The Erythropoietin Receptor of Rat Erythroid Progenitor Cells

CHARACTERIZATION AND AFFINITY CROSS-LINKAGE

(Received for publication, March 3, 1987)

Patrick Mayeux, Claudine Billat, and Robert Jacquot
From the Laboratoire de Physiologie Animale, Faculte des Sciences, F 51062 Reims Cedex, France

Commercially available 125I-labeled erythropoietin, obtained by genetic engineering from a human gene, was used to characterize receptors for this hormone on the cell surface of rat erythroid progenitor cells. A low number of high affinity binding sites (487 ± 32 sites/cell, \( K_d = 167 \pm 14 \) pm) were found. Nonerythroid cells and erythrocytes did not exhibit specific binding. The high affinity binding was reversible and displaced by unlabeled erythropoietin, but not by other hormones and growth factors. After incubation at 37 °C, nearly 35% of the specifically bound hormone was internalized, as judged by resistance to acidic buffer treatment. Thus, binding showed characteristics of a hormone-receptor association. 125I-Erythropoietin-labeled cells were treated with the bifunctional reagent disuccinimidyl suberate. Analysis of the cellular extracts by polyacrylamide gel electrophoresis under denaturing and reducing conditions revealed that erythropoietin can be cross-linked to two molecules of 94 and 78 kDa, respectively. Both labeled bands disappeared when the cells were labeled in the presence of an excess of unlabeled erythropoietin. Under nonreducing conditions, a cross-linked band of 230–255 kDa was observed. The relationships between these bands are discussed.

Erythropoietin, a hormone produced essentially by the kidney in adult mammals, controls erythropoiesis. Its plasma concentrations are of the order of 10 pm (1), and anemia increases both its circulating levels and the accumulation of its messenger RNA (2–4), (for a review on erythropoietin, see Ref. 5). Until recently, it has been difficult to purify large amounts of this hormone and to obtain populations of reactive cells; therefore, the modes of action of erythropoietin are still poorly understood.

Erythropoietin was initially extracted from urine of anemic patients (6), but now the isolation of its gene allows the production of important quantities of the pure hormone (7, 8). It is a heavily glycosylated protein of 166 amino acid residues, with a \( M_r = 34,000 \). Erythropoietin is necessary for survival, proliferation, and differentiation of "late" erythroid progenitor cells, the erythroid colony-forming units, and perhaps of their immediate descendants; it is likely that earlier progenitors, the erythroid burst forming units, are reactive to erythropoietin, as well as perhaps certain pluripotent hemopoietic stem cells (for a discussion, see Refs. 9 and 10). Erythroblasts are apparently able to complete their matura-

\[ K_d = 167 \pm 14 \text{ pm} \]

 tion in the absence of erythropoietin.

Recently, methods have been described for the preparation of erythroid progenitors from hemopoietic populations, either normal (11–13) or infected with the anemic strain of the Friend virus (14). Using the latter material, Krantz and Goldwasser (15) demonstrated the existence of specific binding of [3H]-erythropoietin and found 660 binding sites/cell with a high binding affinity (\( K_d = 5.2 \text{ nM} \)). We describe the preparation from rat fetal livers of cell suspensions essentially made of erythroid progenitors which depend on erythropoietin for proliferation and differentiation (13, 16). These cells were used in this work to test the binding of radioiodinated erythropoietin; we demonstrate the existence of specific and reversible high affinity binding and performed cross-linkage experiments of the hormone with its presumed receptor.

MATERIALS AND METHODS

Chemicals—Pure erythropoietin, produced from an isolated human erythropoietin gene by Kirin-Amgen, Inc. and purchased from Amersham Corp., had a specific activity of 70,000 units/mg. Its labeled derivative, (3-[125I]iodotyrosyl)erythropoietin, was also purchased from Amersham Corp.; six batches were used with specific radioactivities at delivery ranging from 400 to 1,200 Ci/mmol. In some experiments, pig nonradioactive erythropoietin (1,010 units/mg of protein; Centre National de Transfusion Sanguine, Paris) was also used and gave results completely superimposable to, and therefore combined with, those obtained with pure human hormone. Bovine insulin was from Behring Diagnostics; pig platelet-derived growth factor was from Bioprocesing LTD; and mouse epidermal growth factor and phorbol 12-myristate 13-acetate were from Sigma. Bovine platelet-derived growth factor purified as described in Ref. 17 was a generous gift from Dr. M. Felix (University of Strasbourg). Rat interleukin 3 obtained by genetic engineering was a generous gift from Dr. A. J. Kapel (University of Canberra, Canberra, Australia) and was in solution in a culture medium at 200 units/ml (1 unit/ml is the concentration producing half-maximal stimulation in a bone marrow cell proliferation assay). DMSO, Percoll, and RPMI 1640 medium were from Pierce Chemical Co., Pharmaceuticals P-L Biochemicals, and GIBCO, respectively. Inhibitors of proteases were from Sigma.

Preparation of Cells—The preparation of erythroid progenitors from rat fetal livers has been previously described (13, 16). Briefly, suspensions of erythroid cells, obtained by mild mechanical dissociation of livers followed by filtration on a nylon sieve, were treated with rabbit antiserum against rat erythrocytes in the presence of 10% (v/v) guinea pig serum as a source of complement (this procedure produces the lysis of the erythrocytes and of the large majority of the erythroblasts); the remaining intact cells were washed and layered on the top of a discontinuous gradient of Percoll. The cells with a density >1.055 were recovered and washed three times before use. These cells represent roughly 3.8% of the initial cell suspension; over 85% are morphologically undifferentiated, and 65% produce erythroid colonies (mainly erythroid colony-forming units) after culture in semisolid media. Their proliferation and differentiation are strictly erythropoietin-dependent (16).

Rat thymocytes and splenocytes were also prepared by mechanical

The abbreviations used are: DSS, disuccinimidyl suberate; SDS, sodium dodecyl sulfate.
dissociation followed by filtration on a nylon sieve (50-μm mesh). Splenocytes were isolated by centrifugation on Percoll. Circulating rat erythrocytes and leukocytes were also prepared by centrifugation on a density gradient.

Murine erythroleukemic (Friend) cell line 745 was maintained by resuscitation every 3–4 days in RPMI 1640 medium containing 10% heat-inactivated bovine fetal serum and increasing concentrations of sodium dodecyl sulfate (at least ×10, and generally ×50) of radioactive erythropoietin in the presence or absence of an excess of the non-labeled hormone (durations and temperatures of incubations are given under "Results"). Incubations were ended by the addition of 4 ml of ice-cold RPMI 1640 medium; the cells were recovered after 5 min of centrifugation at 180 × g and washed three more times, and we verified that the last supernatant fraction was not radioactive. Radioactivity bound was determined by γ-counting. As usual, high affinity (specific) bound radioactivity was calculated as the difference between total (absence of nonlabeled erythropoietin) and nonspecific (presence of nonlabeled erythropoietin) bound radioactivities.

**Determination of Cell Surface-bound 125I-Erythropoietin**—Rat erythroid progenitor cells (1–10 × 10⁶) were incubated in 100 μl RPMI 1640 medium containing 10% heat-inactivated bovine fetal serum and increasing concentrations of radioactive erythropoietin in the presence or absence of an excess of the non-labeled hormone (durations and temperatures of incubations are given under "Results"). Incubations were ended by the addition of 4 ml of ice-cold RPMI 1640 medium; the cells were recovered after 5 min of centrifugation at 180 × g and washed three more times, and we verified that the last supernatant fraction was not radioactive. Radioactivity bound was determined by γ-counting. As usual, high affinity (specific) bound radioactivity was calculated as the difference between total (absence of nonlabeled erythropoietin) and nonspecific (presence of nonlabeled erythropoietin) bound radioactivities.

**Comparison of the Binding Affinities of Radioactive and Native Erythropoietin**—Rat erythroid progenitors from rat fetal liver, and binding was proportional to the cell concentration (Fig. 1). As illustrated in Fig. 2A, in the presence of 140 pm labeled hormone, equilibrium was reached at 37 °C after 1-h of incubation; and the radioactivity specifically bound remained constant for at least 4 h. When an excess (5 nm) of nonlabeled hormone was added after 1 h of incubation, the radioactivity specifically bound decreased, and 90% of it was displaced after 4 h of chase. By plotting the logarithm of the bound radioactivity as a function of chase duration (Fig. 2B), two phases were evidenced: an initial rapid exchange during which roughly 70% of the specifically bound radioactivity was released (t₅₀ = 30 min) and a slower and prolonged dissociation (t₅₀ = 3–4 h). Dissociation kinetics were also studied by transferring cells, prelabeled for 1 h, in a ligand-free medium at 37 °C; the results were essentially the same (not shown).

**Characteristics of Specific Binding of 125I-Erythropoietin**—As shown in Fig. 3, at least 85% of the labeled erythropoietin could be specifically bound. Moreover, even when more

---

**RESULTS**

**Kinetics of High Affinity Binding of 125I-Erythropoietin**

125I-Erythropoietin was bound with high affinity by the erythroid progenitors from rat fetal liver, and binding was proportional to the cell concentration (Fig. 1). As illustrated in Fig. 2A, in the presence of 140 pm labeled hormone, equilibrium was reached at 37 °C after 1-h of incubation; and the radioactivity specifically bound remained constant for at least 4 h. When an excess (5 nm) of nonlabeled hormone was added after 1 h of incubation, the radioactivity specifically bound decreased, and 90% of it was displaced after 4 h of chase. By plotting the logarithm of the bound radioactivity as a function of chase duration (Fig. 2B), two phases were evidenced: an initial rapid exchange during which roughly 70% of the specifically bound radioactivity was released (t₅₀ = 30 min) and a slower and prolonged dissociation (t₅₀ = 3–4 h). Dissociation kinetics were also studied by transferring cells, prelabeled for 1 h, in a ligand-free medium at 37 °C; the results were essentially the same (not shown).

---

**Characteristics of Specific Binding of 125I-Erythropoietin**—As shown in Fig. 3, at least 85% of the labeled erythropoietin could be specifically bound. Moreover, even when more
Rat Erythropoietin Receptor

A

Association and dissociation kinetics of \(^{125}\text{I}\)-erythropoietin high affinity binding. A, results of a representative experiment. Rat erythroid progenitor cells were incubated for 1 h at 37 °C with 140 pM \(^{125}\text{I}\)-erythropoietin, and incubation was continued in the absence (○) or presence (●) of 5 nM nonlabeled erythropoietin. Each point represents high affinity bound radioactivity per \(5 \times 10^6\) cells. B, dissociation kinetics in semilogarithmic representation. Each point is the mean ± S.D. of three independent experiments. During the first hour of incubation, the standard deviations were too small to be drawn.

FIG. 2. Association and dissociation kinetics of \(^{125}\text{I}\)-erythropoietin high affinity binding. A, results of a representative experiment. Rat erythroid progenitor cells were incubated for 1 h at 37 °C with 140 pM \(^{125}\text{I}\)-erythropoietin, and incubation was continued in the absence (○) or presence (●) of 5 nM nonlabeled erythropoietin. Each point represents high affinity bound radioactivity per \(5 \times 10^6\) cells. B, dissociation kinetics in semilogarithmic representation. Each point is the mean ± S.D. of three independent experiments. During the first hour of incubation, the standard deviations were too small to be drawn.

FIG. 3. Maximum binding capacity of \(^{125}\text{I}\)-erythropoietin. As described in text, a solution containing \(10^4\) cpm of \(^{125}\text{I}\)-erythropoietin was progressively depleted by sequential incubations at 37 °C with murine erythroleukemic cells: adjusted control medium (●) and test medium (○) (see "Materials and Methods"). Each point represents the mean ± S.D. of three assays.

FIG. 3. Maximum binding capacity of \(^{125}\text{I}\)-erythropoietin. As described in text, a solution containing \(10^4\) cpm of \(^{125}\text{I}\)-erythropoietin was progressively depleted by sequential incubations at 37 °C with murine erythroleukemic cells: adjusted control medium (●) and test medium (○) (see "Materials and Methods"). Each point represents the mean ± S.D. of three assays.

than 40% of the hormone was removed by preceding incubations, the radioactivity specifically bound did not differ significantly from that obtained with the corresponding adjusted control medium. In what follows, we shall consider that the maximal binding capacity of the labeled erythropoietin is 100%; even if this value is overestimated by 15%, such an error does not affect the results significantly.

Comparison of the binding affinities of the labeled and nonlabeled hormones by self-displacement yielded two straight and parallel displacement curves, suggesting that the affinities are very similar (Fig. 4).

Comparison of the binding affinities of the labeled and nonlabeled hormones by self-displacement yielded two straight and parallel displacement curves, suggesting that the affinities are very similar (Fig. 4).

The number of binding sites and the affinity were evaluated by Scatchard plots (22). Fig. 5 presents the results of a typical experiment. As judged by the linearity of the representation, the binding sites belong to one category; their number per cell was 485 ± 32 (mean ± S.D., \(n = 3\)). The apparent dissociation constant at equilibrium was \(K_d = 167 ± 14\) pM (mean ± S.D., \(n = 3\)).

Gel filtration (Ultrogel AcA 54) or SDS-polyacrylamide gel electrophoresis of the medium after incubation did not reveal significant degradation of the labeled hormone (not shown).

Specificity of Binding

Cellular Specificity—\(^{125}\text{I}\)-erythropoietin (200 pM) was incubated with several cell types (Table I); among them, only the erythroid progenitors bound the hormone specifically.

Molecular Specificity—Among the substances tested (Table
ERYTHROPOIETIN. Each value is the mean ± S.D. of six determinations made in at least two independent experiments. The results are expressed as counts/minute specifically bound by $5 \times 10^6$ cells, except for erythrocytes, where 5 μl of packed cells were used.

TABLE I

| Cells                          | $^{125}$I-Erythropoietin specifically bound |
|-------------------------------|--------------------------------------------|
| Erythroid progenitor cells (rat) | 272 ± 537                                  |
| Thymocytes (rat)               | 30 ± 16                                    |
| Splenocytes (rat)              | 47 ± 18                                    |
| Erythrocytes (rat)             | 5 ± 23                                     |
| Peripheral blood leukocytes (rat) | 39 ± 22                                   |
| Human erythrocytes             | 21 ± 17                                    |

Specificity of $^{125}$I-erythropoietin binding to erythroid progenitor cells

Each result is the mean ± S.D. of four values obtained in two independent experiments. The cells ($5 \times 10^6$) were incubated with 200 pm $^{125}$I-erythropoietin and the indicated competitors for 2 h at 37 °C. In each experiment, the results are expressed as percent of $^{125}$I-erythropoietin binding in the absence of competitor.

| Competitor                  | Concentration | Binding REP$^*$ FC % of control |
|-----------------------------|---------------|--------------------------------|
| None (control)              |               | 100 ± 3                        |
| Erythropoietin              | 5 mM          | 19 ± 3                         |
| Erythropoietin              | 10 nM         | 127 ± 3                        |
| Insulin                     | 200 nM        | 105 ± 8                        |
| EGF                         | 200 nM        | 101 ± 5                        |
| PDGF                        | 30 nM         | 108 ± 2                        |
| FGF                         | 100 nM        | 104 ± 7                        |
| PMA                         | 100 nM        | 112 ± 6                        |
| IL-3                        | 100 units/ml  | 79 ± 8                         |

$^*$ REP, rat erythroid progenitors; FC, Friend cells; ND, not determined; EGF, epidermal growth factor; PDGF, platelet-derived growth factor; FGF, fibroblast growth factor; PMA, phorbol 12-myristate 13-acetate; IL-3, interleukin 3.

II), only the nonlabeled erythropoietin was able to modify significantly the specific binding $^{125}$I-erythropoietin to rat erythroid progenitors. A similar specificity was observed when using Friend cells.

Internalization

Hormones bound to cell-surface receptors are generally eluted with acidic buffers (23, 24). Here, over 90% of the radioactivity specifically bound after 6 h of incubation of the cells at 0 °C in the presence of the labeled hormone could be recovered by this method. After 30 min of incubation at 37 °C, only 63 ± 3% (mean ± S.D. n = 3) were eluted and 62 ± 5% (mean ± S.D., n = 7) after 2 h incubation.

Affinity Cross-Linkage

After cross-linkage, the analysis of the cellular extract by polyacrylamide gel electrophoresis after treatment by β-mercaptoethanol revealed the existence of three labeled bands (Fig. 6), with the major one corresponding to the free hormone. The efficiency of the cross-linking procedure was rather low, as already observed by others (25). The two labeled bands of high molecular masses were observed only after DSS treatment and were not observed when nonlabeled erythropoietin was in excess during the cell incubation; their molecular masses, determined by comparison with markers stained with Coomassie Blue after either simple (7.5% polyacrylamide) or gradient (5–15% polyacrylamide) gel electrophoresis, were 128 ± 4 kDa and 112 ± 4 kDa (mean ± S.D., n = 3). When protease inhibitors were omitted during cell lysis, the two labeled bands were attenuated, with the 112-kDa band more than the 128-kDa one (Fig. 6, lane A).

In the absence of reductant, the cross-linked material migrated as a single band of higher molecular mass (Fig. 7). By comparison with unreduced molecular mass markers on adjacent lanes, this molecular mass was estimated to be 230–255 kDa. It should be noticed that, under these conditions of electrophoresis, a fraction of the radioactivity did not penetrate the gel; and attempts to dissociate these aggregates with high concentrations of urea or butanol or with acetone precipitation (−20 °C) were unsuccessful. In one experiment, the two bands of lower molecular mass (usually seen in the presence of reductant) were also observed, together with the 230–255-kDa band (not shown).

DISCUSSION

Weiss et al. (26) have shown, with fluorescent antibodies, that only 1–2% of the rat bone marrow hemopoietic cells bind erythropoietin. This very low percentage prevents biochemical evaluation of erythropoietin binding by whole suspension of bone marrow. Mouse erythroid progenitor cells transformed by the anemic strain of Friend virus are erythropoietin-dependent for their differentiation, and they bind erythropoietin specifically (15). In this work, rat normal erythroid progenitors prepared from fetal liver by a technique (13, 16) derived from the procedure of Cantor et al. (27) bind this hormone specifically, with a high affinity, and reversibly. Iodinated erythropoietin has been claimed to be inactive (10); and as emphasized by Nicola and Metcalf for granulocyte colony-stimulating factor (19), preservation of the biological activity depends largely on the conditions of iodination. The labeled preparations we tested were active, but this activity was perhaps due to the presence of nonlabeled molecules since...
the highest specific radioactivity used (1200 Ci/mol) corresponds statistically to less than one atom of iodine/erythropoietin molecule and there are 4 tyrosines potentially available for iodination/molecule (17). It was therefore important to show by self-displacement analysis that the affinities of the native and labeled hormones were the same.

Among the cells we tested, only the rat erythroid progenitors (Table I) and the murine erythroleukemic Friend cells (20) possessed high affinity receptors for erythropoietin. Cells from other hemopoietic lineages, either normal (Table I) or neoplastic (K 562, HL 60, L1210) (20), did not bind the hormone. Mature erythrocytes (rat or human) had no receptors for erythropoietin. Mature erythrocytes (rat or human) had no receptors for erythropoietin. Cells of the white (20) and rat normal erythroid progenitors. More likely, the discrepancy reflects different properties of the labeled ligands. In our system, half-maximal biological stimulation was obtained at around 40 pM erythropoietin (16); at this concentration, roughly 20% of the receptors are occupied. Maximal stimulation was observed at around 200 pM erythropoietin, a value close to $K_d$ for specific binding.

Internalization was apparently modest (only some 35% after 2 h at 37 °C), and SDS-polyacrylamide gel electrophoresis of the bound radioactivity did not reveal substantial hormone degradation (not shown). Nevertheless, as the full biological activity of the iodinated hormone was not proven, it is possible that its internalization and/or degradation was not representative of that of native erythropoietin.

The bifunctional reagent DSS has been often used to crosslink hormones and receptors (32-37). In our system, two bands were revealed by electrophoresis. These bands, which were not observed in the absence of DSS or in the presence of a large excess of nonlabeled erythropoietin, may represent cross-linked hormone-receptor complexes. The difference between their molecular masses (16 kDa) cannot be attributed to the cross-linkage of a supplementary molecule of the ligand (34 kDa). The fact that the 112-kDa band seems more affected by the presence of proteases than the 128-kDa band precludes a degradative filiation between them. By analogy with what was described for the insulin receptor (38), we suppose that specific binding of erythropoietin occurs only on the 94-kDa subunit for the following reasons. 1) Scatchard plots suggest that there is only one category of binding sites; and 2) as in the case of the insulin receptor, cross-linkage is stronger on one subunit (the 94-kDa protein). According to this hypothesis, the 78-kDa subunit should be close enough to the binding sites of the 94-kDa subunit to allow some cross-linkage with specifically bound erythropoietin. In the absence of a single band of cross-linked material was observed; as some proteins behave anomalously in their nonreduced form, the estimated molecular mass of this band (230-255 kDa) may be inaccurate. This band was never observed in the presence of $\beta$-mercaptoethanol and might represent a disulfide-bonded association of the two proteins (94 and 78 kDa, respectively, when hormone-free) observed under reducing conditions. However, as a large fraction of the radioactivity did not enter the gel under nonreducing conditions, we cannot draw any definite conclusion. In one experiment, the cross-linked sub-

**Fig. 7. Affinity cross-linkage of $^{125}$I-erythropoietin: electrophoresis of a non-reduced extract.** The cellular extract was prepared as described for Fig. 6 (lane C), but without $\beta$-mercaptoethanol. Electrophoresis was carried out on a 6.5% polyacrylamide gel. Molecular mass markers were unreduced high and low molecular mass markers from Pharmacia P-L Biochemicals and *Escherichia coli* $\beta$-galactosidase (Sigma). TOP indicates the top of the resolving gel.
units were observed in the absence of β-mercaptoethanol, together with the 230-kDa form; this may be an artifact during the preparation of the cellular extracts, but it should be noted that free (nonreduced) subunits of the insulin receptor were observed in the membrane of various cells and that their existence may have a physiological significance (39).

Acknowledgements—We wish to thank Dr. J. M. Félix (University of Strasbourg) for the gift of platelet-derived growth factor, epidermal growth factor, and fibroblast growth factor, and Dr. A. J. Hapel (University of Cambridge) for the gift of recombinant rat interleukin 3. We are also grateful to Martine Decarme, Yvette Longis, and Marie-Line Sowa for excellent technical assistance and to Michéle Subtil-Francois for typing the manuscript.

Addendum—While this manuscript was being revised, Sawyer et al. (40) published a report on the characterization of erythropoietin receptors in Friend virus-infected erythroid cells. Our results are in general agreement with theirs except for two points. 1) The total number of receptors reported by these authors is larger than what we found in rat erythroid progenitors; this difference could probably be explained both by the use of different types of cells (normal rat erythroid progenitors versus mouse erythroid cells transformed by Friend virus) and by the fact that we probably slightly underestimated the number of receptors per erythroid progenitor. 2) Sawyer et al. report the presence of two sets of receptors \( K_d = 0.07-0.18 \) and \( 0.55-1.3 \) nM, respectively. In our system, no other set of receptors could be characterized even when hormone concentrations were increased to 5 nM. Comparisons of the \( K_d \) values of the dissociation kinetics suggest that the receptor we describe corresponds to the high affinity receptor described by Sawyer et al. Whether the discrepancy comes from the use of different biological materials or of different experimental procedures remains to be established.

REFERENCES
1. Keck, H. P., and Goldwasser, E. (1981) Ann. Intern. Med. 97, 44-47
2. Carnot, P., and Deflandre, C. (1906) C. R. Hebld. Sances Acad. Sci. Ser. M. 3, 384-387
3. Bondurant, M. C., and Koury, M. J. (1986) Mol. Cell. Biol. 6, 2731-2733
4. Beru, N., McDonald, J., Lacombe, C., and Goldwasser, E. (1986) Mol. Cell. Biol. 6, 2571-2575
5. Spivak, J. L. (1986) Int. J. Cell Cloning 4, 139-166
6. Miyake, T., Kung, C. K.-H., and Goldwasser, E. (1977). J. Biol. Chem. 252, 5558-5564
7. Jacobs, K., Shoemaker, C., Rudersdorf, R., Neill, S. D., Kaufman, R. J., Mufson, A., Seehra, J., Jones, S. S., Hewick, R., Fritsch, E. F., Kawakita, M., Shimizu, T., and Miyake, T. (1985). Nature 313, 806-809
8. Lin, F. K., Suggs, S., Lin, C. H., Browne, J. K., Smalling, R., Egie, J. C., Chen, K. K., Fox, G., Martin, F., Stabinsky, Z., Bradfaw, S. M., Lai, P. H., and Goldwasser, E. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 7580-7584
9. Goldwasser, E. (1982) J. Cell. Physiol. 1, (suppl.) 133-135
10. Goldwasser, E. (1984) Blood Cells 10, 147-162
11. Bonancou-Tzedaki, S. A., Sohi, M., and Arnstein, H. R. V. (1981) Cell Differ. 10, 267-279
12. Nijhof, W., and Wierenga, P. K. (1983) J. Cell Biol. 96, 386-392
13. Mayeux, P., Billat, C., Félix, J. M., and Jacquot, R. (1986) Cell Differ. 18, 17-26
14. Koury, M. J., Bondurant, M. C., Duncan, D. T., Krantz, S. B., and Hankins, W. D. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 635-639
15. Krantz, S. B., and Goldwasser, E. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 7574-7578
16. Mayeux, P., Félix, J. M., Billat, C., and Jacquot, R. (1986) Exp. Hematol. 14, 801-806
17. Pettmann, B., Weibel, M., Sassen-Brenner, M., and Labourdette, G. (1986) FEBS Lett. 189, 102-108
18. Calvo, J. C., Radicella, J. P., and Charreau, E. H. (1983) Biochem. J. 212, 259-264
19. Nicola, N. A., and Metcalf, D. F. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 3765-3769
20. Mayeux, P., Billat, C., and Jacquot, R. (1987) FEBS Lett. 211, 229-233
21. Laemmli, U. K. (1970) Nature 227, 680-685
22. Scatchard, G. (1949) Ann. N. Y. Acad. Sci. 51, 660-672
23. Guilbert, L. J., and Stanley, E. R. (1986) J. Biol. Chem. 261, 4024-4032
24. Tanigimoto, M., Feinman, R., Kohase, M., and Vilecek, J. (1986) Arch. Biochem. Biophys. 249, 563-568
25. Nicola, N. A., and Peterson, L. (1986) J. Biol. Chem. 261, 12384-12389
26. Weiss, T. L., Kung, C. K. H., and Goldwasser, E. (1985) J. Cell. Biochem. 27, 57-65
27. Cantor, L. N., Morris, A. J., Marks, P. A., and Rikitkind, R. A. (1972) Proc. Natl. Acad. Sci. U. S. A. 69, 1337-1341
28. Baciu, I., Ivanof, L., Pavel, T., Marina, C., Zirbo, M., and Zdrenkova, C. (1985) Rev. Zool. Morphol. Embryol. Physiol. 22, 227-231
29. Brown, K. D., Dicker, P., and Rosengurt, E. (1979) Biochem. Biophys. Res. Commun. 86, 1037-1043
30. Shoyab, M., De Larco, J. E., and Todaro, G. J. (1979) Nature 279, 387-391
31. Chen, B. D.-M., Lin, H.-S., and Hsu, S. (1983) J. Cell. Physiol. 116, 207-212
32. Pilch, P. F., and Czech, M. P. (1979) J. Biol. Chem. 254, 3375-3381
33. Walker, F., and Burgess, A. W. (1985) EMBO J. 4, 933-939
34. Massagué, J., and Liao, B. (1985) J. Biol. Chem. 260, 2636-2645
35. Park, L. S., Friend, D., Gillis, S., and Urdal, D. L. (1986) J. Biol. Chem. 261, 4177-4183
36. Park, L. S., Friend, D., Gillis, S., and Urdal, D. L. (1986) J. Biol. Chem. 261, 205-210
37. Bovizar, Z., Poucheau-Peron, M., Taboulet, J., Moukhtar, M. S., and Milhaud, G. (1986) Eur. J. Biochem. 155, 141-147
38. Massagué, J., Pilch, P. F., and Czech, M. P. (1981) J. Biol. Chem. 256, 3182-3190
39. Crettaz, M., Jiadal, L., Kasuga, M., and Kahn, C. R. (1984) J. Biol. Chem. 259, 1143-1145
40. Sawyer, S. T., Krantz, S. B., and Goldwasser, E. (1987) J. Biol. Chem. 262, 5554-5562