
THEORETICAL CONSIDERATIONS OF GENETIC REGULATION OF GRANULOPOIESIS^{1, 2}

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ABSTRACT

In a theoretical model describing the factoral hypotheses of granulopoietic control in terms of modern concepts of genetic regulation, the sequential nature of the differentiation process is explained as co-induction of operons or "gene clusters." The full derepression of the operator gene is possible in the presence of both a primary inducing "factor," and a "co-inducer" produced by the last structural gene in the preceding operon. The "co-inducer" and "factor" act together to remove the repressor substance and to allow full functioning of the operon.

When the inducer is removed or inactivated, feedback repression of earlier steps in the maturation sequence is noted. It is probable that this takes place at the translational level or during enzyme synthesis. Finally, the possibility exists that primary control of granulopoiesis occurs at the transcriptional or translational level. The presence of only two distinct factors ("maturation" and "release") would be prejudicial to translational control, but at present little evidence either supporting or refuting transcriptional control is available.

INTRODUCTION

During the past ten years, the study of blood-cell development, hemopoiesis, has made significant strides forward. Computer simulation and mathematical

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analysis of myelopoietic stem-cell kinetics have put forth feasible models for experimental study. However, little direct correlation of kinetic models with molecular events in the cell cycle has been made. This paper relates the kinetic models of Cronkite and Vincent (1970) and of King-Smith and Morley (1970) describing granulocyte development with evidence of factors controlling proliferation and differentiation of granulocytic precursors (Foster *et al.*, 1968; Robinson, Stanley, and Metcalf, 1969; Graham and McMahon, 1971; Graham and Morrison, 1970; Graham, Earney, McMahon, and Tjan, In press).

CONTROL OF GRANULOPOIESIS

King-Smith and his coworkers (King-Smith and Morley, 1970; Morley, King-Smith, and Stohlman, 1970) have clearly demonstrated by computer simulation the probability of a feedback control of granulopoiesis, and have evaluated the granulocyte production rate and the time spent by granulocytes in the bone-marrow reserve-pool. They estimate that the obligatory time spent in the bone marrow by granulocytes is about eight days, and that the average total marrow transit-time is 10 to 12 days. They propose that the cyclic fluctuations in peripheral granulocyte levels are due to rises and falls in repressor (or initiator) substances.

Cronkite and Vincent (1970) propose a multiple-loop feedback system for regulation of granulopoiesis. Based on their established minimum transit-time through the marrow reserve pool of 48 hours in severe infection, the diminished reserve pool can increase stem-cell input by negative feedback. This would be too slow to increase the myeloproliferative rate, and Cronkite and Vincent hypothesized that the generative cycle decreases and added mitoses occur.

The concentration of the differentiation stimulus derepresses transcription sites in the stem cells and sets the rate of transcription and/or of mRNA quantity. This establishes the rate of protein synthesis, and thus, cells hypertrophy. Critical size triggers cell division; roughly four divisions occur between stem cell and metamyelocyte. In infection, the stimulus increases, increasing the quantity of mRNA, and thus, the rate of cellular hypertrophy. Cells divide more frequently until the hypothesized substance attains a concentration that inhibits further mitoses (Cronkite and Vincent, 1970).

The existence of serum "factors", which stimulate the release of granulocytes from the bone-marrow reserve-pool, has been known for several years. In fact, it has been possible to induce the appearance of these release factors in serum and with the release of granulocytes from the bone marrow of noninduced animals. A variety of stresses have been used to achieve this effect, including X-ray, leukapheresis, bacterial endotoxin, and assorted drugs, all of which act on the hemopoietic system. These are briefly reviewed by Graham (1969). Although the cytologic effects of these "release factors" are similar, they do not appear to be chemically identical (Bierman, 1964; Gostomzyk *et al.*, 1964).

The existence of a low-molecular-weight (45,000-60,000) protein in blood and urine has been demonstrated by several workers. Metcalf and coworkers (Robinson *et al.*, 1967; Foster *et al.*, 1968; Metcalf and Stanley, 1969; Stanley and Metcalf, 1969) have isolated from human blood and urine a material capable of stimulating production of mature granulocytes in tissue cultures of both normal and leukemic blast cells. We have shown the existence, in the blood of normal rats, of a protein factor which controls proliferation of granulocytic precursors in the bone marrow of abline rats (Graham and Morrison, 1970; Graham and McMahon, 1971; Graham, Earney, McMahon, and Tjan, In press). The factors studied by Sachs (1972), which regulate cellular differentiation in bone marrow and other tissues, are also proteins of the same order of weight and are non-dialyzable. Present evidence suggests that all three of these factors are proteins of about 60,000 MW, based on gel-filtration measurements.

Radiation protection has been produced in mice, presumably due to regeneration of the bone marrow, using the 19S-macroglobulin fraction from homologous or isologous serum (Hanna *et al.*, 1967). The macroglobulin fraction has been shown to stimulate differentiation (maturation) of granulocytic precursors *in vivo*, and to enhance granulocytic colony formation in the spleen of irradiated rats and granulocytic colony formation on soft agar tissue culture (Graham, *et al.*, in prep.). The macroglobulins do not markedly affect the proliferation rate *in vivo*. Survival of mice implanted with the C1498 myelogenous leukemia has been prolonged by treatment with antiserum to the macroglobulin fraction as well as by treatment with the macroglobulins directly (Graham and McMahan, In press).

It is therefore suggested that there are at least two substances normally found in the blood which can stimulate the proliferation and differentiation of granulocytic precursors in the bone marrow. Other factors may be involved in the release of mature granulocytes from the marrow storage compartment.

FUNCTION OF GRANULOPOIETIC FACTORS

Hemopoietic mechanisms must be considered in terms of modern concepts of genetic regulatory mechanisms. These range from the Jacob-Monod operon, the existence of which is widely disputed in mammalian and other eukaryotic cells (Tomkins and Ames, 1967) to blocks in transcription and translation.

The occurrence of clustering of functionally related genes in higher (eukaryotic) organisms as simulating the prokaryotic operon is discussed by Tomkins and Ames (1967), and the possibility is raised that regulation of genetic expression may occur at any level, from inactivation of an entire chromosome to translational control of specific enzyme systems. For these reasons, it is necessary to consider several levels in deriving a model for regulation of a developmental process in higher animals.

Study of the granulopoietic pathway is most advantageous if the process is divided into cellular compartments with finite limits that can be readily measured. Boggs (1965) postulated such a compartmentalized scheme using the following divisions.

Stem cell—primitive, highly undifferentiated (and unrecognizable) cells, which give rise to mature granulocytes. These might be further subdivided into committed and noncommitted groups.

Mitotic—cells which divide mitotically, corresponding to classical myeloblasts, promyelocytes, and myelocytes.

Maturation—those cells corresponding to metamyelocytes and ring, or stab, forms, which have lost the capacity for mitotic division, but which continue differentiation into mature granulocytes.

Storage—the bone-marrow reserve of mature segmented granulocytes.

According to this scheme, control factors could direct the overall flow of cells through the pathway of compartments, or could regulate the movement of cells from one compartment to the next. The compartmental model may be refined to include many subdivisions of the major groups, but this serves only to confuse the basic problem of determining at what level regulation takes place.

Jacob and Monod's (1961) concept of the operon and regulator genes, which repress or derepress a series of structural genes, has raised the possibility that differentiation, or cellular maturation, is an expression of a highly organized series of operons. The operon concept in prokaryotes has been supported by many studies of the histidine and lactose operons in bacteria and fungi (Ames and Hartman, 1963; Goldsberger and Berberich, 1965). An extension of the Jacob and Monod (1961) concept to involve an organized series of operons in the control of mammalian granulocyte differentiation is possible. Here, it may be useful to substitute the term "gene cluster" for operon.

FIGURE 1

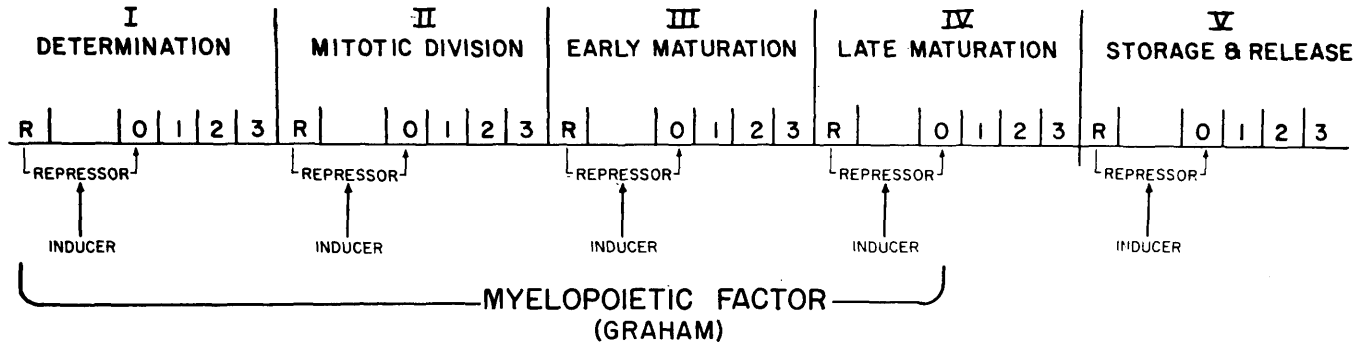
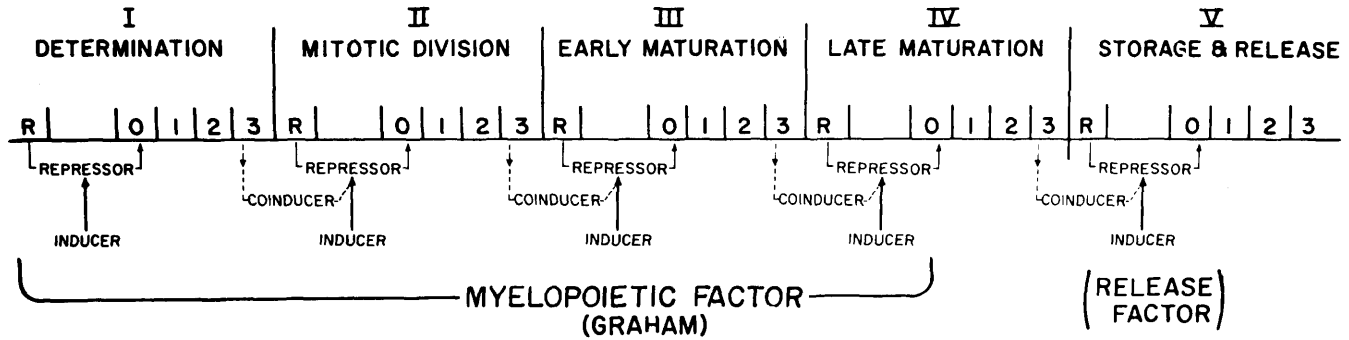


FIGURE 2



- FIGURE 1. Proposed basic multiple-operon model for granulopoietic control. Induction of operons I-IV may involve a single inducer, while operon V is induced by "release factors".
- FIGURE 2. Schematic representation of co-induction phenomenon in granulopoietic regulation.

As outlined in Figure 1, multiple operons function in a sequential pattern, with structural genes controlling individual enzymatic steps in the maturation process. Specific inducers may be postulated for each operon, although at least the fourth and fifth are extramedullary in origin. Differences in concentration of a single inducer would also explain the induction of the first four operons, although the release factors appear quite distinct.

Arbitrarily, I have assigned the first operon to the early sequence of events committing a mesenchymal stem cell to the granulocytic lineage. The second would then direct the series of mitotic cell divisions (or the mitotic compartment). The third and fourth operons would of necessity function to control the maturation process. A fifth operon would regulate release of mature granulocytes into the peripheral blood stream. The interrelationship between granulopoiesis and erythropoiesis may be explained through the first, or determination, operon, which directs the uncommitted stem cell through the initial steps of differentiation while the genes controlling erythroid development are being repressed.

A further extension of the operon concept is required. A system of co-induction may be described to correlate the sequential nature of the maturation process with the evidence of compartmentalization (Boggs, 1965; King-Smith, 1970; Morley, 1970; Cronkite, 1970). The primary inducer is a substance similar to Graham's maturation factor. In order for the full derepression of the "operon" to occur, the presence of a "co-inducer" molecule, produced by the last structural gene of the preceding operon (or gene cluster) is necessary. This "co-inducer" acts in concert with the maturation factor to remove or inactivate the repressor substance (fig. 2).

The work of Graham and McMahon (1971), which indicates that inactivation of a "maturation factor" causes an initial block in the late stages of differentiation and subsequently inhibits earlier stages in the developmental sequence, suggests the presence of a feedback-repression mechanism. However, there seems to be no experimental mechanism for separating a possible feedback repression from the equally feasible explanation of increasing levels of a factor which sequentially activates earlier operons in the pathway. If quantitative differences in the inducer are responsible for the sequencing of the differentiation mechanism, it would be expected that low-level inactivation would "turn off" the last step first and that the earlier stages would be repressed, progressively, as the level of inactivation increased.

If the factors controlling proliferation, differentiation, and release of granulocytes from the bone marrow function as inducers of specific operons (gene clusters), they must be present in normal animals at all times to maintain normal cell levels. It has already been demonstrated that this is true for the proliferation and maturation factors (Graham and Morrison, 1970; Graham and McMahon, 1971). While "release factors" seem to be induced by physiologically stressing treatments, it is suggested that this is merely a quantitative induction and that the substances are normally present in low quantity (Bierman, 1964; Graham *et al.*, 1969).

TRANSCRIPTIONAL OR TRANSLATIONAL CONTROL

The possibility exists that regulation of granulopoiesis takes place at the transcription or translational level, although the latter would seem to be ruled out by the failure to isolate relatively large numbers of inducing or repressing factors. Thus, it would be essential to control multiple operations in the differentiation process with a single regulator. Control at the transcriptional level is much more difficult to rule out, because increasing quantities of a single inducer could sequentially start the transcription of mRNA at various sites on the DNA. Intuitively, the simpler DNA-level regulation system would seem to be more likely, but efforts to demonstrate experimentally the level of regulatory mechanisms have been slow.

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