Mapping Human Autosomes: Evidence Supporting Assignment of Rhesus to the Short Arm of Chromosome No. 1

Abstract. *Rh-negative erythrocytes were found in the blood of an Rh-positive man suffering from myelofibrosis. Nucleated hemopoietic precursors were also circulating in his blood, and these cells had an abnormal chromosome complement from which identifiable chromosome segments had been deleted. Correlation of the serological and cytogenetic findings, combined with previous data, indicates that the Rhesus blood group locus is on the distal portion of the short arm of chromosome No. 1.*

Cancer cells can sometimes be shown to differ from the normal cells of the host in respect to some genetically determined trait. Because cancer cells also may differ from normal in their chromosome complement, correlation of phenotypic with cytogenetic change is of potential value in chromosome mapping. Our report is the first, however, in which this form of so-called deletion mapping has been successful in the localization of a human gene to a particular chromosome region.

A normally developed 45-year-old man entered the hospital because of excessive fatigue and weakness. He had splenic and hepatic enlargement, decreased erythrocyte survival time with splenic sequestration of cells, anemia (hemoglobin 6.0 g per 100 ml of blood), leukopenia, and a leukoerythroblastic peripheral blood film. The histology of his iliac bone marrow was consistent with the diagnosis of myelofibrosis. (Although this rare myeloproliferative disorder is not usually classified as cancer, it is often viewed as a preleukemic state.) Blood transfusion had not been given when the following studies were conducted.

Blood typing showed the patient's circulating erythrocytes to consist of a mixture of Rh-positive and Rh-negative cells. When first detected, the Rh-positive cells constituted 13 percent of the total; 15 months later, 7 percent; and 19 months after the original examination, only 1 to 2 percent. His Rh-positive erythrocytes were separable by means of antiserum to C (anti-C) or antiserum to D (anti-D), and in both cases the cells remaining unagglutinated were C- and D-negative. After removal of the Rh-positive cells by agglutination with anti-D, the remaining Rh-negative cells reacted with antiserum to c and antiserum to e in a manner comparable to those of an Rh-negative individual, and they also reacted strongly with antiserum to f.

The probable genotype of the Rh-positive mother of the patient is R^1R^1; quantitative studies of her erythrocytes with anti-C and anti-D revealed that they reacted like known R^1R^1 cells. The patient's father is Rh-negative, so that by constitution the patient is heterozygous R^1r. The population of Rh-positive erythrocytes in his circulation thus reflects the genetic endowment of the zygote from which he developed. The Rh-negative erythrocytes in his blood cannot be explained by any conventional mode of inheritance, and chimerism or dispermy are excluded as possible explanations for the mosaicism. His Rh-negative cells must have arisen as result of some somatic change affecting expression of the R^1 gene.

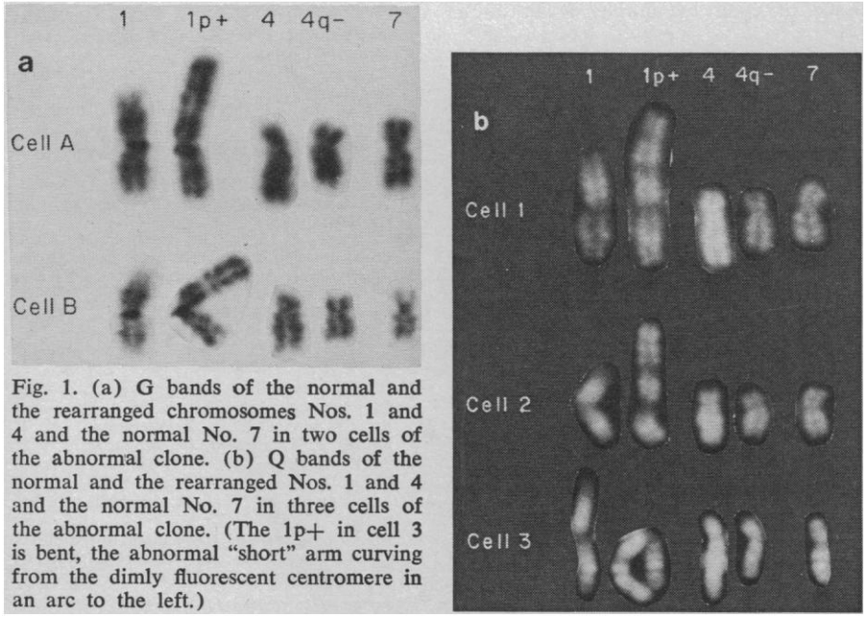

Fig. 1. (a) G bands of the normal and the rearranged chromosomes Nos. 1 and 4 and the normal No. 7 in two cells of the abnormal clone. (b) Q bands of the normal and the rearranged Nos. 1 and 4 and the normal No. 7 in three cells of the abnormal clone. (The 1p+ in cell 3 is bent, the abnormal "short" arm curving from the dimly fluorescent centromere in an arc to the left.)

In testing for other erythrocyte antigens, no evidence of an admixture of cell types was found. As to the Duffy blood groups, all the cells in both the Rh-positive and Rh-negative populations were Fy(a – b+), and in quantitative studies they reacted strongly with antiserums to Fy^b and to Fy3. In other major blood groups, the patient is heterozygous only at the ABO and the MNSs loci. He has the genotype *BO* (his mother is *OO*), and cells lacking the B antigen were not present. Because loss of an *O* gene would be undetectable, mosaicism at the ABO locus, though undetected, could not be excluded. The blood grouping tests would have revealed mosaicism at the MNSs locus, but it was not found. The erythrocytes were not polyagglutinable, and they aggregated normally when suspended in Polybrene solution, evidence that their sialic acid content was within the normal range. No irregular blood group antibodies were detected in the patient's serum. Both the Rh-positive and the Rh-negative cells had two types of the enzyme phosphoglucomutase-1 (genotype PGM_1^1/PGM_1^2).

Cytogenetic studies of dividing cells from the patient's circulating blood were made when 7 percent of his circulating erythrocytes were Rh-positive. Chromosomes of cells that were in spontaneous division after 1 day of incubation and also of cells that had been stimulated by phytohemagglutinin and were in division after 3 days were examined. A cytogenetic mosaicism consisting of two discrete populations of cells was detected in both cases. One population of cells had a normal chromosome complement, the other an abnormal. Among the cells dividing spontaneously 1 day after their removal from the circulation, only 1 of the 57 examined was normal (46,XY), whereas among the cells in division after 3 days (which are assumed to be predominantly phytohemagglutinin-stimulated lymphocytes), 24 of 29 cells were normal.

The chromosome complement of the

abnormal cells was only 45. A study of the rearranged chromosomes by Q-, G-, and C-staining techniques (*1*) showed that each cell contained the same complex and unbalanced translocation affecting chromosomes No. 1 and No. 4, and probably No. 7. The chromosome arms visibly affected were 1p, which had gained length, and 4q, which had lost length. Only one No. 7 chromosome was present in the complement. Comparison of the lengths of 1p and 4q of the normal and of the aberrant chromosomes revealed that the translocation was not a simple reciprocal one. The combined lengths of the rearranged 1p and 4q was greater than the combined lengths of the normal 1p and 4q, indicating the addition of chromosome material to 1p. Q- and G-banding (Fig. 1, a and b) indicated that the aberrant No. 1 chromosome was intact from 1qter to 1p32. Beyond that followed (i) a lightly stained region in which a narrow, more intensely stained, band was sometimes seen; (ii) two prominent, intense bands in tandem; (iii) a light band; and (iv) an intense terminal segment. Q- and G-banding of the aberrant No. 4 indicated that it was intact from 4pter to 4q28, beyond which was a narrow lightly stained band.

Based on these observations, the following postulates are made regarding the translocation and the composition of the two aberrant chromosome arms. Breaks occurred in 1p at or distal to 1p32 and in 4q near 4q28. The distal segment of 4q was translocated to 1p where it forms the distal, intensely staining segment of the 1p+. The two prominent intense bands on the aberrant 1p attached in tandem proximal to this terminal segment are derived from 7q and comprise at least the region 7q11 to 7q32. This interpretation, implying insertion into the 1p+ of a segment of 7q, appears warranted both because the two intense bands resemble the two dark bands of 7q more than any other chromosome segment of the normal complement and also because a No. 7 chromosome is missing from the complement; besides, both Nos. 9 in this clone, the only other chromosome that could have contributed similar tandem dark staining bands, were found to be intact by Q-, G-, and C-staining techniques. The three segments still to be accounted for by this interpretation of the rearrangement are 1p distal to its breakpoint, 7p and the centromeric region of No. 7, and the terminal segment of 7q distal to its more distal breakpoint. We postulate that the first two of these three segments were deleted from the complement, and that the third, distal 7q, was translocated to the broken end of 4q, thereby providing 4q with a telomere. Alternatively, it could be postulated that the distal segment of 7q is lost and the distal segment of 1p is translocated to 4q. Against this second interpretation is the fact that the pale band at the end of the aberrant 4q is too short to be the segment of 1p distal to the apparent 1p breakpoint (unless yet another more distal break in 1p is postulated to have occurred). Our interpretation of the translocation showing the postulated breakpoints is presented in Fig. 2.

In this patient, the blood-borne cells that divided spontaneously in culture may be viewed as hemopoietic precursors which, by the nature of his disease, arose in the bone marrow or sites of extramedullary hemopoiesis. The majority of these cells had an aberrant karyotype, and the majority of the

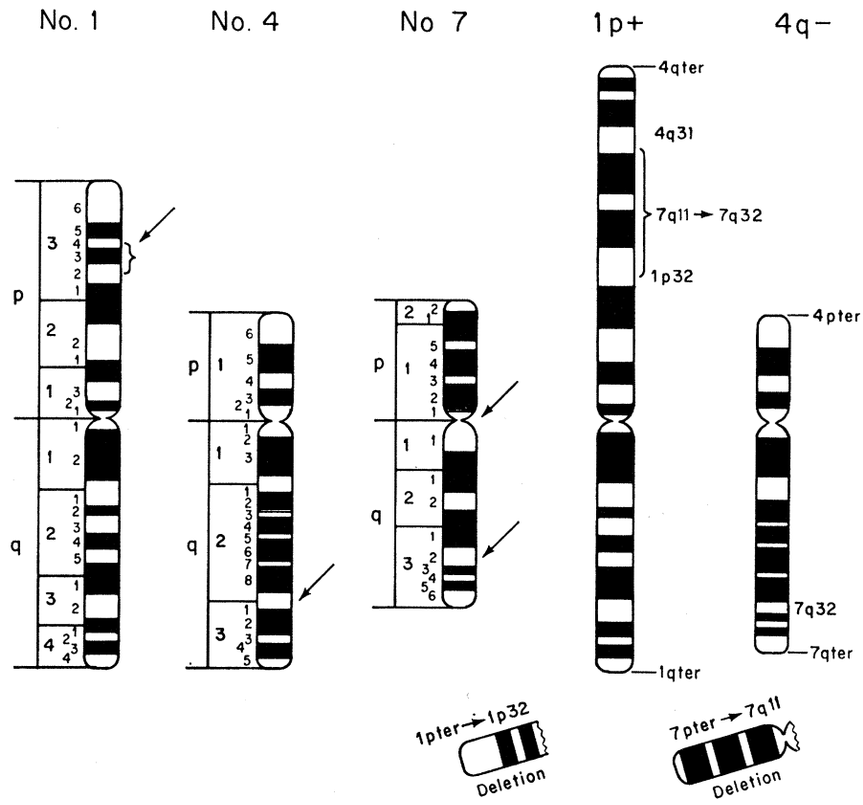

Fig. 2. Interpretation of the chromosome rearrangements in terms of the Paris Conference cytogenetic nomenclature (*1*). The diagram shows (left to right) the band patterns of the normal chromosomes No. 1, 4, and 7 and the rearranged No. 1 (1p+) and No. 4 (4q−), where p is the short arm; q, the long arm; +, increase in length; −, decrease in length; and ter, terminus of an arm. Numbers refer to chromosomes, chromosome regions, and chromosome bands; thus, 1p32 indicates chromosome No. 1, short arm, region 3, band 2. The arrows indicate sites of postulated breakage in the cell which became the progenitor of the abnormal clone of cells. The band pattern in the rearranged No. 1 is intact from the distal end of its long arm (1qter) past the centromere to at least the second band in region 1p3 (band 1p32), but it is abnormal beyond that. (Although we are uncertain as to the exact breakpoint in 1p3, the diagram shows that the segment deleted included band 1p33. However, we cannot exclude the possibility that band 1p33 and possibly even 1p34 were retained.) At that point, a segment probably of the long arm of a No. 7, from band 7q11 to 7q32, was attached. (The orientation of this segment could not be determined; that is, whether band 7q11 or band 7q32 was attached at the break in 1p3.) A segment which appears to be that from 4q (distal to the breakpoint at 4q31) was attached distal to the translocated segment of No. 7. Thus, deleted from the chromosome complement were: a segment of 1p distal to band 1p32, the entire 7p, the centromere of No. 7, and the segment of 7q from the centromere to band 11. The band pattern of the rearranged No. 4 was intact from the terminus of its short arm past the centromere to band 4q31. At this point was attached a very short segment of chromosome which stained lightly with Giemsa and showed pale fluorescence with quinacrine; it is presumed to be the terminal segment of 7q from band 32.

blood erythrocytes had lost an R^1 gene complex. In the absence of other information, it could have been assumed from this study that the locus for the Rh blood group is either on the short arm of chromosome No. 1 or the short arm or proximal portion of the long arm of No. 7, because, along with an Rh locus, these segments had been deleted from the major population of the man's dividing blood cells. But Ruddle *et al.* (*2*) have already deduced that the Rh locus is on chromosome No. 1, although evidence available until now had pointed to the long arm of this long chromosome as its position (*2*). Because the chromosome rearrangement in our patient resulted in deletion of a segment of chromosome No. 1, our present observations are well explained by the hypothesis that the Rh locus is located on the deleted segment, that is, on the distal segment of the short arm at some point between band 1p32 and the end of the arm (*3*).

Douglas *et al.* (*4*), using cell hybridization methods, presented evidence that PGM_1 is on 1p somewhere distal to band 1p32. The PGM_1 locus was not deleted by the chromosome rearrangement in the mutant clone of our patient, whereas the Rh locus was. We conclude therefore that the Rh locus lies distal to PGM_1 (*5*).

W. L. MARSH
R. S. K. CHAGANTI
New York Blood Center,
310 East 67 Street, New York 10021
FRANK H. GARDNER
Presbyterian–University of Pennsylvania Medical Center, Philadelphia
KLAUS MAYER
Memorial Sloan-Kettering Cancer Center, New York
PETER C. NOWELL
University of Pennsylvania School of Medicine, Philadelphia
JAMES GERMAN
New York Blood Center,
310 East 67 Street, New York

References and Notes

1. "Paris conference (1971): Standardization in human cytogenetics," *Birth Defects: Original Article Series* (The National Foundation, New York, 1972), vol. 8, No. 7.
2. F. H. Ruddle, F. Ricciuti, F. McMorris, F. A. Tischfield, Jr., R. Creagan, G. Darlington, T. Chen, *Science* **176**, 1429 (1972).
3. It is of additional interest that the patient's Rh-negative erythrocytes gave double-dose rather than single-dose serological reactions with antiserum to c and antiserum to e. If our hypothesis is correct, the Rh locus when hemizygous is fully expressed as though two genes were present.
4. G. R. Douglas, P. J. McAlpine, J. L. Hamerton, *Genetics* **74**, s65 (1973).
5. The inability to rule out mosaicism at the Duffy and the ABO loci in this patient makes it impossible to conclude from the present study alone that these loci are not on one of the deleted segments of chromosome No. 1 or No. 7. [However, Duffy has, as just stated, already been assigned to the No. 1 (*4*).] Demonstrable absence in our patient of mosaicism at the MNSs locus eliminates the possibility that it is on either of the deleted segments. Furthermore, deletion mapping in a child with a constitutional chromosome imbalance has shown that the MNSs locus is on the proximal portion of the long arm of No. 2. [J. German and R. S. K. Chaganti, *Science* **182**, 1261 (1973)].
6. We thank J. Fleming and J. Jensen (cytogenetics); and M. Blank, R. Øyen, S. Self, and J. Tegoli (immunohematology) for technical assistance. Supported from PHS grants HD04134, HL09011, AM11188, and CA12779, and by grants from the American Cancer Society and the John A. Hartford Foundation.

17 October 1973; revised 12 December 1973

Calcification on an Unstable Substrate: Marginal Growth in the Mollusk Pecten diegensis

Abstract. *Observations of the growing margin of* Pecten diegensis *show that calcification can occur on an unstable substrate, although this initial shell material is quite disordered. Later growth on the inner surface of the disordered material soon becomes ordered, which suggests that the calcification process benefits from the stability of a mineralized substrate, but does not utilize the substrate as a physical or stereochemical template.*

The formation of mollusk shells has been the object of speculation and study for a very long time, but the process is still imperfectly understood (*1*).

Investigations of the mineralogy and structure of shells, culminating in the classic work of Bøggild (*2*), have shown that many different structural arrangements of calcite and aragonite are utilized by mollusks, and that most shells are built of two or more layers with different structures.

Shells grow by the accumulation of new material on a substrate which usually consists of the parts of the shell that are in contact with the tissues of the mantle. The new shell material is usually of the same mineralogy and structural arrangement as the older shell which serves as its substrate; this led to the concept that the substrate plays the dominant role in the calcification process, serving as a crystal template to direct the precipitation of calcium carbonate from a solution maintained by the mantle tissues.

This concept has proved to be overly simplistic; it is now recognized that the organic component of molluscan shells is a precursor to mineralization, and that it may serve as a matrix to control the nucleation, mineralogy, and growth direction of the calcium carbonate (*3–5*). It also appears, based on experiments in shell regeneration, that the mantle can vary the type of organic

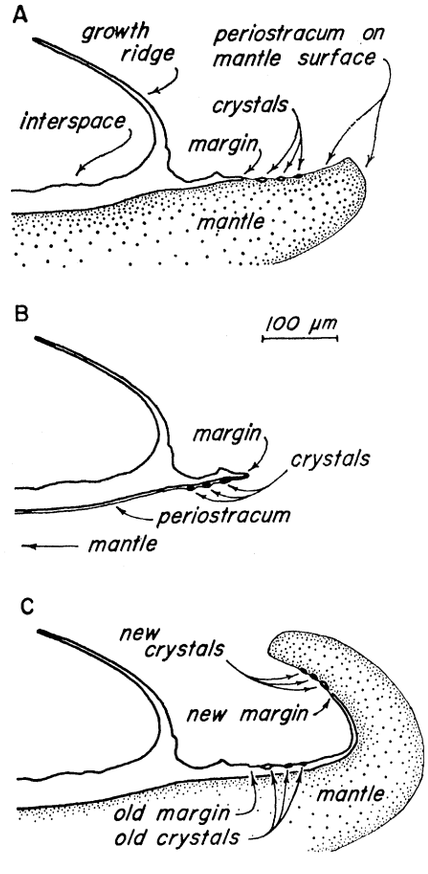

Fig. 1. Growth of the shell margin in *Pecten diegensis*; scale as shown. The direction of growth is to the right. (A) Mantle extended, with crystals forming under a thin periostracum on its surface and eventually coalescing to form the margin of the growing shell. (B) Mantle withdrawn into the shell but retaining contact with the periostracum; crystals not yet integrated into the margin are carried under the shell by the periostracum. (C) Several hours later; crystals observed in (A) and (B) have coalesced (in the same relative positions) into solid shell, and the mantle has moved out and up to begin forming a growth ridge. The interpretation is based on time lapse photomicrography and radial sections through shells.