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Direct evidence for interaction between human erythroid progenitor cells and a hemoglobin switching activity present in fetal sheep serum

(environment–cell interaction/humoral factor)

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ABSTRACT **An activity that induces Hb F to Hb A switching in human cells is present in fetal sheep serum. To test directly the role of cell-to-environment interactions in hemoglobin switching and to define the level of erythroid cell differentiation at which this activity operates, colony transfer experiments were done. Clones grown in the presence of switching activity-containing medium (fetal sheep serum) or control medium (fetal calf serum) were transferred, at the 16- to 30-cell stage, to either fetal sheep serum or fetal calf serum plates and Hb F synthesis was determined in the fully mature erythroid bursts. Fetal calf serum-to-fetal calf serum transfers produced colonies with the high Hb F levels characteristic of undisturbed fetal calf serum-grown clones. Fetal sheep serum-to-fetal calf serum transfers resulted in significant decrease in Hb F synthesis, revealing an interaction between hemoglobin switching activity and cells at an early stage of progenitor cell development. The reduction of Hb F synthesis in fetal calf serum-to-fetal sheep serum transfers indicated that hemoglobin switching activity interacts with cells at later stages of progenitor cell development. Maximal decrease in Hb F synthesis was observed in fetal sheep serum-to-fetal sheep serum transfers, indicating that optimal effects on Hb switching are obtained when the environment that induces Hb switching is present throughout the development of progenitor cells. By splitting single early clones into two parts and transferring them to either a fetal sheep serum or a fetal calf serum environment, these interactions were further demonstrated in the progeny of a single erythroid burst-forming unit. Since all clone transfers were done on cell-free plates, the results of fetal calf serum-to-fetal sheep serum and of fetal sheep serum-to-fetal sheep serum transfers indicated that the switching activity does not require helper cells for its action. These studies show directly that (*i*) Hb F synthesis is controlled at the level of progenitors and (*ii*) it involves interactions between progenitor cells and their environment.**

In man, fetal hemoglobin (Hb F; $\alpha_2\gamma_2$) is synthesized in the fetus and it is replaced by adult hemoglobin (Hb A; $\alpha_2\beta_2$) after birth. Hb F continues to be synthesized in a few cells of every adult person (1, 2), and its level is elevated in several disorders of erythropoiesis, especially β-chain hemoglobinopathies (3). The mechanism that controls the switch from Hb F to Hb A formation during ontogeny and the synthesis of Hb F in the adult remains speculative. Most likely, this control is accomplished during early erythroid differentiation, in the compartment of committed erythroid progenitor cells (4–12).

During a search for environmental factors that might influence the switch from Hb F to Hb A formation, we found that fetal sheep serum contains an activity that has a profound effect on Hb F synthesis in colonies produced by human erythroid progenitors (13). This activity inhibits Hb F synthesis in cultures of cells of adult persons with or without a hemoglobinopathy. It induces Hb F-to-Hb A switching in cultures of erythroid progenitor cells from neonates, but it fails to induce Hb F-to-Hb A switching in cultures of progenitor cells from human fetuses (13). The detection of this activity, which we preliminarily designate as "hemoglobin switching activity," provides an opportunity to test directly whether interactions between environment and erythroid progenitor cells control the expression of fetal hemoglobin in man. This was attempted with the culture experiments reported here.

In human erythrogenesis, early progenitor cells, called burst-forming units [bfu(e)], give rise to progeny known as colony-forming units [cfu(e)], from which the erythroblasts are formed. cfu(e) and bfu(e) can be assayed in clonal cultures (14, 15). bfu(e) produce multilobed colonies that appear late in culture and are designated erythroid bursts. Our experimental design is to culture bfu(e) in a given environment until the clones have reached a size equivalent to four or five cell divisions and then transfer the clones to a different environment and test whether these changes of environment have affected Hb F synthesis in the mature erythroblasts comprising the clones. Transfer from an environment that does not induce switching of Hb to an environment that does so tests whether Hb switching activity interacts with late erythroid progenitors. Transfer from an environment that induces Hb switching to an environment that does not examines whether Hb switching activity interacts with early erythroid progenitors. The results of the experiments show that the expression of Hb F in mature erythroid cells is indeed controlled interactively at the level of erythroid progenitors.

METHODS

Cells were obtained from peripheral blood or bone marrow of adult persons with activated synthesis of fetal hemoglobin. From these samples, mononuclear cells were isolated through Ficoll–sodium metrisoate (Lymphoprep; Nyegaard, Oslo, Norway) centrifugation and used for clonal cultures. Cells were cultured at 1×10^5/ml in methylcellulose plates in the presence of erythropoietin (2 international units/ml)/10 mM 2-mercaptoethenol/0.1% bovine serum albumin/30% (vol/vol) serum. Control medium contained fetal calf serum previously tested for support of erythroid colony growth. Fetal sheep serum previously tested for support of erythroid colony growth and for induction of Hb F-to-Hb A switching in clonal cultures was the source of medium containing Hb switching activity.

The fetal sheep serum or fetal calf serum plates used for colony transfers were monitored for colony growth, and erythroid bursts were ascertained, at an early stage of their development,

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Abbreviations: bfu(e), burst-forming unit(s); cfu(e), colony-forming unit(s).

by their tendency to form a multilobed clone. Bursts, composed of 16–30 cells each, were lifted from the plate by using an ultrathin pipette and implanted on a methylcellulose plate containing fetal calf serum or fetal sheep serum. All the other components in the latter plates were as described above, but there were no cells other than those transferred. In each experiment, at least 20 bursts were used for each combination of transfers (from fetal calf serum to fetal sheep serum, from fetal calf serum to fetal calf serum, from fetal sheep serum to fetal sheep serum, from fetal sheep serum to fetal calf serum). A similar number of control bursts were marked and left in the initial plates to grow undisturbed. When the bursts had reached full maturation (as judged by the degree of hemoglobinization of erythroblasts), they were used for the detection of the synthesized globin chain species by globin chain biosynthesis.

In experiments testing the effects of hemoglobin switching activity on Hb F synthesis in cfu(e) colonies, bone marrow cells were used for culture and the supporting medium was plasma clots. The method used was as described (4, 16), with the exception that cultures were done in either fetal sheep serum or fetal calf serum and 5- to 6-day cfu(e) colonies were used for globin biosynthesis.

For experiments testing Hb F synthesis in erythroblasts of various degrees of maturity, peripheral blood bfu(e) cultures were used. Erythroid bursts of a desired culture day were classified to three levels of maturity (immature, intermediate, mature), using the criteria described before (17–19). Bursts of a given degree of maturity were lifted from the plates and pooled, and their erythroblasts were used for globin biosynthesis.

For measurements of globin biosynthesis, previously described methods were used (13, 18). Cells were incubated with [^{3}H]leucine and lysates of erythroblasts were analyzed by isoelectric focusing. Globin chains were separated and globin chain synthesis was measured from densitometric tracings of the resultant fluorograms (13, 18).

RESULTS

Transfers of Erythroid Bursts. The manipulation of transfer of erythroid clones did not significantly affect clone viability; replating efficiency (i.e., the proportion of transferred clones that survived and matured) was >90%, in both transfers to fetal calf serum-containing and to fetal sheep serum-containing medium. The transferred clones reached full hemoglobinization with 1 or 2 days' delay, compared with clones left in the initial plates to grow undisturbed. Their average size was 1,000–2,000 cells. Since the clones were transferred when they were composed of 16–30 cells, each cell forming a clone had undergone four or five divisions before transfer. On average, the cells of each transferred clone completed five or six divisions after transfer. If, during the development of a clone, the last three divisions are maturational (i.e., divisions of erythroblasts), the remaining divisions are proliferative or differentiative divisions of bfu(e) and cfu(e) progenitors. We assume that before transfer the cells of a clone were bfu(e) and that the initial divisions after transfer produced cells that were bfu(e) or cfu(e).

Clones initially grown in fetal calf serum plates were transferred to fetal sheep serum plates (inductive of Hb F-to-Hb A switching environment) or were transferred to fetal calf serum plates (noninductive environment) or were left *in situ* to grow undisturbed. The fetal calf serum-to-fetal calf serum transfers served as the controls of fetal calf serum-to-fetal sheep serum transfers. The effects of these changes in environment on Hb F synthesis are shown in Figs. 1 and 2. In the fetal calf serum-to-fetal calf serum transfers, Hb F synthesis was higher than in fetal calf serum undisturbed bursts, indicating that the transfer

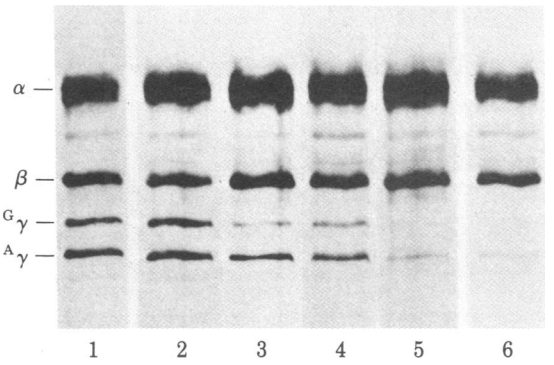

FIG. 1. Fluorogram of an isoelectric focusing gel containing [^{3}H]-leucine-labeled hemolysates from peripheral blood bfu(e) cultures of a person with homozygous β^+ thalassemia. Lanes: 1, control erythroid bursts grown in fetal calf serum plates; 2, bursts initially grown in fetal calf serum plates and subsequently transferred to fetal calf serum plates; 3, bursts initially grown in fetal calf serum and then transferred to fetal sheep serum plates; 4, bursts initially grown in fetal sheep serum and then transferred to fetal calf serum; 5, bursts initially grown in fetal sheep serum and then transferred to fetal sheep serum; 6, control bursts grown in fetal sheep serum undisturbed. The striking decrease in Hb F synthesis in clones grown in fetal sheep serum containing hemoglobin switching activity should be noted (compare lanes 1 and 6) as should the decrease in γ-chain synthesis in fetal calf serum-to-fetal sheep serum (lane 3) and fetal sheep serum-to-fetal calf serum (lane 4) transfers. The maximal decrease in Hb F synthesis in these transfer experiments was found in the fetal sheep serum-to-fetal sheep serum transfers (lane 5).

manipulation per se can increase Hb F synthesis. However, in the fetal calf serum-to-fetal sheep serum transfers, there was a consistent decrease in γ-chain synthesis (compared with the level of Hb F in fetal calf serum-to-fetal calf serum transfers or in the bursts that were left to grow in fetal calf serum undisturbed). In these transfers from the noninductive to the inductive environment, the level of Hb F synthesis was approximately one-third of the level in controls.

The results of transfers of clones initially grown in fetal sheep serum are shown in Fig. 3. Such clones were transferred to a noninductive environment (fetal calf serum) or to an environment inductive of Hb switching (fetal sheep serum) or were left to grow in fetal sheep serum undisturbed. In the clones transferred from fetal sheep serum to fetal sheep serum, Hb F synthesis was as low as in the fetal sheep serum-grown undisturbed bursts (Figs. 1 and 3). Hb F synthesis in the fetal sheep serum-to-fetal calf serum transfers was significantly less than in fetal

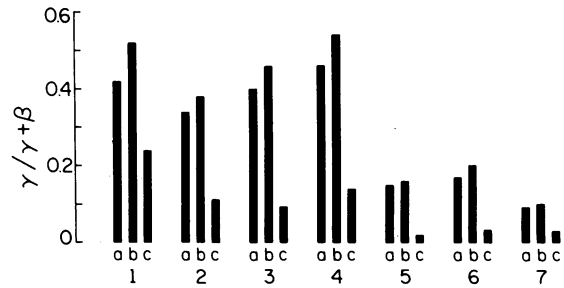

FIG. 2. Hb F synthesis (expressed as the $\gamma/\gamma+\beta$ ratio) after transfer of erythroid bursts initially grown in a noninductive environment (fetal calf serum). Bars: a, control bursts left in the plates to grow undisturbed; b, fetal calf serum-to-fetal calf serum transfers; c, transfers from fetal calf serum to fetal sheep serum. Each experimental value is based on globin biosynthesis done on cohorts of 20–50 bursts. Numbers on the horizontal axis represent different experiments. The consistent decrease in the $\gamma/\gamma+\beta$ ratio when the erythroid bursts are transferred from a noninductive environment to an environment inductive of hemoglobin switching should be noted (bars c).

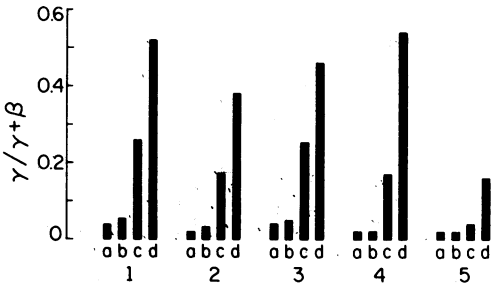

FIG. 3. Hb F synthesis after transfer of erythroid bursts initially grown in an environment that induces Hb switching (fetal sheep serum). Bars: a, control bursts left to grow in fetal sheep serum undisturbed; b, transfers of erythroid bursts from fetal sheep serum to fetal sheep serum; c, transfers of erythroid bursts from fetal sheep serum to a noninductive environment (fetal calf serum); d, control transfers from fetal calf serum to fetal calf serum. It should be noted that bursts initially grown in an inductive environment and subsequently transferred to a noninductive environment (bars c) have significantly lower levels of γ-chain synthesis compared with control bursts (bars d) and also that the decrease in the $\gamma/\gamma+\beta$ ratio in fetal sheep serum-to-fetal sheep serum transfers is as striking as in the fetal sheep serum-grown undisturbed bursts.

calf serum-to-fetal calf serum controls (Fig. 3), indicating that exposure of bfu(e) to an inductive environment influences Hb F synthesis in their progeny even if these cells are transferred and left to mature in a noninductive environment.

Transfers of Divided Clones. Early erythroid bursts comprised of about 50 cells were identified in the plates and were split *in situ* in halves comprised of more or less equal numbers of cells. One-half of the clone was transferred to a fetal sheep serum plate; the other half was transferred to a fetal calf serum plate. These halves were left to grow until mature erythroid colonies were formed. Ninety-four percent of these divided and transferred parts of the clones survived and matured.

Results of globin biosynthesis in the divided parts of β^+ thalassemia clones are presented in Table 1. The findings show that

Table 1. Divided clones: Globin biosynthesis in parts transferred to inductive or noninductive environments

Clone no.	First growth medium*	$\gamma/\gamma+\beta$ synthesis of clone transferred†	
		Fetal calf serum	Fetal sheep serum
1	Fetal calf serum	0.86	0.50
2	Fetal calf serum	0.86	0.58
3	Fetal calf serum	0.82	0.61
4	Fetal calf serum	0.79	0.50
5	Fetal calf serum	0.78	0.29
6	Fetal calf serum	0.77	0.53
7	Fetal calf serum	0.69	0.23
8	Fetal calf serum	0.50	0.15
9	Fetal calf serum	0.50	0.12
10	Fetal calf serum	0.45	0.10
11	Fetal calf serum	0.40	0.10
12	Fetal calf serum	0.30	0.09
13	Fetal sheep serum	0.25	0.03
14	Fetal sheep serum	0.25	0.01
15	Fetal sheep serum	0.22	0.05
16	Fetal sheep serum	0.20	0.02
17	Fetal sheep serum	0.17	0.06

*Medium in which clones were grown to the 50-cell stage.
†At the 50-cell stage, clones were divided into two portions one of which was transferred to fetal calf serum and the other, to fetal sheep serum. The $\gamma/\gamma+\beta$ synthesis ratio was determined when the transferred clones were mature.

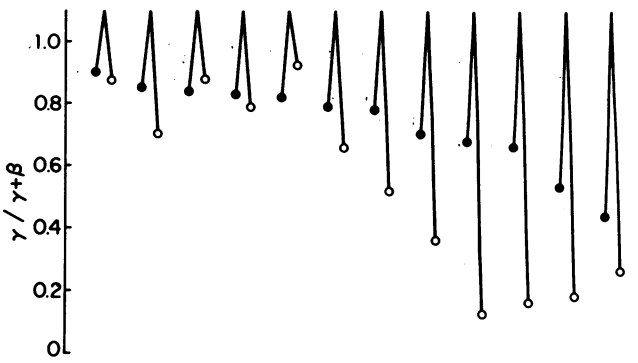

FIG. 4. Single neonatal-origin erythroid [bfu(e)] clones composed of about 50 cells were split into two parts. One part was transferred to a fetal calf serum plate (●), the other part, to a fetal sheep serum plate (○). Globin biosynthesis (expressed as $\gamma/\gamma+\beta$ ratio) was determined when the transferred clones were mature. The neonatal bfu(e) respond to the switching factor in fetal sheep serum and there is heterogeneity in the degree of their response.

the fetal sheep serum-grown part of a clone synthesizes less Hb F than the fetal calf serum-grown part.

Studies of divided bursts were also done using neonatal bfu(e). Neonatal erythroid bursts initially grown in fetal calf serum were divided at about the 50-cell stage into two parts, and one was grown in fetal calf serum while the other was grown in fetal sheep serum. As shown in Fig. 4, in 9 of 12 clones, there was a decrement in Hb F synthesis in the part of the clone that was transferred to the inductive environment (fetal sheep serum).

Studies of cfu(e) and Erythroblasts. To test whether cfu(e) are sensitive to Hb switching activity, experiments were done using bone marrow cells from persons who had an activated Hb F synthesis. Bone marrow cells were grown in fetal calf serum or in fetal sheep serum, and Hb F synthesis was determined in cfu(e) clones. cfu(e) colonies grown in fetal calf serum showed increased Hb F synthesis (Fig. 5); a decrease in Hb F was characteristic of cfu(e) colonies grown in fetal sheep serum, suggesting that the cfu(e) is one of the target cells of Hb switching activity.

bfu(e) were cultured in fetal calf serum or in fetal sheep serum, and Hb F synthesis was measured in cohorts of clones composed of immature erythroblasts, of erythroblasts of "intermediate" degree of maturity, or of fully mature erythroblasts.

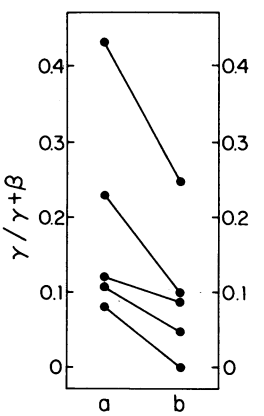

FIG. 5. Effect of hemoglobin switching activity on cfu(e) colonies. Cells of donors (with activated *in vivo* Hb F synthesis) were grown in fetal calf serum medium (points a) or in fetal sheep serum medium (points b) and synthesis of Hb F was measured in the 5- to 6-day cfu(e) colonies. The consistent decrease in $\gamma/\gamma+\beta$ ratios in the fetal sheep serum-grown cfu(e) should be noted.

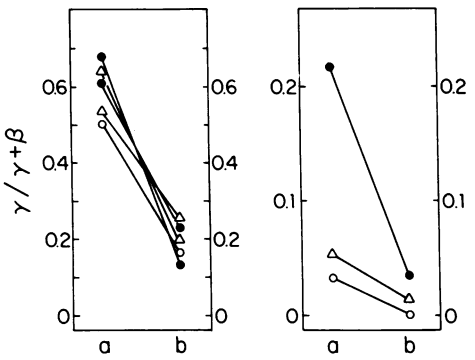

FIG. 6. Synthesis of γ chains in erythroblasts of various degrees of maturity. Cultures of peripheral blood bfu(e) were carried out in the fetal calf serum noninductive environment (points a) or in the fetal sheep serum inductive environment (points b). Erythroid bursts were classified according to their degree of hemoglobinization as immature (●), composed mainly of proerythroblasts; fully mature (○); or of intermediate degree of maturity (△). (*A*) β^+ thalassemia bfu(e) cultures. (*B*) Cultures of cells of a person with homozygous Hb S disease. Low synthesis of γ chains is characteristic of fetal sheep serum-grown immature erythroblasts.

The fetal calf serum-derived immature erythroblasts synthesized high levels of γ-globin (Fig. 6), in agreement with previous findings (17–19). In the fetal sheep serum-derived immature erythroblasts, γ-globin synthesis was as low as in the population of mature erythroblasts (Fig. 6), suggesting that the early erythroblasts formed by fetal sheep serum-grown progenitors are already programmed to synthesize low levels of Hb F.

DISCUSSION

The results we report here provide direct evidence (*i*) that the control of Hb F synthesis in man is accomplished at the level of progenitors, (*ii*) that this control involves interactions between progenitor cells and their environment, and (*iii*) that the interactions are of direct nature and do not require the presence of accessory or helper cells.

In the transfers from fetal sheep serum to fetal calf serum, clones initially grown in an environment inductive of Hb F-to-Hb A switching were transferred to a noninductive environment. These experiments tested whether the hemoglobin switching activity acts during the first four or five divisions of the cells forming the erythroid bursts. A consistent decline in the $\gamma/\gamma+\beta$ ratio in fetal sheep serum-to-fetal calf serum transferred bursts was noted, suggesting that exposure of bfu(e) to fetal sheep serum changes the program of the cell; this change in program is manifested later, when the erythroblasts are formed during the growth of the clone in an environment noninductive of switching. These results directly support the concept of interactive control of the Hb F phenotype at the level of progenitor cells. The fetal sheep serum-to-fetal calf serum transfers are informative from other points of view. Since early exposure of the progenitors produces effects of Hb F synthesis on erythroblasts (though the latter are born in a noninductive environment), the Hb switching activity of fetal sheep serum cannot induce Hb switching by acting exclusively at the level of erythroblasts. For the same reasons, the activity cannot induce Hb switching secondarily through the mechanism of maturational modulation of Hb F in erythroblasts (17–20). This conclusion is also supported by the results of measurements of Hb F in immature and mature erythroblasts of clones grown in fetal sheep serum.

In the transfers from fetal calf serum to fetal sheep serum, clones cultured initially in an environment lacking the hemoglobin switching activity were subsequently transferred to and grown in an environment containing this activity. These transfers resulted in decreased synthesis of Hb F, indicating that the Hb switching activity interacts with cells at later stages of erythroid cell differentiation and induces changes in their Hb F program. Additional evidence for interaction between late progenitors and the switching activity was obtained by comparison of Hb F synthesis in cfu(e) grown in fetal calf serum or in fetal sheep serum, which showed that this late progenitor is responsive to Hb switching activity.

In these experiments, the maximal decrease in Hb F synthesis was observed when clones initially grown in fetal sheep serum were transferred to fetal sheep serum. Hb F in the clones so transferred was as low as in the fetal sheep serum-grown undisturbed bursts. The difference in Hb F synthesis between fetal sheep serum-to-fetal sheep serum transfers and fetal sheep serum-to-fetal calf serum or fetal calf serum-to-fetal sheep serum transfers suggests that Hb switching activity has an optimal effect when it acts throughout *in vitro* development of the erythroid progenitors. In support of this conclusion are the experiments with cfu(e) colonies. Although Hb F in the fetal sheep serum-grown cfu(e) was significantly lower than in fetal calf serum-grown cfu(e), Hb F synthesis in the former was still elevated, while in fetal sheep serum-grown bfu(e) colonies of the same bone marrow donors, Hb F was barely measurable (data not shown). This difference in Hb F between the fetal sheep serum-grown cfu(e) and bfu(e) colonies may be explained if optimal effects on Hb F synthesis are obtained when the Hb switching activity operates throughout progenitor cell development.

For clonal culture of hemopoietic cells, mononuclear cells are plated in semisolid medium containing components (such as erythropoietin and burst-promoting activity) that trigger growth of erythroid progenitors. Only a small fraction of the plated cells are progenitors. Mature terminal cells of the various lineages and accessory or helper cells are also seeded in culture, and the latter cells presumably influence progenitor cell growth. Under such culture conditions, it is difficult to assess whether an environmental factor inducing Hb switching exerts its effect by acting directly on progenitors or by activating an accessory cell present in the plates. In the transfer experiments, however, single clones are transferred to cell-free plates (i.e., plates that contain no other cells than those of the transferred bursts). Under such cell-free conditions, we observed that Hb F decreases in the fetal calf serum-to-fetal sheep serum transfers and Hb F synthesis is almost completely inhibited in fetal sheep serum-to-fetal sheep serum transfers. From these findings, we conclude that the hemoglobin switching activity exerts its effect by acting directly on progenitors. This conclusion is further supported by the results we obtained with transfers of divided bursts.

Previously, we presented evidence, based on immunofluorescence analyses of bursts, that the same bfu(e) can provide either F-producing or non-F-producing progeny (4, 12, 20). Other investigators, however, have suggested that in the adult person different bfu(e) produce Hb F-expressing or Hb F-non-expressing cells (21). The findings obtained with divided bursts are of relevance to this question, since they show directly that one-half of the progeny of a bfu(e) can develop into a high F-producing clone while the other half develops into a low F-producing (or non-F-producing) clone, depending on the environmental stimulus.

The results obtained with the divided neonatal bfu(e) clones are of interest because they show (*i*) that neonatal bfu(e) do respond to the hemoglobin switching activity of fetal sheep serum

and (*ii*) that there is heterogeneity in the degree of their responses. Both observations are in accord with the hypothesis (13) that Hb switching reflects an intrinsic change in the ability of progenitor cells to respond to Hb switching activities in their environment. Inherent to this hypothesis is the prediction of qualitative heterogeneity of the erythroid progenitors that are produced during the time hemoglobin switching takes place *in vivo*.

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1. Boyer, S. H., Belding, T. K., Margolet, L. & Noyes, A. N. (1974) *Science* **188**, 361–363.
2. Wood, W. G., Stamatoyannopoulos, G., Lim, G. & Nute, P. E. (1975) *Blood* **46**, 671–682.
3. Weatherall, D. J. & Clegg, J. B. (1981) *The Thalassaemia Syndromes* (Blackwell, Oxford).
4. Papayannopoulou, T., Brice, M. & Stamatoyannopoulos, G. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 2923–2927.
5. Nienhuis, A. W. & Benz, E. J., Jr. (1977) *N. Engl. J. Med.* **297**, 1318–1328.
6. Nienhuis, A. W. & Benz, E. J., Jr. (1977) *N. Engl. J. Med.* **297**, 1430–1436.
7. Kidoguchi, K., Ogawa, M., Karam, J. D. & Martin, A. D. (1978) *Blood* **52**, 1115–1124.
8. Clarke, B. J., Nathan, D. G., Alter, B. P., Forget, B. G., Hillman, D. G. & Housman, D. (1979) *Blood* **54**, 805–817.
9. Kidoguchi, K., Ogawa, M. & Karam, J. D. (1979) *J. Clin. Invest.* **63**, 804–806.
10. Fauser, A. A. & Messner, H. A. (1979) *Blood* **54**, 1384–1394.
11. Darbre, P. D., Adamson, J. W., Wood, W. G., Weatherall, D. J. & Robinson, J. S. (1979) *Br. J. Haematol.* **41**, 459–475.
12. Stamatoyannopoulos, G. & Papayannopoulou, T. (1979) in *Cellular and Molecular Regulation of Hemoglobin Switching*, eds. Stamatoyannopoulos, G. & Nienhuis, A. W. (Grune & Stratton, New York), pp. 323–349.
13. Papayannopoulou, T., Kurachi, S., Nakamoto, B., Zanzani, E. D. & Stamatoyannopoulos, G. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 6579–6583.
14. Axelrad, A. A., McLeod, D. L., Shreeve, M. M. & Heath, D. S. (1974) in *Hemopoiesis in Culture*, ed. Robinson, W. A. (GPO, Washington, DC), pp. 226–237.
15. Iscove, N. N. & Sieber, F. (1975) *Exp. Hematol.* **3**, 32–43.
16. Papayannopoulou, T., Brice, M. & Stamatoyannopoulos, G. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 2033–2037.
17. Papayannopoulou, T., Kalmantis, T. & Stamatoyannopoulos, G. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 6420–6424.
18. Papayannopoulou, T., Kurachi, S., Brice, M., Nakamoto, B. & Stamatoyannopoulos, G. (1981) *Blood* **57**, 531–536.
19. Farquhar, M. N., Turner, P. A., Papayannopoulou, T., Brice, M., Nienhuis, A. W. & Stamatoyannopoulos, G. (1981) *Dev. Biol.* **85**, 403–408.
20. Stamatoyannopoulos, G., Kurnit, D. M. & Papayannopoulou, T. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 7005–7009.
21. Alter, B. P., Jackson, B. T., Lipton, J. M., Piasecki, G. J., Jackson, P. L., Kudisch, M. & Nathan, D. G. (1981) *J. Clin. Invest.* **67**, 458–466.