the midgut by day 7 suggests that digestion and passage of the blood meal creates conditions in the midgut that resemble the nutrient-depleted environment of stationary cultures. Thus the differentiation of promastigotes into an infective stage appears to occur in response to adverse growth conditions. This is consistent with the biology of other protozoa. Encystation of organisms such as *Entamoeba* and *Giardia*, as well as other trypanosomatids such as *Leptomonas* and *Crithidia*, occurs in response to nutrient deficiency and enables these parasites to survive conditions that would kill the dividing, noninfective forms. Transformation of cultured *Trypanosoma cruzi* epimastigotes into infective stage trypomastigotes occurs during the stationary phase (*7*). The avirulence of dividing promastigotes does not prevent effective transmission, since flies usually do not take another meal until the previous meal has been digested and passed. The development of infective promastigotes in nondividing populations coincides with the time at which another meal is sought by the fly. Parasites found in mouthparts are never in active division (*2*). The generation of an infective stage in the midgut before their migration to mouthparts ensures that parasites which are transmitted during feeding by the fly are preadapted to survival in the vertebrate.

In conclusion, *Leishmania* promastigotes sequentially develop into an infective stage both in culture and in the sandfly. The immediate implication of these findings is that investigators using cultured promastigotes must take their potential biological heterogeneity into account. More important, the easy generation of infective and noninfective stages in vitro will enable study of the antigenic, biochemical, and physiological changes that accompany transition of these parasites from invertebrate to vertebrate environments.

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Sequential Interaction of Glia Maturation Factor with Insulin

Abstract. *Astroblasts in culture proliferated when exposed to glia maturation factor for at least 2 hours and then to insulin, but not when exposed in the reverse order. The sequential relation suggests that glia maturation factor is a competence factor.*

Glia maturation factor (GMF) is a potent mitogen as well as maturation agent for astrocytes (*1–5*). When GMF is added to density-arrested cultures of rat astroblasts, the cells proliferate and then undergo chemical differentiation. The mitogenic influence of GMF is evident in both confluent and sparse cultures (*6*). Although the presence of fetal calf serum is necessary for this mitogenicity, serum components can restore part of the effect (*6*). We recently observed (*6*) that the growth rate of astroblasts in the serum-free medium N2 (*7*) supplemented with GMF approaches that in 10 percent serum alone. We now report that the mitogenic effect of GMF and medium N2 can be reproduced if all the ingredients of N2 but insulin are omitted and that the same effect is obtained if the cells are exposed

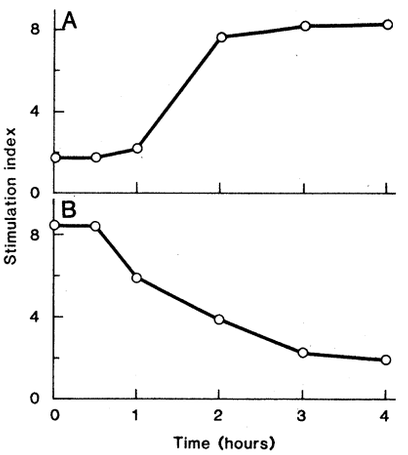

Fig. 1. Time dependence of the establishment and decay of GMF-induced competence. Rat astroblasts were cultured and sequentially stimulated by GMF and insulin as described for condition 7 in the legend to Table 1, except that in (A) the length of exposure to GMF varied and in (B) the cells were exposed to GMF for 2 hours and the interval between the withdrawal of GMF and the beginning of insulin exposure varied. During the interval the cells were cultured in the base medium without serum. Fibronectin was included in all serum-free media. Each value is the mean for quadruplicate samples and has a standard deviation of 5 percent or less.

Table 1. Glia maturation factor as a competence factor for astroblast proliferation. In each experimental condition the base medium was used with various additives. Medium N2 contained insulin (5 μg/ml), transferrin (100 μg/ml), 20 n*M* progesterone, 100 μ*M* putrescine, and 30 n*M* selenium (*7*). When alone or in combination with GMF, insulin was used at a concentration of 5 μg/ml. The GMF, a sample from bovine brain purified 10,000-fold (*6*, *8*), was used at a concentration of 200 ng/ml. In conditions 1 through 6 the base medium and additives were renewed at 2 days. In condition 7 the monolayer culture was stimulated with GMF for 4 hours, washed twice with the base medium, and immediately switched to insulin, which was subsequently renewed at 2 days. In condition 8 the cells were exposed to insulin for 4 hours and then to GMF. The total exposure period for all cultures was 4 days. The stimulation index is the ratio of the final number of cells to the initial number, as determined with a Coulter counter, the initial number being 8.4×10^3 cells per square centimeter. Each value is the mean for quadruplicate samples and has a standard deviation of 5 percent or less. Human serum fibronectin (2 μg/ml) was included in all media lacking fetal calf serum.

Condition	Stimulation index	Condition	Stimulation index
1. No additives	1.55	5. Insulin	1.91
2. Medium N2	1.96	6. GMF	2.26
3. Medium N2 + GMF	9.56	7. First GMF, then insulin	8.95
4. Insulin + GMF	9.41	8. First insulin, then GMF	2.26

first to GMF and then to insulin, but not if they are exposed to these substances in the reverse order.

Homogeneous populations of rat astroblasts were obtained (*8*) and seeded sparsely in secondary culture in plastic wells in a base medium (a 1:1 mixture of medium F12 and Dulbecco's modified Eagle medium) supplemented with 10 percent fetal calf serum. One day later the cells were starved of serum for 48 hours and exposed to the base medium containing various additives (Table 1). When the culture was exposed to GMF for 4 hours, washed, and then exposed to insulin for 4 days, the mitogenic effect was similar to that of N2 combined with GMF. The effect was not observed if the culture was first exposed to insulin for 4 hours and then to GMF. Medium N2, insulin, or GMF alone did not have this effect. The minimum length of exposure to GMF necessary to elicit the effect was 2 hours (Fig. 1A). After GMF was removed, the culture had to be stimulated by insulin within 1/2 hour or mitogenicity was reduced (Fig. 1B).

The sequential relation described above suggests that GMF functions as a competence factor; namely, that it renders cells potentially able to leave the quiescent G_0 state and enter the cell cycle. Insulin, a progression factor (*9*), propels the cycle thereafter. Competence factors have to be present for a short time only, whereas progression factors must be continuously present for cells to proliferate (*9*). Pledger *et al.* (*10*) showed platelet-derived growth factor (PDGF) to be a competence factor. This growth factor differs from GMF in being a basic protein. While the minimum time required for cells to become competent in the presence of PDGF is about the same as in GMF, the competent state induced by PDGF is stable for at least 13 hours (*10*). The difference in stability could be due to the factors themselves, the cell type (PDGF was tested on BALB/c mouse 3T3 fibroblasts), or both.

Since GMF and glial cells are both found in the brain, elucidation of the mechanism of GMF action should help to show how the nervous tissue regulates the proliferation and maturation of its own cells.

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Appearance of a New Nucleosomal Protein During Differentiation of Human Leukemia (HL-60) Cells

Abstract. *A 60-kilodalton protein was identified in chromatin digested by micrococcal nuclease during retinoic acid–induced differentiation of human leukemia (HL-60) cells to mature-like granulocytes. The protein was not detected in a retinoic acid–resistant variant of the HL-60 cell line treated with retinoic acid, in HL-60 cells induced with dimethyl sulfoxide, or in normal human granulocytes. This protein may have an important role in the regulation of retinoic acid–induced leukemic cell differentiation.*

Human leukemias are viewed as clonal diseases resulting from blockage at specific stages of cell differentiation. A conceptual approach to treatment is to induce leukemic cells to undergo terminal differentiation and to inhibit their proliferation (*1*). Human promyelocytic leukemia (HL-60) cells (*2*) can be induced to differentiate to functionally mature granulocytes by a physiologically tolerable dose of retinoic acid (RA) (*3*). Induction of HL-60 cells to undergo differentiation may be triggered by RA-induced intracellular factors responsible for rearrangement in the macromolecular structure of chromatin. In this study the protein components of monomer nucleosomes (mononucleosomes) isolated from micrococcal nuclease–digested chromatin during RA induction were analyzed. A nucleosomal protein with a molecular weight of approximately 60 kilodaltons (kD) (protein P60) was identified that quantitatively corresponded to the extent of RA-induced HL-60 cell differentiation. Protein P60 was not detected in an RA-resistant HL-60 cell line (*4*), in HL-60 cells induced with 1.3 percent dimethyl sulfoxide (DMSO), or in freshly prepared human granulocytes.

Eukaryotic chromatin consists of a string of repeating subunits. Each subunit, termed a nucleosome, has DNA strands wrapped on the surface of a histone octamer core particle that is linked to other particles by internucleosomal DNA, called linker DNA (*5, 6*). The primary site of micrococcal nuclease degradation under limited conditions is on the linker DNA structures (*7*). Digestion of chromatin structure with micrococcal nuclease therefore results in the release of mononucleosomal particles as well as dimer, trimer, and oligomer particles from the chromatin string. These particles can be separated by sucrose density gradient centrifugation (*8*) or by polyacrylamide gel electrophoresis (*9*). We attempted to determine whether any change in mononucleosomal protein components occurs in HL-60 cells during RA-induced cell differentiation.

Approximately 40 and 95 percent of HL-60 cells (2×10^5 cells per milliliter) were induced to undergo terminal differentiation when cultured with RA (0.5 μM) or DMSO (1.3 percent) for 3 days and 6 days, respectively. The differentiated cells demonstrated morphological and functional maturation, as assessed by their ability to reduce nitroblue tetrazolium dye (*10*) and to phagocytose latex beads (*11*).

At various times cells (1×10^9) were harvested and fractionated. Nuclei were isolated and treated with 0.5 percent Nonidet P-40 before chromatin purification in a buffer system of low ionic strength (*12*). Approximately 15 percent of chromatin DNA was digested by micrococcal nuclease (the method is described in the legend to Fig. 1). The released nucleosomal particles were isolated by centrifugation in a linear density gradient of 5 to 28.8 percent sucrose. Three major categories of nucleosomes were separated in addition to the nondigested residual chromatin fragments that sedimented on the bottom of the gradient (Fig. 1). Superimposed sedimentation profiles obtained from noninduced cells and RA-induced cells revealed that monomer particles from cells induced for 6 days sedimented slightly faster than those from noninduced cells.

The monomer fractions were pooled