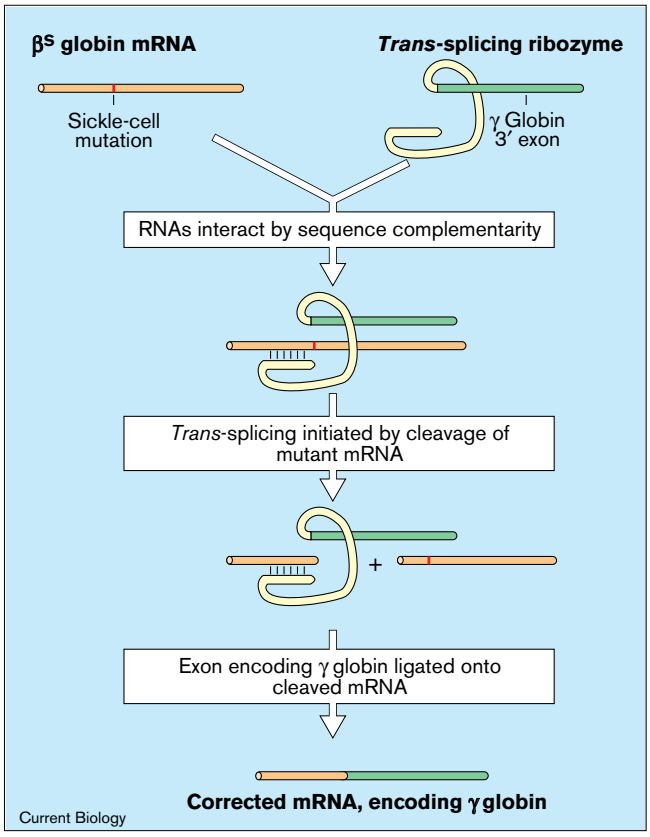
The inherited disorders of haemoglobin, particularly the thalassaemias and sickle cell anaemia, are the commonest monogenic diseases and are likely to pose an increasingly serious public health problem for many countries in the new millennium [1]. Control of these disorders by prenatal diagnosis is not acceptable to some countries, and although they can be cured by bone-marrow transplantation, this is possible only if there is a matching donor available. Symptomatic treatment is expensive and not entirely satisfactory. Hence, particularly as their molecular pathology is so well understood, there has been great interest in exploring more definitive ways of treating these disorders by genetic manipulation.

The development of gene therapy for the haemoglobin disorders poses formidable problems [2]. Human adult haemoglobin, haemoglobin A, is made up of two pairs of globin-chain subunits, α and β, which must be synthesised in approximately equal amounts; any imbalance of globin-chain production leads to a thalassaemic phenotype. Furthermore, a high level of gene expression, producing somewhere in the region of 15 picograms of haemoglobin per red blood cell, is required. And although much is known about the regulation of the α and β globin gene clusters, it is still not absolutely clear whether all the sequences have been identified that are required for tightly regulated, high level expression over a long period.

There has been considerable success in obtaining high-level human globin gene expression in transgenic animals [3,4], but despite a great deal of work this has not been achieved using any of the currently available vectors for gene transfer. To obtain a useful clinical result, it would be necessary to transfect a relatively large stem-cell population. Here, again, it has been very difficult to develop gene-transfer systems of the appropriate efficiency. And although there have been successes in correcting the β globin disorders *in vitro* by site-directed recombination, the level of efficiency is so low as to render this impractical, at least with current technology [5].

There is, however, a completely different approach to the control of the β globin disorders [6,7]. In fetal life, the major haemoglobin is haemoglobin F, which has two α chains and two γ chains ($\alpha_2\gamma_2$). Over the first few months after birth, γ chains are replaced by β chains, reflecting a switch from haemoglobin F to haemoglobin A production. It has been known for a long time that those patients with β thalassaemia or sickle cell anaemia who synthesise a relatively large amount of haemoglobin F after the neonatal period have a milder clinical disorder. In the case of sickle cell anaemia, haemoglobin F interferes with the polymerisation of sickle haemoglobin and hence reduces the rate of intravascular sickling. In β thalassaemia, augmented γ chain

Figure 1



Ribozyme-mediated repair of sickle β globin transcripts in human erythrocyte precursors. The figure outlines the scheme for converting β^S globin messenger RNAs into transcripts encoding γ globin, as reported recently by Lan *et al.* [10].

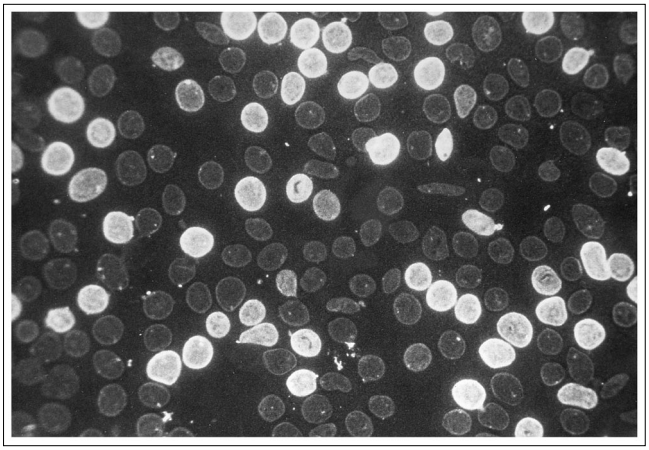
production reduces the overall level of globin chain imbalance caused by defective β chain synthesis. The ameliorating effects of unusually high levels of fetal haemoglobin on these diseases have been highlighted by a series of 'experiments of nature', in which it has been observed that patients who also inherit genes that result in high levels of γ chain synthesis in adult life have a much milder illness [7,8]. The encouraging results of efforts directed at the pharmacological manipulation of fetal haemoglobin in the haemoglobin disorders are reviewed elsewhere [9].

Lan *et al.* [10] have recently described a novel approach to the correction of the molecular defect that underlies sickle cell anaemia, which combines the double benefit of deleting the sickle-cell (β^S) mutation and, potentially, augmenting fetal haemoglobin levels in the red cells. Their ingenious experiment exploited the catalytic properties of ribozymes to alter the defective mRNA produced by the mutant β haemoglobin gene, and is outlined in Figure 1. First, a source of nucleated red cell precursors was obtained by growing erythroid colonies from the peripheral blood of patients with sickle cell anaemia. A *trans*-splicing group 1 ribozyme was then used to alter the mutant β globin transcripts in these cells. In short, Lan *et al.* [10] created a *trans*-splicing ribozyme that was able to convert the β^S globin transcripts into RNAs encoding γ globin. Remarkably, sequence analysis of eight different cDNA subclones derived from blood cells that were taken from sickle cell anaemia patients and treated in this way demonstrated that, in each case, the ribozyme had correctly spliced its γ globin 3′ exon onto nucleotide 61 of the β globin target transcript and, in the process, had maintained an open reading frame for translation of the mRNA. These experiments suggest that *trans*-splicing ribozymes are able to correct mutant globin transcripts in primary human red cell precursors at a high level of fidelity.

As Lan *et al.* [10] point out, RNA repair of this type could be particularly appropriate for the treatment of sickle cell disease, because not only is the sickle mutation removed by the ribozyme but, at the same time, the globin chain that is encoded by the hybrid RNA product has the anti-sickling properties of the γ chains of fetal haemoglobin. Lan *et al.* also suggest that it may not be necessary to repair 100% of the transcripts; patients with sickle cell disease that express γ globin at 10–20% of the β^S globin level in most of their red cells tend to have a milder disease.

This approach has other potential advantages. In adult life, haemoglobin F production is uneven between different red-cell precursors (Figure 2). Because haemoglobin F interferes with sickling, those red cells that produce relatively more fetal haemoglobin undergo intense selection in the circulation of patients with sickle-cell anaemia, and have a longer survival [6,7]. A population of precursors treated in this way would thus be expected to expand in the blood, simply by selective amplification.

Figure 2



The distribution of haemoglobins F and S in the blood of a patient with sickle cell anaemia. This preparation was treated with a fluorescent antibody that labels γ globin chains. The unequal distribution of haemoglobin F among the red cell population is clearly shown, and in the centre of the field there is an irreversibly sickled cell which contains no detectable fetal haemoglobin.

Although these results are very encouraging, there are formidable problems to be overcome before the fruits of the elegant experiment carried out by Lan *et al.* [10] can be applied in clinical practice. First, it is not clear how it would be possible to scale up the operation such that a sufficient number of red-cell precursors could be treated *in vivo* to obtain a useful clinical effect. And as this form of therapy is directed at transcription products, rather than the defective gene itself, how often would it have to be repeated to provide effective long-term therapy?

There is another problem which is not just confined to this particular approach, but which is relevant to any form of gene therapy for sickle cell anaemia. It has been believed for a long time, both from the results of *in vitro* viscosity studies and from clinical observations, that to control sickling, either *in vitro* in artificial mixtures of sickle cells and normal cells or in patients, it is necessary to reduce the sickle cell population to somewhere below 30% of the total; above this level there is a rapid increase in blood viscosity on deoxygenation [11]. Thus, at least theoretically, there is a danger in adding a normal — that is non-sickling — population of red cells to the blood of a patient with sickle cell anaemia unless it constitutes more than about 70% of the total cell population. At any lower frequency, the non-sickling cells may increase blood viscosity without sufficiently reducing the overall numbers of sickling cells, and hence exacerbate the disorder.

Indeed, this last concern has formed the basis for current policies regarding blood transfusion in sickle cell anaemia. Patients with serious complications are treated by exchange transfusion to reduce the proportion of sickleable cells to below 30% of the red cell population, and conventional transfusion is given only if the haemoglobin level is unusually low. If long-term transfusion is embarked on, it is designed to suppress endogenous red cell production to maintain a low level of sickle cells in the blood. These policies may have to be revised as the result of more recent clinical trials suggesting that it may be safe to transfuse children with this disease to a haemoglobin level of about 10 grams per decilitre [12] with normal red cells. However, even patients who have unusually high haemoglobin and haemoglobin F levels and are protected against some features of sickle cell anaemia, like those of Eastern Saudi Arabia, still have bone disease and other severe complications [13].

There is, therefore, a potential danger in any form of gene therapy that might produce a population of non-sickleable cells in the blood of a patient with sickle cell anaemia. Until more is known about the relationship between the haemoglobin S and haemoglobin F levels and the clinical course, and the effects of maintaining higher haemoglobin levels by transfusion without reducing the numbers of sickle cells to the levels that have hitherto been advocated, it will not be possible to predict the effects of correcting the sickling defect in a limited sub-population of red cells. Despite these concerns, however, this new approach to the correction of monogenic disease through ribozyme-mediated repair is an interesting addition to the gene therapy armamentarium, and one which should certainly be explored further.

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