the effects of FGF in the embryo (*3, 4*). It is increasingly important to know what parts of the embryo contain FGF, what parts release it, and what parts are capable of responding to FGF. The pattern of release and response should explain many of the features of signaling during gastrulation and neurulation.

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Activation of Developmentally Mutated Human Globin Genes by Cell Fusion

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Human fetal globin genes are not expressed in hybrid cells produced by the fusion of normal human lymphocytes with mouse erythroleukemia cells. In contrast, when lymphocytes from persons with globin gene developmental mutations (hereditary persistence of fetal hemoglobin) are used for these fusions, fetal globin is expressed in the hybrid cells. Thus, mutations of developmental origin can be reconstituted in vitro by fusing mutant lymphoid cells with differentiated cell lines of the proper lineage. This system can readily be used for analyses, such as globin gene methylation, that normally require large numbers of pure nucleated erythroid cells, which are difficult to obtain.

DURING HUMAN ONTOGENY THERE is a switch from embryonic to fetal globin production early in development and a switch from fetal to adult globin production around birth (*1*). In a group of genetic mutations known as hereditary persistence of fetal hemoglobin (HPFH), the latter switch is never completed, thus resulting in fetal globin production in adult life. HPFH mutations are of two general types: those characterized by deletions in the globin cluster (deletion HPFH) and those that consist of point mutations in the promoters of the fetal globin genes (promoter mutation HPFH) (*1*).

The mechanism whereby HPFH mutations activate fetal hemoglobin production in adult red cells is not yet known, and the functional molecular analysis of the affected cells is severely hampered by a lack of amenable model systems. HPFH-mutated genes are expressed in terminally differentiated cells, the erythroblasts; bone marrow cells and purified erythroblasts are required to perform functional studies of chromatin or structural studies such as DNA methylation. The same limitations apply to studies of all other human mutations expressed in the terminally differentiated cells of inaccessible tissues. For example, for studies of mutations expressed in the liver or the nervous system, hepatic or neural cells are required for functional studies.

Certain quiescent genes can be activated by cell fusion (*2*). With HPFH as a model, we used this approach to activate human developmental mutations and thereby facilitate their analysis. After transfer into mouse erythroleukemia (MEL) cells, normal lymphoid chromosomes express only adult and not fetal globin (*3*). We therefore examined whether lymphoid chromosomes containing HPFH mutations would express the fetal globin genes after chromosomal transfer into MEL cells.

We used two deletion HPFH mutations, HPFH-1 and HPFH-2 (*1*). In both cases there are large deletions in the β globin gene cluster (>150 kb), which remove the δ and

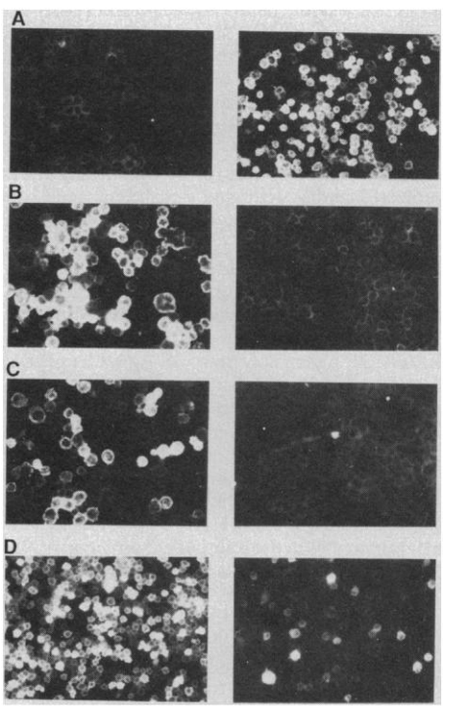

Fig. 1. Immunofluorescent labeling of lymphoid × MEL hybrid cells with monoclonal antibodies to the γ and β chains. Hybrids contain (**A**) the normal human β globin locus, (**B**) the HPFH-2 mutation locus, (**C**) the HPFH-1 locus from a HPFH-1/HPFH-2 compound heterozygote, and (**D**) the $-117\ ^{A}\gamma$ mutation locus.

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β globin genes but leave the $^{G}\gamma$ and $^{A}\gamma$ genes intact. In two separate experiments, Epstein-Barr virus (EBV)–transformed HPFH lymphocytes were fused with MEL cells (*4*), and cells that contained chromosome 11 were selected and analyzed (*5*). These lymphocytes were obtained from HPFH heterozygotes and thus contained the normal as well as the HPFH globin loci. Six hybrids that contained human chromosome 11 were rescued. Globin gene expression in these hybrids was analyzed with fluorescently labeled monoclonal antibodies specific to γ and β globin (Fig. 1), with isoelectric focusing of globin chains (Fig. 2, A and B), and with S1 nuclease mapping (Fig. 2, C and D). The chromosomal composition of the hybrids was determined by DNA blotting (Fig. 3). Hybrids that contained only the HPFH chromosomes (Fig. 3, A and B) expressed only γ globin (Fig. 1, B and C, and Fig. 2C). Heterozygous hybrids containing both the HPFH and the normal chromosomes expressed both γ and β globins (Fig. 2, A and C, and Fig. 3B). In contrast, hybrids that contained only wild-type chromosomes expressed only β globin (Fig. 1A and Fig. 2, A and C). Thus, unlike wild-type γ globin genes, γ genes from HPFH deletion mutations become activated after chromosomal transfer into MEL cells. We tested whether γ genes of nonhemopoietic mutant cells would also be activated in the hybrids by repeating the experiment with fibroblasts that had a deletion mutation. Hybrids obtained from the fusion of transformed HPFH fibroblasts with MEL cells also express fetal globin (Fig. 2C). Hence, the cellular source of these developmental mutations does not appear to be important for their activation in hybrid cells.

The mechanisms that underlie deletion HPFH and promoter mutation HPFH are thought to be different (*1, 6*). Mutations that affect the $^{A}\gamma$ gene promoter result in expression of $^{A}\gamma$ genes in adult life, whereas mutations of the $^{G}\gamma$ promoter lead to $^{G}\gamma$ HPFH (*1*). We therefore tested whether the promoter mutation HPFH γ genes would also be activated in lymphoid × MEL hybrid cells. We fused EBV-transformed lymphocytes heterozygous for an HPFH promoter mutation (a G to A transition in the

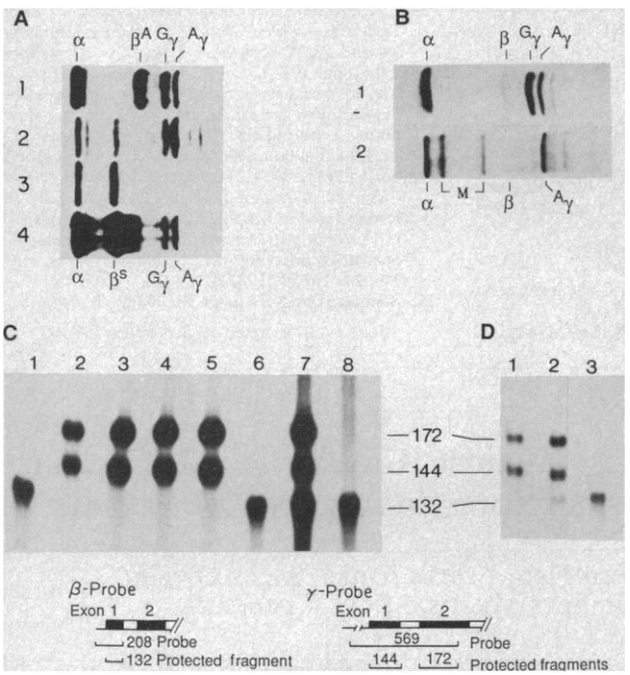

Fig. 2. Human globin gene expression in hybrids and controls. (**A** and **B**) Isoelectric focusing of globin chains. A-1, Mixed cord and adult blood controls; A-2, hybrid containing the β^s and the HPFH-2 chromosome and expressing β^s, $^{G}\gamma$, and $^{A}\gamma$ chains; A-3, hybrid with only the β^s chromosome and expressing only β^s chains; A-4, hemolysate of β^s/HPFH-2 compound heterozygote; B-1, control newborn hemolysate; and B-2, hybrid having only the −117 $^{A}\gamma$ HPFH chromosome. M indicates coprecipitated mouse globin chains. (**C** and **D**) S1 nuclease mapping of globin mRNA. Sizes are indicated in nucleotides. C-1, β mRNA; C-2, γ mRNA controls; C-3 and C-5, lymphoid × MEL hybrids that contain the HPFH-1 and HPFH-2 loci, respectively; C-4, fibroblast × MEL hybrid that contains the HPFH-1 locus; C-6 and C-7, hybrids that contain only the normal (C-6) or both the normal and HPFH (C-7) chromosomes from an HPFH-2 heterozygote; C-8, MEL × normal lymphoid hybrid showing only β mRNA; D-1, γ mRNA control; D-2 and D-3, lymphoid × MEL hybrids that contain the HPFH (D-2) and the normal (D-3) chromosome of a −117 $^{A}\gamma$ HPFH heterozygote.

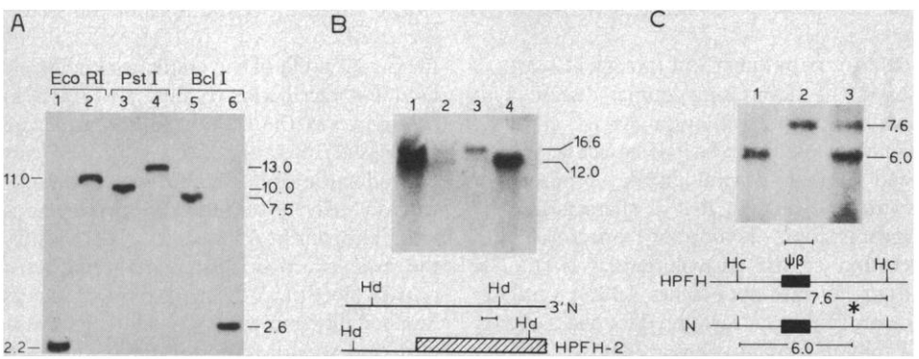

Fig. 3. DNA blots of lymphoid × MEL hybrids. Sizes are indicated in kilobases. (**A**) The HPFH-2 locus after digestion with Eco RI, Pst I, and Bcl I and hybridization with probe 3D (*9*). Lanes 2, 4, and 6, normal γ genes. Lanes 1, 3, and 5, hybrids containing the HPFH-1 locus. (**B**) The HPFH-2 locus after Hind III digestion and hybridization with probe 3D (indicated by the bar). DNA from HPFH-2/β^s heterozygotes (lane 1), hybrid containing both the β^s and the HPFH chromosomes (lane 2), hybrid containing only the HPFH-2 chromosome (lane 3), and normal DNA control (lane 4). (**C**) Distinction between hybrids carrying the normal or the −117 $^{A}\gamma$ HPFH chromosomes by use of linked polymorphisms. In this family, the normal β globin locus can be identified by virtue of a polymorphic Hinc II site, shown by the asterisk. Hybridization of Hinc II–digested DNA with a probe derived from the ψβ region (shown by the bar) (*6*) yields a 6-kb fragment for the normal chromosome and the 7.6-kb fragment for the −117 $^{A}\gamma$ HPFH locus. Hybrids contain the normal (lane 1) or the −117 $^{A}\gamma$ HPFH (lane 2) chromosome from the −117 $^{A}\gamma$ HPFH heterozygote; genomic DNA from this person (lane 3). Abbreviations: Hc, Hinc II; Hd, Hind III; and N, normal locus.

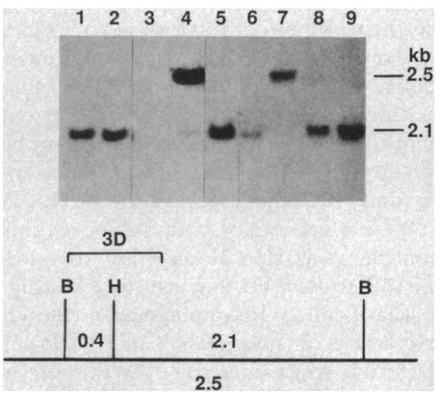

Fig. 4. Analysis of human globin gene methylation in MEL × HPFH lymphoid hybrids. The site analyzed is located 3′ to the β globin gene cluster and is detected with a probe derived from a region immediately 3′ to the breakpoint of the HPFH-1 deletion (*9*). An interpretation of the digestion patterns is shown in the diagram. DNA was from K562 cells (lane 1), MEL × fetal erythroid hybrid cells (lane 2), MEL cells (lane 3), normal lymphocytes (lane 4), HPFH-2 × MEL hybrid cells (lane 5), HPFH-1 × MEL hybrid cells (lane 6), −117 $^{A}\gamma$ HPFH lymphocytes (lane 7), MEL × −117 $^{A}\gamma$ HPFH hybrid cells (lane 8), and normal × MEL hybrid cells (lane 9). Bracket indicates position of probe 3D. Abbreviations: B, Bam HI; and H, Hpa II.

distal CAAT box at the −117 position of the $^{A}\gamma$ globin gene) (*6*) with MEL cells. Six hybrids that contained chromosome 11 were analyzed. Three hybrids contained the wild-type chromosome and expressed only adult human globin (Fig. 2D). The other three hybrids contained only the HPFH chromosome (Fig. 3C) and expressed γ globin (Fig. 2, B and D), providing evidence that nondeletion globin developmental mutations are activated in the environment of erythroleukemia cells (*7*).

The activation of both deletion and promoter HPFH-mutated genes, but not wild-type fetal globin genes, in these hybrids suggests that the HPFH lesions affect developmental expression by promoting interactions between the γ genes and the adult erythroid environment. A detailed structural and functional analysis of mutant chromatin may lend insight into how this is brought about. It is difficult to obtain sufficient numbers of pure nucleated cells needed for a study of this nature. In addition, cells with developmental mutations like HPFH are usually heterozygous; the presence of normal genes in the homologous chromosome complicates structural analysis. However, hybrids hemizygous for a mutant chromosome can be easily obtained (either directly or through cell subcloning) in large numbers. We performed DNA methylation analysis on a sequence brought into the vicinity of the γ globin genes by the HPFH deletions. This region has an erythroid-specific methylation pattern (*8, 9*) and acts as an enhancer in transient gene transfer assays (*9*). Juxtaposition of this region with the γ globin genes causes the activation of the γ globin genes in cells with HPFH deletion mutations (*1, 9*). When Bam HI–digested human DNA is hybridized with a probe specific for this region, a fragment of approximately 2.5 kb is seen. When the Hpa II restriction site located within this region is unmethylated, Hpa II digestion truncates this 2.5-kb Bam HI fragment to 2 kb (Fig. 4, lanes 1 and 2). In lymphoid cells, a 2.5-kb fragment is obtained after Bam HI–Hpa II digestion, which indicates that this site is methylated (Fig. 4, lanes 4 and 7). In wild-type lymphoid × MEL hybrids, however, this site was unmethylated in that a 2-kb band was present (Fig. 4, lane 9). In both deletion HPFH × MEL hybrids and promoter mutation HPFH × MEL hybrids this position was also undermethylated (Fig. 4, lanes 5, 6, and 8). Thus, in these cells the juxtaposed enhancer region, normally located 100 kb downstream of the globin cluster, acquired an erythroid-specific methylation pattern.

These observations, like those from nuclease sensitivity studies (*10*), imply that the structure adopted by the human globin cluster within the MEL cell is representative of that seen in normal human erythroid cells. Hybrids of this sort should therefore prove useful in functional analyses of globin gene developmental mutants. Indeed, by fusing lymphocytes from mutant individuals with suitably differentiated, established cell lines, one may be able to activate and therefore study other human developmental mutations.

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Protection of Cattle Against Rinderpest with Vaccinia Virus Recombinants Expressing the HA or F Gene

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Rinderpest is a highly contagious ruminant viral disease manifested by a rapid course and greater than 90% mortality. Infectious vaccinia virus recombinants were constructed that express either the hemagglutinin or the fusion gene of rinderpest virus. All cattle vaccinated with either recombinant or with the combined recombinants produced neutralizing antibodies against rinderpest virus and were protected against the disease when challenged with more than 1000 times the lethal dose of the virus.

RINDERPEST, OR CATTLE PLAGUE, IS a devastating animal disease with serious ecological, social, and economic consequences. It is an acute, febrile, and contagious viral disease, primarily of cattle and buffalo, that is characterized by inflammation, hemorrhage, necrosis, and erosion of the gastrointestinal tract. The mortality rate approaches 100%; until recently rinderpest accounted for a loss of over 2 million animals per year (*1*).

The Plowright tissue culture vaccine (PTCV) provides protective immunity for the practical life of the animal and is widely used for vaccination against rinderpest in preference to the caprinized and lapinized vaccines (*2*). Because of economic problems in production of PTCV and logistical problems in delivery to the field, rinderpest is again rampant in Africa and Asia (*3*). Thus, the most effective rinderpest vaccine must possess more practical characteristics such as heat stability and ease of production, transport, and administration in the field, which are our reasons for using vaccinia virus recombinant vaccines.

The causative agent of the disease, the rinderpest virus (RPV), is enveloped and has a single-stranded RNA genome with a negative polarity. The virus is in the family Paramyxoviridae and is a member of the morbillivirus group, as are measles virus of humans, distemper virus of dogs, and peste-des-petits-ruminants virus of goats and sheep. In paramyxoviruses, the hemaggluti-

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