contrast, the same sequence shows many differences when compared among humans, chimpanzees, gorillas, and orangutans. Confronted with the mathematically rather awkward situation of dealing with the absence of variation, they calculate the minimum amount of nucleotide diversity that must exist in this intron in order to allow their observation of no variation to lie within the 95% confidence interval of the expected. They then go on to estimate a time to the common ancestor of the Y chromosome by calculations that, surprisingly, do not take the N_e of males into account. The date they come up with is 270,000 years ago, more recent than can be envisioned for the origin of *Homo erectus*. It thus fits with the recent mitochondrial date of the modern human gene pool.

Could there be other explanations for this observation? Peter Goodfellow and his group (5), as well as others, have studied the evolution of another gene on the Y chromosome, the *SRY*, which is directly responsible for inducing maleness. They find that when this gene is compared among species, it evolves very rapidly. Some parts of the protein even show such a high number of amino acid replacement substitutions relative to silent substitutions that one must conclude either that part of the protein has no function at all or that its evolution is driven by directional selection—a very rare phenomenon among protein-coding genes. Yet, within species very little variation is found. How could this be? A possible explanation is the absence of recombination on the Y chromosome. Normally, a mutation that is selected will, on its way to fixation, be broken loose from accompanying regions on the chromosome where it initially occurred by recombination. Not so on the Y chromosome. Here, a selected allele will drag with it all the rest of the nonrecombining part of the Y chromosome in potentially gigantic selective sweeps. This could homogenize the gene pool within the species, yet allow rapid evolution between species. So does the finding of Dorit *et al.* reflect a lack of recombination on human Y chromosomes rather than say something about our past?

A recent paper by Nachman and Aquadro (6) is of relevance to this question. They have compared the amount of within-species polymorphism among mice for a noncoding region flanking the *Sry* gene and related it to the amount of between-species divergence for the same sequence. They compared this ratio to the same ratio for the rapidly evolving control region of the mtDNA in the same mice. If any of the two loci were the victims of a selective sweep in the recent past, this would show up in the form of a reduced intraspecific polymorphism. The results show that they cannot reject the hypothesis that the *Sry*, and thus the entire nonrecombining part of the Y chromosome, evolves neutrally. Therefore, there seems to be no reason to believe that selection acts on the Y chromosome.

Of course, the mtDNA data, as well as the *ZFY* data, could both be explained by selective sweeps. However, when two loci rather than one tell the same story, the selectional explanation becomes less likely. But more work is still needed on how to calculate the coalescence time when no variation is observed. It is also of importance to see to what extent the *ZFY* intron varies among nonhuman primates. Other regions in the human genome, so small that recombination over the time span relevant for human history will not be a major factor, should also be examined to show whether our recent common ancestry is a genome-wide phenomenon. So far, we have just seen the small beginning of what someone has called the biggest archaeological excavation of all times: the quest into the genome to reveal our past.

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The Biophysics of Sickle Cell Hydroxyurea Therapy

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Hydroxyurea has recently been shown to be effective for treating adult patients with sickle cell disease (*1*). This approach, pioneered by Platt and colleagues (*2*), represents the first specific therapy for this class of genetic diseases (*3*). The sequence of pathological events in sickle cell disease is (i) the polymerization of the abnormal hemoglobin S to form a viscous gel upon deoxygenation of the red cells in the tissues, (ii) a large decrease in the deformability of the cells, (iii) occlusion of the vessels of the microcirculation by the stiffened cells, (iv) insufficient oxygen delivery, and finally, (v) tissue damage, which may cause episodes of severe pain known as a "sickle cell crisis." This sequence is less likely in patients with an unusually high fraction of "F cells." F cells contain about 20% fetal hemoglobin (hemoglobin F) and 80% hemoglobin S, whereas "S cells" contain only hemoglobin S. The principal therapeutic effect of hydroxyurea in sickle cell patients is believed to be the increase in the fraction of F cells, from about 30% to about 50% (*1*). Although the biochemical mechanism by which hydroxyurea stimulates hemoglobin F synthesis is not yet known, the biophysical mechanism of how hemoglobin F has such a profound inhibitory effect on polymerization in F cells is well understood. The story of this mechanism is an example of physics and physical chemistry providing direct and deep insights into the molecular pathology of a human disease (*4*).

The net effect of hemoglobin F in F cells is simply to dilute the hemoglobin S. How does such a small decrease in hemoglobin S concentration have a therapeutic effect? Kinetics hold the key (*5*). Upon deoxygenation of sickle red cells, there is a pronounced delay before the explosive appearance of polymer (see figure). If the delay time is less than the ~1 s transit time of a red cell through the microcirculation, polymerization will occur while the cell is squeezing through small vessels, and blockage may occur (see figure). If the delay time is longer than the 10 to 20 s that it takes the cell to return to the lungs to be reoxygenated, polymerization will not occur. Although polymerization takes place in almost all sickle cells at venous oxygen pressures at equilibrium in vitro, there is no polymerization in the vast majority of cells in vivo because the delay time is longer than 10 to 20 s (*6*).

The delay time is incredibly sensitive to solution conditions, inversely proportional to about the 30th power of the initial hemoglobin S concentration (*5*). In contrast, ordinary chemical reaction rates depend on the first or second power of the reactant concentration. Consequently, a small decrease in the concentration of hemoglobin S has a large effect on the delay time. Decreasing the intracellular hemoglobin S concentration to delay polymerization is

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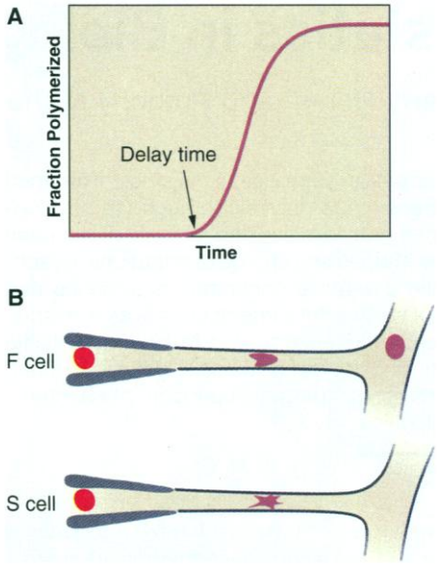
therefore an obvious strategy for the treatment of sickle cell disease (*5*, *7*), and that is precisely how hemoglobin F works.

What is the molecular explanation of the delay time and its enormous concentration dependence? Because polymerization is a highly cooperative process, it behaves like a two-phase system with a well-defined critical concentration or solubility (*8*). Polymers therefore form by some kind of nucleation mechanism. Conventional nucleation mechanisms fail, however, to explain the delay period. A novel, double nucleation mechanism provides the answer (*9*). In this mechanism, the first polymer molecule in a given solution volume forms by "homogeneous" nucleation. This molecule then serves as a template for the "heterogeneous" nucleation of additional polymers. The more polymerized hemoglobin, the larger is the surface area available for heterogeneous nucleation. In this way, heterogeneous nucleation provides the autocatalysis that is responsible for the delay period. The mechanism also readily explains the high concentration dependence of the delay time: The apparent reaction order is simply half the size of the critical nuclei. The general features of the double nucleation mechanism can be literally "seen" in fuzzy optical images of growing polymers, including branching at high supersaturation, indicating heterogeneous nucleation, and elongation only at low supersaturation where heterogeneous nucleation is shut down (*10*).

Predicting exactly how much the delay time is increased by hemoglobin F requires some explanation of the structure and thermodynamics of polymer formation. Hemoglobin is a tetramer consisting of two pairs of identical subunits, α and β (or γ in the case of hemoglobin F). The $\beta 6$ (Glu) $\rightarrow$ (Val) mutation in hemoglobin S creates a "sticky" hydrophobic patch on the molecular surface resulting in the polymerization of deoxygenated or partially deoxygenated tetramers in the T (deoxy) quaternary conformation. In a mixture of hemoglobin S ($\alpha_2\beta_2^S$) and hemoglobin F ($\alpha_2\gamma_2$), there is a third tetrameric species, the hybrid molecule $\alpha_2\beta^S\gamma$ (*11*). A key result from equilibrium studies is that there is very little copolymerization of either $\alpha_2\beta^S\gamma$ or $\alpha_2\gamma_2$ tetramers (*4*, *12*), which can be understood from the structure of the polymer (*13*). Tetramers containing either one or two γ subunits, therefore, appear to act as virtually inert diluents.

In F cells, the net effect of the 20% hemoglobin F is, then, to dilute the $\alpha_2\beta_2^S$ (polymerizing) tetramers by about 35% (from the binomial distribution: 0.64 $\alpha_2\beta_2^S$, 0.32 $\alpha_2\beta^S\gamma$, and 0.04 $\alpha_2\gamma_2$). A naïve calculation suggests that dilution of 1/0.64 increases the delay time in F cells by about $(1/0.64)^{30} \approx 10^6$-fold compared to the experimental value of "only" $\sim 10^3$ (*7*). However, this simple calculation neglects the fact that the nonpolymerizing molecules containing γ subunits take up space in the highly concentrated red cell and thereby increase the effective thermodynamic concentration (activity) of the polymerizing $\alpha_2\beta_2^S$ tetramers (*8*, *14*). This effect can be dealt with quantitatively by evaluating the supersaturation of the solution in terms of activities instead of concentrations. The reciprocal delay time depends on the activity supersaturation raised to about the seventh power, indicating a nucleus size of about 14 tetramers, and the resulting increase in the delay time is calculated to be very close to the experimental value of $\sim 10^3$ (*15*).



Delay time of hemoglobin S polymerization, and transit of red cells through the microcirculation. (**A**) Kinetic progress curve for the polymerization of hemoglobin S. (**B**) (**Top**) An F cell passes through a capillary and escapes without intracellular polymerization because the delay time is longer than the transit time. (**Bottom**) In an "S cell," the delay time is shorter than the capillary transit time, and polymerization and cellular deformation ("sickling") occur while the cell is in the capillary, with the possibility of occluding the vessel.

The critical concentration for polymerization (sickling) in a typical F cell is about 0.3 g/cm^3, the estimated solubility at mixed venous oxygen pressures (*4*) (total intracellular hemoglobin concentrations vary from 0.25 to 0.5 g/cm^3). F cells with total hemoglobin concentrations less than 0.3 g/cm^3 will never sickle at this pressure. F cells with total hemoglobin concentrations greater than 0.3 g/cm^3 would sickle at equilibrium but do not sickle in vivo because of the $\sim 10^3$-fold increase in the delay time. This increase is sufficient to allow almost all F cells to escape the microcirculation and be reoxygenated in the lungs before any significant polymerization has begun (unless, of course, they are trapped in a vessel occluded by another cell) (see figure). The larger the number of F cells, the larger the number of cells that do not sickle in vivo. That the additional F cells produced by hydroxyurea are slowly sickling or nonsickling is supported by a joint (Harvard, Johns Hopkins, and NIH) pilot study on the distribution of intracellular delay times in patients before and after hydroxyurea therapy (*16*).

There are many remaining problems in understanding the effects of hydroxyurea. The most pressing challenge for the molecular biologist is to unravel the genetic mechanism of the stimulation of hemoglobin F synthesis. For the biophysical scientist, it will be important to determine the effect of hydroxyurea on the distributions of critical concentrations and delay times in F cell populations, and also to establish with even greater certainty whether hydroxyurea does more to influence intracellular polymerization than just increase the number of F cells. Addressing these problems may lead to an even more effective therapy for sickle cell disease.

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