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# Synchrony of gene expression and the differentiation of myeloid leukemic cells: reversion from constitutive to inducible protein synthesis

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**There are mutant myeloid leukemic cells that cannot be induced to differentiate in serum-free culture medium, or medium with calf serum by the macrophage and granulocyte differentiation-inducing protein (MGI-2) that induces differentiation in normal myeloid cells. These mutants can be induced to differentiate by MGI-2 in medium with mouse serum. The mechanism of this induction of differentiation has been analysed by using two-dimensional gel electrophoresis to study changes in the synthesis of cytoplasmic proteins. In calf serum, 46 of the protein changes that were induced by MGI-2 in normally differentiating cells were constitutive in the differentiation-defective mutant cells. Treatment with mouse serum reverted 13 of these proteins from the constitutive to the non-constitutive state. This reversion was associated with a gain of inducibility for various differentiation-associated properties, so that 23 proteins were induced by MGI-2 for the same type of change as in normal differentiation. A normal developmental program requires synchrony of gene expression. The existence of constitutive instead of inducible gene expression can produce asynchrony in this program and thus produce blocks in differentiation. The results indicate that it is possible to treat these mutant cells so as to induce the reversion of specific proteins from the constitutive to the non-constitutive state, and that this can then restore the synchrony required for induction of differentiation. It is suggested that this mechanism may also allow induction of differentiation in other types of differentiation-defective cells.**
*Key words:* differentiation-defective mutants/gene complementation/myeloid leukemia

## Introduction

The growth and differentiation of normal myeloid haematopoietic precursor cells are controlled by a family of macrophage and granulocyte inducing (MGI) proteins. Some of these proteins (MGI-1) induce cell growth (multiplication) and other proteins (MGI-2) induce differentiation (reviewed in Sachs, 1978, 1980, 1982; Lotem and Sachs, 1982). MGI-1 has also been referred to as mashran gm (Ichikawa *et al.*, 1967), colony stimulating factor (CSF) (Metcalf, 1969), colony stimulating activity (CSA) (Austin *et al.*, 1971) and MGI (Landau and Sachs, 1971). There are clones of myeloid leukemic cells that differ from normal myeloid precursors in that they no longer require MGI-1 for growth. Some of these clones can be induced to differentiate to mature macrophages or granulocytes by the normal myeloid differentiation-inducing protein MGI-2, and there are also mutant clones that are either partly or almost completely blocked in their ability to be induced to differentiate by MGI-2 (reviewed in Sachs, 1974, 1978, 1980, 1982). The mature cells derived from the differentiation of leukemic cells are no longer malignant *in vivo* (Fibach and Sachs, 1975; Lotem and Sachs, 1981).

Changes in the synthesis of specific proteins during differentiation have been analysed by two-dimensional gel electrophoresis in a number of cell systems (Alton and Lodish, 1977; Storti *et al.*, 1978; Levison *et al.*, 1978; Waring and Mahowold, 1979; Reeves and Cserjesi, 1979). We have also used this approach to study the synthesis of specific proteins in normal myeloid precursors and the different types of myeloid leukemic clones. The results have shown the existence of constitutive gene expression in the leukemic cells (Sachs, 1980; Liebermann *et al.*, 1980; Hoffman-Liebermann *et al.*, 1981a; Cohen and Sachs, 1981). All the leukemic clones were constitutive for changes in the synthesis of specific proteins that were induced in the normal cells after treatment with MGI-1. These constitutive protein changes have been called $C_{leuk}$; constitutive for leukemia. There were other protein changes that were induced by MGI-2 in normal myeloid precursors and in leukemic cells that can be induced to differentiate normally, and were constitutive in the differentiation-defective leukemic cells. The highest number of these constitutive changes was found in clones with the lowest ability to differentiate. These constitutive protein changes have been called $C_{def}$; constitutive for differentiation-defective (Lieberman *et al.*, 1980).

The protein changes during growth and differentiation of normal myeloid precursors seem to be induced as a series of parallel multiple pathways of gene expression (Liebermann *et al.*, 1980). The presence of constitutive instead of inducible gene expression for some pathways can produce asynchrony in the co-ordination required for the normal developmental program. In cells with $C_{def}$ this asynchrony can produce blocks in differentiation (Sachs, 1980). The present experiments show that constitutive $C_{def}$ protein changes in differentiation-defective leukemic cells can be made to revert to the normally inducible state. The data indicate that this reversion then restores the ability of the cells to be induced for various differentiation-associated properties by the normal differentiation-inducing protein MGI-2.

## Results

### *Protein changes induced by MGI-2 in calf serum*

Two clones of myeloid leukemic cells were used in the present experiments. One of these clones (no. 11) can be induced to differentiate by MGI-2 for the differentiation-associated properties of $C_3$ and Fc rosettes and the synthesis of lysozyme in medium with calf serum (Table I) or no serum (Symonds and Sachs, 1982a, 1982b). The cells of this leukemic clone can also be induced by MGI-2 to form mature cells. This clone will be referred to as $MGI^+D^+$ clone 11 ($MGI^+$ to indicate that the cells can be induced to differentiate by MGI-2; $D^+$ for differentiation to mature cells) (Sachs, 1980). The other clone (no. 1) could not be induced by MGI-2 to form $C_3$ or FC rosettes or to synthesize lysozyme in medium with calf serum (Table I) or no serum (Symonds and Sachs, 1982a, 1982b). This clone will be referred to as $MGI^-D^-$ clone 1.

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**Table I.** Induction of Fc and $C_3$ rosettes and lysozyme synthesis in clone 1 by mouse serum plus MGI-2

| Clone type | Clone no. | Serum | MGI-2 | Cells with rosettes (%) | | Lysozyme (μg equiv./ 5 x $10^6$ cells) |
|---|---|---|---|---|---|---|
| | | | | Fc | $C_3$ | |
| $MGI^-D^-$ | 1 | Calf | − | 0.5 ± 0.2 | 0.6 ± 0.2 | 0 |
| | | | + | 0.6 ± 0.1 | 0.5 ± 0.2 | 0 |
| | | Mouse | − | 0.5 ± 0.1 | 0.4 ± 0.1 | 0 |
| | | | + | 2.0 ± 0.3 | 2.8 ± 0.4 | 0.4 ± 0.1 |
| $MGI^+D^+$ | 11 | Calf or mouse | − | 0.6 ± 0.2 | 0.5 ± 0.1 | 0 |
| | | Calf or mouse | + | 14.0 ± 1.7 | 19.1 ± 2.3 | 0.8 ± 0.1 |

Cells were cultured for 2 days with (+) or without (−) MGI-2 in medium with 1% of the serum type shown, and the frequency of cells with rosettes and amount of lysozyme were then determined.

These clones were cultured with different materials added to the culture medium and changes in the cytoplasmic proteins analysed by two-dimensional gel electrophoresis after labeling with [$^{35}$S]methionine for 3 h at the end of 2 days of incubation.

One hundred and eighty proteins were scored. In cells cultured with calf serum there were 92 protein changes in $MGI^+D^+$ clone 11 incubated with MGI-2 for 2 days (Figure 1, group I – VI) and 10 protein changes in $MGI^-D^-$ clone 1 incubated with MGI-2 for the same period (Figure 1, groups II and III). The 10 changes in clone 1 were a subset of the 92 changes in clone 11. Most (85%) of the protein changes in clone 11 and all those induced in clone 1 by 2 days were decreases (Figure 1).

In the cells cultured in calf serum, 46 of the 92 protein changes induced by MGI-2 in clone 11 were constitutively expressed in clone 1 cells that had not been incubated with MGI-2. These proteins have been labelled in Figure 1 by the letter C. They are of the type called $C_{def}$ (Liebermann *et al.*, 1980).

*Protein changes induced by mouse serum: reversion from constitutive to non-constitutive state*

Incubation of $MGI^-D^-$ clone 1 with mouse serum instead of calf serum without adding MGI-2, did not induce the formation of $C_3$ or Fc rosettes, or the synthesis of lysozyme (Table I). However, mouse serum induced changes in the synthesis of certain proteins. These included 13 proteins that were induced to revert from the constitutive to the non-constitutive state (Figure 1, groups IV and V). Mouse serum thus induced this reversion in 28% (13 out of 46) of the constitutive protein changes in clone 1. In addition, mouse serum induced four protein changes that were also induced by MGI-2 in calf serum (Figure 1, group III) and one protein change to a level similar to that found in uninduced clone 11 (Figure 1, group IV, no. 5). In contrast to these changes induced by mouse serum compared with calf serum in $MGI^-D^-$ clone 1, there were no protein changes induced by mouse serum in $MGI^+D^+$ clone 11 (Figure 1).

*Reversion from constitutive to non-constitutive state and the induction of differentiation*

Treatment of clone 1 with mouse serum and MGI-2, but not with either treatment alone, induced the formation of $C_3$ and Fc rosettes and the synthesis of lysozyme (Table I). The protein changes associated with this differentiation that were found only after both treatments included nine constitutive proteins in group V that were induced to increase by mouse serum and thus reverted to the non-constitutive state, and were then decreased by MGI-2 as in normal differentiation. Mouse serum plus MGI-2 also induced the same type of change as in normal differentiation in eight constitutive and six non-constitutive proteins in group VI (Figures 1 and 2). The constitutive proteins in group VI were at a lower level in clone 1 than in their non-constitutive state in clone 11 and they were induced to decrease further. At 2 days after the combined treatment in clone 1 they were therefore lower than after treatment of clone 11 with MGI-2 (Figure 1). $MGI^+D^+$ clone 11 showed the same protein changes with mouse serum and MGI-2 as those induced by treatment only with MGI-2 (Figure 1).

The results show that reversion from the constitutive to the non-constitutive state in 13 proteins in clone 1 was associated with induction of differentiation, so that 23 proteins were induced by MGI-2 for the same type of change in clone 1 as in clone 11. However, not all the 46 proteins that were in the constitutive state in clone 1 were induced to change by both treatments (Figures 1 and 3). This can explain why these treatments induced the formation of $C_3$ and Fc rosettes and the synthesis of lysozyme, but did not induce the formation of mature cells in clone 1 (Symonds and Sachs, 1982a).

There were also three other proteins that were originally at a higher level in clone 1 than in clone 11, and which were reduced in clone 1 after both treatments to the same level as in untreated clone 11 (Figure 1, group VII). These proteins did not change during differentiation of clone 11, and they may be proteins whose initial level also plays a role in regulating the induction of differentiation.

## Discussion

Differentiation-defective ($MGI^-D^-$) clones of myeloid leukemic cells that are blocked in their ability to be induced to differentiate by the normal myeloid differentiation-inducing protein MGI-2, can be induced for some differentiation-associated properties by combined treatment with various compounds (Krystosek and Sachs, 1976; Lotem and Sachs, 1979; Symonds and Sachs, 1982a). Among these compounds are the tumor-promoting phorbol ester 12-O-tetradecanoyl-phorbol-13-acetate (TPA) which can regulate gene expression both at the level of mRNA production and mRNA transla-

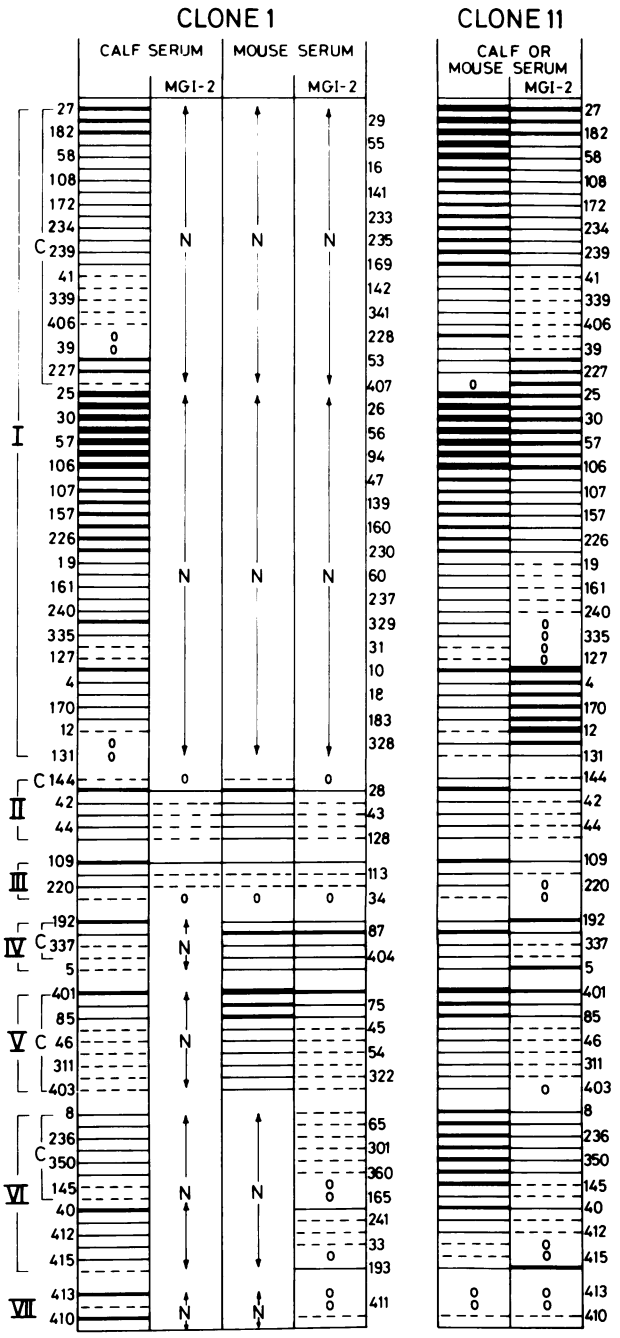


**Fig. 1.** Schematic summary of protein changes in $MGI^-D^-$ clone 1 and $MGI^+D^+$ clone 11 incubated in calf serum or mouse serum with or without MGI-2 for 2 days. The numbers of the proteins are not continuous and have been rearranged into groups. Differences in the thickness of the solid line indicate differences in the relative synthesis of the polypeptides with the thickest line showing the highest synthesis; a dashed line indicates a lower synthesis than the thinnest solid line; and 0 indicates that the polypeptide was not detected. (C), proteins that were in the constitutive state in clone 1 in calf serum. (N), no change in synthesis compared with the preceding column.

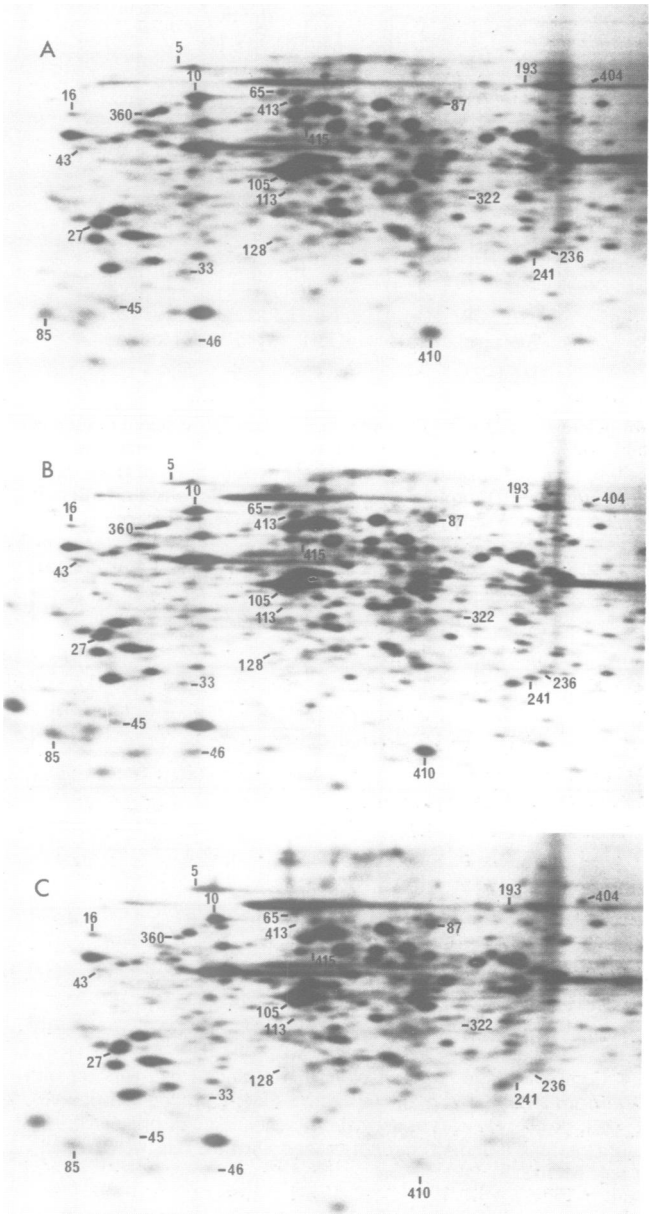


**Fig. 2.** Two-dimensional gel electrophoresis of proteins from clone 1. **(A)**, in calf serum; **(B)**, in mouse serum; **(C)**, in mouse serum and MGI-2. The isoelectric focusing dimension is horizontal with the basic end at the right and the acidic end at the left. Protein numbers correspond to those shown in Figure 1. Protein no. 105 is actin.

tion (Hoffman-Liebermann *et al.*, 1981b), and which induced differentiation in a differentiation-defective clone after treatment together with MGI-2, but not after treatment together with lipopolysaccharide (LPS) or dexamethasone (Lotem and Sachs, 1979). However, in the case of mouse serum, differentiation was induced in such clones together with MGI-2, LPS or dexamethasone (Symonds and Sachs, 1982a). The present results have shown that this more general effect of treatment with mouse serum included the reversion of 13 proteins from the constitutive to the non-constitutive state.

Treatment with mouse serum and MGI-2 resulted in different types of complementation of gene expression (Figure 4). Nine out of the 13 constitutive proteins that were induced to revert by mouse serum were induced to change by MGI-2 as in normal differentiation, and there were also other types of changes that were induced only by the combined treatment (Figure 4). It will be of interest to characterise the compound(s) in mouse serum, also found in rat serum (Symonds and Sachs, 1982a), that is responsible for this effect. Such combined treatment may also induce differentiation in differentiation-defective clones of erythroleukemic (Friend,

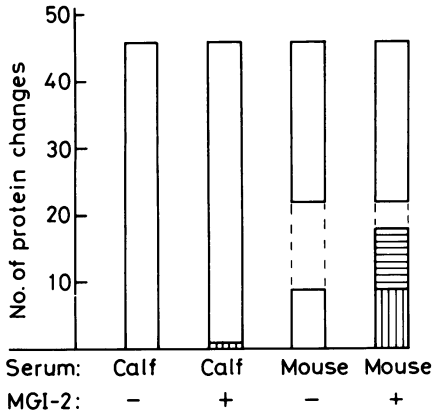


**Fig. 3.** Changes in proteins that were in the constitutive state in untreated clone 1 in calf serum. (▭), constitutive state; (⋯), reversion from constitutive to non-constitutive state; (▤), change induced from the reverted state, (▥), proteins that were lower in the constitutive than in the non-constitutive state and were induced to decrease further.

| No. of proteins | Protein changes: Mouse serum | Protein changes: Mouse serum + MGI-2 | Type of complementation |
|---|---|---|---|
| 9 | Increase | Decrease | Mouse serum → MGI-2 |
| 8 | No Change | Decrease | Mouse serum + MGI-2 |
| 5 | No Change | Decrease | Mouse serum + MGI-2 |
| 1 | No Change | Increase | |

**Fig. 4.** Complementation of gene expression in clone 1 by treatment with mouse serum and MGI-2. (▭), constitutive state; (⋯), non-constitutive state; (▨), protein changes in normal differentiation. All these proteins (Figure 1, groups V and VI) did not change when the cells were treated with only MGI-2.

1978; Marks and Rifkind, 1978) and other types of cells.

The presence of constitutive instead of inducible gene expression can produce asynchrony in the co-ordination required for the normal developmental program and thus produce blocks in differentiation (Sachs, 1980; Liebermann *et al.*, 1980). The present results indicate that it is possible to revert constitutive to inducible gene expression, and that this then restores the synchrony required for induction of differentiation. Not all the constitutive protein changes were induced to revert by the treatment used, and this can explain why the cells were induced for some but not for all the changes that occur in normal differentiation. The use of other compounds may allow reversion of the other constitutive proteins so that the right combination of compounds may permit the complete differentiation of these cells.

Studies with the genes *c-myc* and *c-mos* have shown the existence of DNA rearrangements in certain lymphomas and myelomas, and indicate that these rearrangements can activate these genes (Hayward *et al.*, 1981; Payne *et al.*, 1981; Calame *et al.*, 1982; Harris *et al.*, 1982; Rechavi *et al.*, 1982; Taub *et al.*, 1982). In one myeloma this DNA rearrangement with *c-mos* seemed to involve an insertion sequence (IS)-like element (Rechavi *et al.*, 1982). It will be of interest to determine whether the changes from inducible to constitutive gene expression in the myeloid leukemic cells, $C_{leuk}$ and $C_{def}$ (Liebermann *et al.*, 1980), which are associated with specific chromosome changes (Azumi and Sachs, 1977), may be due to similar DNA rearrangements in addition to changes in gene dosage (Sachs, 1974; Azumi and Sachs, 1977) and whether reversion to the non-constitutive state may be due to reversion of such rearrangements.

## Materials and methods

*Cells, cell culture and induction of differentiation*

$MGI^-D^-$ clone 1 was derived from an X irradiation-induced myeloid leukemia (Lotem and Sachs, 1974) and $MGI^+D^+$ clone 11 from a spontaneous myeloid leukemia (Fibach *et al.*, 1973). Cells were routinely cultured in Eagle's medium with a 4-fold concentration of amino acids and vitamins (H-21, Grand Island Biological Co., NY) (EM) and 10% fetal calf serum (which will be referred to as calf serum) from Grand Island Biological Co., NY. Mouse serum was made from blood collected from the orbital sinus of 2–4 months old ICR mice (Symonds and Sachs, 1982a). At least two batches of each serum type were tested and similar results were obtained with each batch. All sera were inactivated at 56°C for 30 min, and the concentration of serum used in the experiments was 1% (Symonds and Sachs, 1982a, 1982c). The source of MGI-2 was serum-free conditioned medium from Krebs ascites tumor cells (Lotem and Sachs, 1979). Cells were seeded to give a final density of 1–1.5 x $10^6$ cells/ml at the time of harvest. They were incubated with different materials added to the culture medium for 2 days, and at the end of this period they were analysed for the differentiation-associated properties of $C_3$ and Fc rosettes and synthesis of lysozyme (Lotem and Sachs, 1974, 1977; Krystosek and Sachs, 1976) and for cytoplasmic protein changes.

*Labelling of cells, two-dimensional gel electrophoresis and analysis of protein changes*

Cells were pulse labelled with [$^{35}$S]methionine (Radiochemical Centre, Amersham) for the final 3 h of the 2 day incubation and cytoplasmic extracts prepared as described (Hoffman-Liebermann and Sachs, 1978). Two-dimensional gel electrophoresis was carried out by the method of O'Farrell (1975), with a pH range of 4.5–7.3, except that the resolving gel in the second dimension was a 7–17% acrylamide gradient (Laemmli, 1970; Maizel, 1971). Gels were prepared for fluorography according to Bonner and Laskey (1974). Autoradiograms of the proteins were made by exposing gels containing 5 x $10^5$ c.p.m. for 48 h to X-ray film (Agfa, Curix RP2). Three cytoplasmic extracts were prepared for each experiment and each extract was run on at least two gels.

For the protein analysis, spots on the fluorogram which changed in intensity relative to neighbouring spots which did not change, were scored as relative protein decreases or increases. Regions of gels corresponding to specific spots were excised and the amount of radioactivity was measured by scintillation counting (Liebermann *et al.*, 1980). The results were in agreement with conclusions drawn by visual inspection. The level of confidence of this analysis, which shows the relative and not the absolute rates of protein synthesis, has been described (Liebermann *et al.*, 1980; Hoffman-Liebermann *et al.*, 1981a). A total of 180 polypeptide spots were scored in the present experiments.

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