Proc. Nat. Acad. Sci. USA
Vol. 73, No. 4, pp. 1232–1236, April 1976
Cell Biology

Erythroid cell differentiation: Murine erythroleukemia cell variant with unique pattern of induction by polar compounds

(Friend cell/dimethylsulfoxide resistance/globin synthesis/globin mRNA/cell culture)

YOSHIRO OHTA, MASAO TANAKA, MASAAKI TERADA, ORLANDO J. MILLER, ARTHUR BANK, PAUL A. MARKS, AND RICHARD A. RIFKIND

Departments of Medicine, Human Genetics and Development, Obstetrics and Gynecology and the Cancer Research Center, Columbia University, College of Physicians and Surgeons, 630 West 168th Street, New York, N.Y. 10032

Contributed by Paul A. Marks, December 29, 1975

ABSTRACT The murine-virus-infected erythroleukemia cell system provides an opportunity to examine regulatory mechanisms controlling cytodifferentiation. A cloned cell line (DR10c3) resistant to the erythropoiesis-inducing effect of dimethylsulfoxide (Me_2SO) was isolated from the Me_2SO-sensitive line DS19. DR10c3 is characterized as follows: (*1*) the uptake of [^{3}H]Me_2SO is similar to that in DS19; (*2*) cell growth with and without Me_2SO is similar to that of DS19; (*3*) resistance is relatively stable; (*4*) the karyotype of DR10c3 reveals an average loss of five chromosomes per cell, but is otherwise similar to that of DS19; (*5*) total protein and globin synthesis by cells cultured 4 days with or without Me_2SO is similar to these syntheses in DS19 cultured without Me_2SO; (*6*) virtually no globin mRNA is detectable after 3 days in Me_2SO, as assayed both by RNA·complementary DNA hybridization and by the heterologous cell-free protein-synthesizing system; (*7*) other polar compounds, *N*-methylpyrrolidinone, 1-methyl-2-piperidone, *N,N*-dimethylacetamide, and *N*-methylacetamide, induce erythroid differentiation in DR10c3, and the accumulation of α- and β-globin chains is indistinguishable from that in DS19; and (*8*) the concentration optima for induction of differentiation by all these compounds are identical for DR10c3 and DS19.

Addition of dimethylsulfoxide (Me_2SO) to murine-virus-infected erythroleukemia cell (MELC) cultures induces erythroid differentiation (1), characterized by the appearance of globin mRNA, synthesis of hemoglobin, cessation of cell division, appearance of erythrocyte membrane antigens, and the morphological changes characteristic of erythroid differentiation in normal mouse hematopoietic tissues (2–5). This paper reports the characterization of a variant of MELC (DR10c3) resistant to the effect of Me_2SO on erythroid differentiation, isolated from cultures of a Me_2SO-inducible cell line, designated DS19.

MATERIALS AND METHODS

MELC strain 745A was provided by Dr. Charlotte Friend and maintained in culture for the past 3 years as described elsewhere (6). Cell line DS19 was cloned from 745A. Cultures were inoculated at 2 to 3 × 10^5 cells per ml, and transferred every 3–4 days. Cell counts were by Coulter counter, corrected by trypan blue exclusion. Slides were prepared by cytocentrifuge, fixed in methanol, stained with benzidine–Wright–Giemsa, and scored for the proportion of benzidine-reactive cells.

To isolate Me_2SO resistant cells, clone DS19 was cultured with 280 mM Me_2SO for 6 days and surviving cells were cloned in semi-solid medium as previously described (6). Clones were passed five times without Me_2SO, six times with 280 mM Me_2SO, and then passaged every 3–4 days without Me_2SO.

The uptake of Me_2SO was determined according to methods previously described (7). Chromosome analysis following 10 min of Colcemid arrest was performed by the method of quinacrine mustard staining according to techniques described elsewhere (8). Chromosomes were identified by their fluorescent banding patterns and arranged according to the standard mouse karyotype (9). Structurally rearranged (marker) chromosomes were given arbitrary numbers of M-1 through M-10.

For determination of total protein or globin synthesis 10^8 cells were incubated in 10 ml of leucine-free Eagle's Basal Medium (GIBCO) with 250 μCi of [^{3}H]leucine (New England Nuclear, specific activity 33.6 Ci/mmol) at 37° for 60 min. Aliquots (25 μl) of the incubation mixture were removed at intervals, 50 μg of bovine serum albumin was added, and the mixture was precipitated with 10% trichloroacetic acid, collected on Millipore filters, washed with 5% trichloroacetic acid, and counted by liquid scintillation with 0.2 ml of formic acid and 10 ml of Bray solution, for total protein synthesis. For globin synthesis cells were lysed and globin chains were isolated by chromatography as described elsewhere (10, 11). Globin mRNA was isolated and assayed by cell-free system and by hybridization with ^{3}H-labeled DNA complementary to globin mRNA ([^{3}H]cDNA) as previously reported (12–14).

RESULTS

Isolation of the Variant. Isolation of a Me_2SO-resistant cell line was accomplished by taking advantage of the fact that Me_2SO-induced differentiation, as normal erythropoiesis, is accompanied by cessation of cell division. Cells still capable of cell division after 6 days in Me_2SO were cloned in semi-solid medium. Seventeen clones were subsequently cultured, in suspension, with 280 mM Me_2SO to determine their sensitivity to this agent. Under these conditions, 85% to 95% of DS19 cells become benzidine-reactive by 5 days of culture. Of the 17 selectively cloned lines, eight displayed fewer than 1% benzidine-reactive cells under the same conditions, which is the same as the spontaneous rate observed in the absence of Me_2SO. Me_2SO-resistant clones were designated DR. One of these, DR10, was arbitrarily chosen for further study, passaged serially six times in 280 mM Me_2SO, and nonselectively subcloned as DR10c1 through c8. DR10c3 has been used for most subsequent studies.

To determine if Me_2SO in excess of 280 mM could increase the extent of erythropoiesis in DR10 cells, four subclones (c3–6) were incubated with 280 mM, 350 mM, 385 mM, 420 mM, and 490 mM Me_2SO, and compared with

Abbreviations: Me_2SO, dimethylsulfoxide; MELC, murine-virus-infected erythroleukemia cells; cDNA, DNA complementary to RNA; *N*-MP, *N*-methylpyrrolidinone.

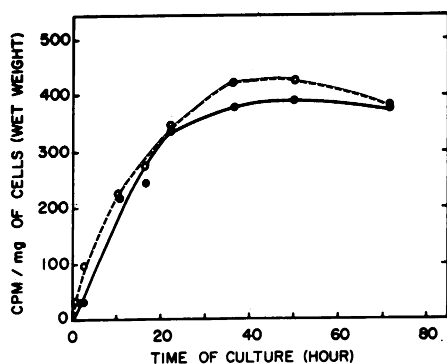

FIG. 1. Uptake of [^{3}H]Me$_2$SO by DS19 and DR10c3 cells, performed as described in the *text*. (●) DS19; (O) DR10c3.

DS19 cells grown under the same conditions. No induction of hemoglobin production was observed in any of the four DR10 clones. Concentrations of Me$_2$SO in excess of 350 mM inhibited cell growth in DS19 and in all the DR10 cell lines.

Permeability. Failure of Me$_2$SO to induce differentiation of DR10c3 may be due to failure to achieve an effective intracellular concentration of Me$_2$SO. It has previously been shown (7) that, for DS19, 14–22 hr is required for intracellular tritium ([^{3}H]Me$_2$SO) to achieve a concentration equal to that in the incubation medium. This observation was confirmed in the present studies; the rate of uptake of tritium from [^{3}H]Me$_2$SO by DR10c3 is similar to that of DS19 (Fig. 1). These data are consistent with the interpretation that resistance of DR10c3 to Me$_2$SO does not reflect a permeability barrier.

Karyotype. DS19 cells have a mean chromosome number of 37.7 per cell with a range of 32–40 (modal value 39) in 24 karyotypes analyzed. DR10c3 cells have a mean of 32.4 chromosomes per cell with a range of 23–37 and no distinct mode. Despite loss of about five chromosomes per cell, DR10c3 was otherwise similar in its karyotype to the parental line; both contain the same normal and rearranged (marker) chromosomes of mouse origin and show similar variation in the number of copies of specific chromosomes. The presence of identical marker chromosomes indicates that the two lines are closely related, as would be expected from their history. The difference in chromosome number between the cell lines was due to variation in the number of copies of several normal and rearranged chromosomes, and not to loss or gain of specific chromosomes. Although loss of Me$_2$SO-inducible erythropoiesis might be due to loss of a specific chromosome, such loss would be difficult to detect in the presence of the multiple, and probably largely random, chromosome changes observed.

Stability of the Variant. DR10c3 cells were passaged 40 times in suspension culture without Me$_2$SO. Cell growth in the presence and in the absence of Me$_2$SO was essentially unchanged from the initial cultures of this clone, and similar to the growth characteristics of DS19 (Fig. 2). Fewer than 3% benzidine-reactive cells were observed in 5 day cultures of these DR10c3 with Me$_2$SO (Fig. 2). Stability of DR10c3 with respect to resistance to induction by Me$_2$SO was examined again after 95 passages without the agent. Growth characteristics were unchanged, but approximately 20% of the cells were benzidine-reactive by 5 days of culture with Me$_2$SO.

At the time of isolation of DR10c3, aliquots were stored in liquid nitrogen. Portions of those frozen cells were thawed after 52 weeks of storage, passaged 10 times without Me$_2$SO,

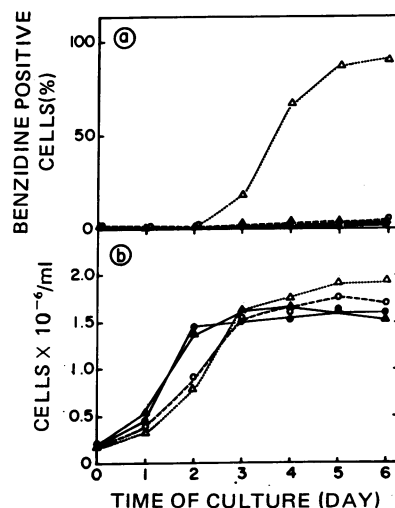

FIG. 2. Cell growth and differentiation of DS19 and DR10c3 cells cultured with and without 280 mM Me$_2$SO. (a) Percent benzidine-reactive cells; (b) cell growth; (●) DR10c3 without Me$_2$SO; (O) DR10c3 with 280 mM Me$_2$SO; (▲) DS19 without Me$_2$SO; (△) DS19 with 280 mM Me$_2$SO.

then tested for responsiveness to Me$_2$SO. Their growth was indistinguishable from that of DS19 or DR10c3 in uninterrupted culture, and they showed fewer than 0.5% benzidine-reactive cells by 5 days of culture with Me$_2$SO. Taken together, these data indicate that DR10c3 is stable for at least 40 passages and in liquid nitrogen for up to 1 year. This variant does show instability in Me$_2$SO-sensitivity after prolonged passage in the absence of the agent.

Protein Synthesis. A decrease in the rate of total protein synthesis is characteristic of differentiation in MELC. In DS19 incubated with Me$_2$SO for 4 days, the rate of protein synthesis is strikingly less than that in uninduced cells (Fig. 3). The rate of protein synthesis in DR10c3, however, was the same whether the cells were cultured with or without Me$_2$SO and was indistinguishable from the rate of DS19 grown without Me$_2$SO (Fig. 3).

DS19 and DR10c3 cells grown with and without Me$_2$SO for 4 days were examined for synthesis of α- and β-globin chains. In DS19 without Me$_2$SO there was no detectable synthesis of either α- or β-globin chains; with Me$_2$SO these cells

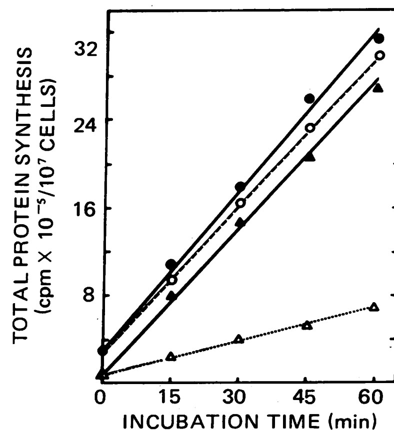

FIG. 3. Rate of protein synthesis ([^{3}H]leucine incorporation) in DS19 and DR10c3 cultured 4 days with and without 280 mM Me$_2$SO. Methods are as described in the *text*. (●) DR10c3 without Me$_2$SO; (O) DR10c3 with 280 mM Me$_2$SO; (▲) DS19 without Me$_2$SO; (△) DS19 with 280 mM Me$_2$SO.

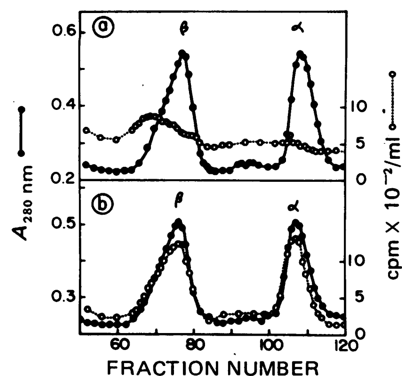

FIG. 4. Globin synthesis in DS19 cells after 4 days with or without Me_2SO. Globin chains were separated by chromatography on carboxymethyl-cellulose urea with carrier strain DBA hemoglobin, as described in the *text*. (a) DS19 without Me_2SO; (b) DS19 with Me_2SO; (●) $A_{280\ nm}$; (O) [^{3}H]leucine.

synthesized DBA mouse α- and β-globin chains (Fig. 4). DR10c3 cells with or without Me_2SO do not synthesize α- or β-globin chains (Fig. 5).

A small peak of radioactivity elutes in the region just before the peak of β-globin (Figs. 4a, 5a, and 5b) in samples prepared from DS19 without Me_2SO and from DR10c3 with and without Me_2SO. This pre-β peak and the β-peak were recovered and rechromatogrammed on Sephadex G-100 to determine whether the pre-β material has the size of globin. Pre-β radioactivity elutes before globin (Fig. 5c), which suggests that it is not globin by the criterion of molecular weight.

Globin mRNA. The fraction of RNA corresponding to 6–16 S was prepared from total RNA from DS19 and DR10c3 cells grown with or without Me_2SO for 3 days, and was assayed for globin mRNA in a Krebs ascites tumor cell-free system (13). RNA from DS19 with Me_2SO stimulated globin synthesis in the cell-free system (Table 1), while RNA from DS19 without Me_2SO and from DR10c3 with or without Me_2SO demonstrated no globin mRNA activity.

Globin mRNA sequences in DS19 or DR10c3 RNA, after 3 days with and without Me_2SO, were determined by hybridization with cDNA prepared with adult DBA mouse reticulocyte globin mRNA (Table 2). The $C_0t_{1/2}$ values* of RNA·cDNA hybridizations were similar for RNA from DS19 without Me_2SO and for DR10c3 cultured either without or with Me_2SO, and were in the range of 200–650. The $C_0t_{1/2}$ values for RNA from DS19 cultured with Me_2SO was 3. These data indicate that DS19 incubated with Me_2SO accumulates globin mRNA to a concentration approximately 100 times higher than that in uninduced DS19 or DR10c3 with or without the agent. An estimate of the number of molecules of globin mRNA per cell can be calculated from the data presented in Table 2. In the uninduced DS19 cells and in treated or untreated DR10c3 cells, there are, on the average, fewer than 50 molecules of globin mRNA per cell. On the other hand, in DS19 cells after 3 days of culture with Me_2SO, there are 4000 globin mRNA molecules per cell, on the average.

Effects of Other Agents. Eight polar compounds which induce differentiation of DS19 (15) were tested with DR10c3. Cultures of DR10c3 cells with any one of four of these compounds (1-methyl-2-piperidone, *N*-methylpyrrolidinone, *N,N*-dimethylacetamide, and *N*-methylacetamide) result in differentiation (Table 3). Dimethylformamide, pyridine-*N*-oxide, *N*-methylformamide, and glycerol were as ineffective as Me_2SO. The optimum concentration for each of the four effective compounds was identical to that for DS19. *N*-methylpyrrolidinone (*N*-MP) was selected as representative of the compounds which induce DR10c3. The growth and differentiation of DR10c3 in the presence of *N*-MP are illustrated in Fig. 6.

In order to determine whether the globin synthesized by DR10c3 with 30 mM *N*-MP is the same as that formed by DS19, these cells were cultured 3 days with 30 mM *N*-MP and then incubated with isotopic leucine, ^{3}H for DR10c3 and ^{14}C for DS19. After 1 hr at 37°, the cultures were mixed together, and globin chains were recovered and separated on carboxymethyl-cellulose urea. The α- and β-globin chains synthesized by DS19 cochromatographed identically with the globin chains produced by DR10c3 cells.

Globin mRNA from DR10c3 incubated with *N*-MP was assayed both in the heterologous cell-free system and by hybridization with globin cDNA. The globin chains synthesized in the cell-free system containing mRNA from DR10c3 with *N*-MP cochromatogrammed identically with normal

* $C_0t_{1/2}$ is the product of the initial concentration of RNA (in moles of nucleotide/liter) and time (in seconds) at the midpoint between the maximum and minimum percent hybridization plateau levels.

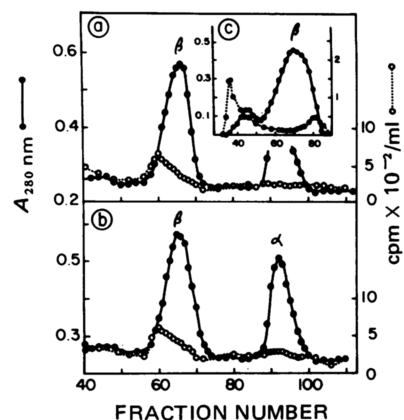

FIG. 5. Globin synthesis in DR10c3, with and without Me_2SO, determined as in Fig. 4. (a) DR10c3 without Me_2SO; (b) DR10c3 with Me_2SO; (c) Sephadex G-100 filtration profile of pooled fractions 50 through 70 of the carboxymethyl-cellulose urea chromatography illustrated in (b); (●) $A_{280\ nm}$; (O) [^{3}H]leucine.

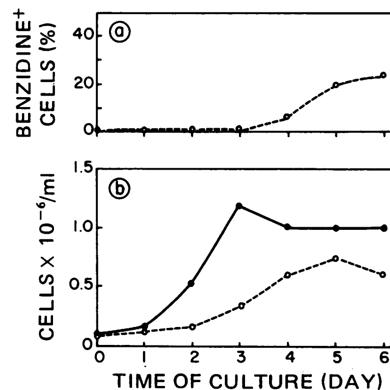

FIG. 6. Cell growth and differentiation of DR10c3 with and without *N*-methyl pyrrolidinone. (a) Percent benzidine-reactive cells; (b) cell growth. (●) DR10c3 without *N*-methylpyrrolidinone; (O) DR10c3 with 30 mM *N*-methylpyrrolidinone.

Table 1. Assay for mRNA for globin in RNA preparations from DS19 and DR10c3 cells

Cell line	Agent*	No. cells $\times 10^{-7}$	6–16S RNA recovered and assayed, μg†	cpm [^{3}H]leucine incorporation into protein	cpm [^{3}H]leucine incorporation into globin‡	
					β	α
DS19	—	3.3	22.0	60,980	<50	200
	—	6.6	44.0	64,880		
	Me_2SO	3.2	11.2	102,820	5,928	3,960
	Me_2SO	6.4	22.4	141,780	8,255	2,600
	N-MP	3.6	18.0	97,705	3,960	3,000
DR10c3	—	2.8	16.4	161.700	<50	190
	—	5.6	32.8	156,980	<50	200
	Me_2SO	3.0	11.2	151,540	<50	170
	Me_2SO	6.0	22.4	256,780	<50	240
	N-MP	3.6	25.0	95,605	1,734	1,107

* Cells were cultured for 3 days with and without either 280 mM Me_2SO or 30 mM *N*-methylpyrrolidinone (*N*-MP).
† RNA was extracted and fractionated as described in the *text*.
‡ The product in cell-free assay system was analyzed by carboxymethyl-cellulose urea chromatography and the activity under α- and β-peaks was calculated to provide the values for [^{3}H]leucine incorporation in α- and β-globin.

adult DBA mouse globin and with globin from DS19 mRNA in the cell-free system. The stimulatory effect of *N*-MP on globin mRNA activity in both lines is shown in Table 1. As determined by hybridization, DS19 cultured 3 days with *N*-MP accumulated approximately 1500 molecules of globin mRNA per cell, while DR10c3 accumulated approximately 600 molecules of globin mRNA per cell under the same conditions (Table 2). In this experiment, DS19 cultures contained 45% benzidine-positive cells, while DR10c3 had 18%, by 5 days of culture with *N*-MP.

DISCUSSION

This paper describes the isolation and characterization of a strain of MELC (DR10c3) resistant to induction of erythroid differentiation by Me_2SO. On the basis of both parentage and karyotype, DR10c3 is closely related to the Me_2SO-sensitive parent cell line. DR10c3 has about five fewer chromosomes per cell than DS19. Paul and Hickey (16) report isolation of a Me_2SO-resistant MELC with fewer chromosomes than the sensitive parental cells. Because of variation among the individual karyotypes, identification of a unique chromosomal deletion or rearrangement, characteristic of DR10c3, is difficult. DR10c3 cannot represent a deletion of the structural genes for globin mRNA, since DR10c3 displays the same low (less than 1%) but definite level of spontaneous erythroid differentiation as does DS19 and can be induced to increase erythroid differentiation by other polar compounds. The similar rate of uptake of [^{3}H]Me_2SO by DR10c3 and DS19 suggests that resistance is not due to selective impermeability. This interpretation is also supported by the similar toxicity of Me_2SO for DR10c3 and DS19.

The compounds which induce differentiation in DR10c3

Table 2. Determination of globin mRNA sequences in RNA from DS19 and DR10c3, by RNA·cDNA hybridization

Cell source of 6–16S RNA	Agent*	No. cells $\times 10^{-8}$	RNA, μg†	$C_0t_{1/2}$	Globin mRNA‡ molecules/cell
DS19	—	6.7	376	300	33
DS19	Me_2SO	3.1	204	3	4000
DR10c3	—	5.6	348	650	12
DR10c3	Me_2SO	6.0	200	200	30
DR10c3 (total cell RNA)	Me_2SO	5.5	3840	3000	42
DS19	*N*-MP	1.8	92	6	1533
DR10c3	*N*-MP	1.8	124	20	620
Reticulocyte 10S RNA§				0.006§	

* Cells were cultured for 3 days with and without either 280 mM Me_2SO or 30 mM *N*-methylpyrrolidinone (*N*-MP).
† RNA recovered from the 6 to 16S fraction, except for line 5 where total cell RNA was used.
‡ Calculation: Since 5×10^{-12} mol = 1 μg of RNA, and Avogadro's number is 6×10^{23} molecules/mol,

$$\mu\text{g of globin mRNA} = \frac{C_0t_{1/2}\text{ of 10S RNA}_{\text{reticulocyte}} \times \mu\text{g of RNA}_{\text{sample}}}{C_0t_{1/2\text{sample}}} \quad [1]$$

$$\text{molecules globin mRNA per cell} = \frac{\mu\text{g of globin mRNA} \times (5 \times 10^{-12}) \times (6 \times 10^{23})}{\text{number of cells}} \quad [2]$$

§ This is the value of $C_0t_{1/2}$ for globin mRNA prepared from adult DBA mouse reticulocytes and used as a standard in these calculations.

Table 3. Effect of polar compounds on the growth and differentiation of DR10c3*

Compound	Concentration, mM	No. cells $\times 10^{-6}$	Benzidine-reactive, %
1-Methyl-2-piperidone	1	0.9	<1
	3	0.3	<1
	10	0.2	25.6
	15	0.2	28.6
	20	Dead	—
N-Methylpyrrolidinone	5	0.7	<1
	10	0.9	2.0
	20	0.6	21.2
	30	0.6	20.4
	40	0.6	<1
	50	0.2	<1
	70	Dead	—
N,N-Dimethyl acetamide	5	0.4	<1
	10	0.5	10.0
	20	0.5	17.0
	30	0.4	10.6
	40	0.2	<1
	50	Dead	—

Compound	Concentration, mM	No. cells $\times 10^{-6}$	Benzidine-reactive, %
N-Methylacetamide	50	1.0	12.6
	60	0.4	<1
	75	0.1	<1
	100	Dead	—
Pyridine-*N*-oxide	3–150	0.8–1.3	<1
	200	0.2	<1
	300	Dead	—
Dimethylformamide	25–200	0.5–0.9	<1
	250	0.1	<1
	300	Dead	—
N-Methylformamide	15–250	1.2–1.4	<1
	280	0.8	<1
Me_2SO	25–280	1.0–1.3	<1
	350	0.6	<1
	420	Dead	—
Glycerol	600–1000	0.6–1.0	<1

* Cells were counted and the benzidine reaction was assayed at day 5 of incubation.

cells have previously been shown to induce DS19 at the same molar concentrations, which are considerably below that required for Me_2SO. The pattern of erythropoiesis induced in DR10c3 is similar to that in DS19, by the criteria of globin mRNA accumulation and the α- and β-globin chains synthesized.

Me_2SO fails to initiate accumulation of globin mRNA sequences in strain DR10c3; this suggests that resistance is not due to a defect in translation. Resistance could reflect an alteration at the level of control of transcription of those genes which constitute the program of erythroid differentiation. It is not possible, however, to eliminate the possibility that resistance is expressed through mRNA processing or turnover. The existence of a strain of MELC resistant to Me_2SO but sensitive to other defined reagents provides an opportunity to investigate the regulatory mechanisms in cytodifferentiation employing a somatic cell genetic approach.

We are indebted to Julia Banks and Chun Mei Tzeng for their invaluable assistance. These studies were supported in part by grants and contracts from the National Institutes of Health (GM-14552, CA-13696, CA-12504, and N01-CB-4-4008) and the National Science Foundation (GB-27388). A.B. is Faculty Research Scholar of the American Cancer Society; M. Tanaka was supported by Training Grant TIAM-05231.

1. Friend, C., Scher, W., Holland, J. G. & Sato, T. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 378–382.
2. Furusawa, M., Ikawa, Y. & Sugano, H. (1974) *Proc. Jpn. Acad.* **47**, 220–224.
3. Marks, P. A., Rifkind, R. A. & Bank, A. (1974) in *Biochemistry of Cell Differentiation* (MTP International Review of Science, University Park Press, Baltimore, Md.), Vol. 9, pp. 129–160.
4. Ross, J., Ikawa, Y. & Leder, P. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 3620–3623.
5. Sato, T., Friend, C. & deHarven, E. (1971) *Cancer Res.* **31**, 1402–1417.
6. Singer, D., Cooper, M., Maniatis, G. M., Marks, P. A. & Rifkind, R. A. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 2668–2670.
7. Levy, J., Terada, M., Rifkind, R. A. & Marks, P. A. (1975) *Proc. Nat. Acad. Sci. USA* **72**, 28–32.
8. Miller, O. J., Miller, D. A., Kouri, R. E., Allderdice, P. W., Dev, V. G., Grewal, M. S. & Hutton, J. J. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 1530–1533.
9. Committee on Standardized Genetic Nomenclature for Mice (1972) *J. Hered.* **63**, 69–72.
10. Bank, A. & Marks, P. A. (1966) *Nature* **212**, 1198–1200.
11. Modell, C. B., Latter, A., Steadman, J. H. & Huehns, E. R. (1969) *Br. J. Haematol.* **17**, 485–501.
12. Ramirez, F., Gambino, R., Maniatis, G. M., Rifkind, R. A., Marks, P. A. & Bank, A. (1975) *J. Biol. Chem.* **250**, 6054–6058.
13. Metafora, S., Terada, M., Dow, L. W., Marks, P. A. & Bank, A. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 1299–1303.
14. Kacian, D. L., Gambino, R., Dow, L. W., Grossbard, E., Natta, C., Ramirez, F., Spiegelman, S., Marks, P. A. & Bank, A. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 1886–1890.
15. Tanaka, M., Levy, J., Terada, M., Breslow, R., Rifkind, R. A. & Marks, P. A. (1975) *Proc. Nat. Acad. Sci. USA* **72**, 1003–1006.
16. Paul, J. & Hickey, I. (1974) *Exp. Cell Res.* **87**, 20–30.