per 2 million cells transfected with 10 μg of plasmid DNA, a value similar to that obtained with pNEO3, a plasmid in which *neo* transcription is driven by the HSV-tk promoter. Twelve clones were randomly selected and cultured for the analysis of *neo* mRNA in exponentially growing cells (asynchronous culture at 35°C) and in cells arrested in G_1 (cultures shifted to 40.5°C) (Fig. 2). When the *neo* mRNA level of exponentially growing cells was compared with that of cells blocked in G_1, increased amounts of *neo* transcripts were seen in asynchronously cycling cells. This observation for all the transformant clones tested indicates that position of the hybrid gene in the host genome was not a major factor in determining the differential *neo* expression between growing and G_1-arrested cells. Nonetheless, the sites of integration and copy number may have some effect on the transcription of the *neo* RNA, since some transformants had more *neo* RNA than others. Therefore, the sequence elements present on a 1.1-kb Pvu II fragment of the hamster H3 gene may be important for its cell cycle regulation.

To further investigate whether the *neo* mRNA expression was regulated during the cell cycle as it is in the endogenous histone H3 genes, we studied three stable transformants that produced different amounts of *neo* mRNA for further analysis. The transformants were synchronized by serum deprivation, and upon addition of fresh medium, were stimulated to progress through the cell cycle (*3, 10*). The rate of DNA synthesis in these synchronized cells was monitored by [^{3}H]thymidine incorporation. *Neo* transcripts were detectable at low levels during the G_1 period (Fig. 3). As cells entered the S phase, the *neo* RNA began to increase in parallel to the increase in histone H3 mRNA accumulation. In all three cases, at the peak, *neo* mRNA increased 6- to 17-fold. In contrast, actin mRNA remained relatively constant throughout the cell cycle. We conclude that the 1.1-kb Pvu II fragment derived from this particular hamster H3 gene contains some elements necessary for regulated temporal expression of the gene during the cell cycle. It is not yet known whether the sequences encoding for the first 20 amino acid residues of the H3 gene play a role in this regulation.

In studying regulated gene expression during the cell cycle, it is important to show that the gene is regulated temporally. (In contrast, growth might be induced by the addition of fresh medium to starving synchronized cells.) We used two independent methods to address this issue. (i) When DNA synthesis was inhibited by the K12 *ts* mutation, the *neo* mRNA levels decreased substantially (Fig. 2). Since the cells were not starved of serum, the result argued against the effect of nutrient on *neo* mRNA. In addition, when DNA synthesis was inhibited by hydroxyurea, similar results were observed. Together, these results reaffirm the coupling between DNA synthesis and the amount of *neo* mRNA. (ii) Where serum-synchronized cells were used, *neo* mRNA increased concurrently with the increase in host cell DNA synthesis and, as with histone mRNA, decreased as host cell DNA synthesis declined (Fig. 3). This result shows that the hybrid gene is regulated in a cell cycle–dependent manner, and that changes were not a result of overall growth stimulation. However, it is also clear that the regulation of the *neo* RNA is not as stringent as that of histone mRNA (Fig. 3), probably because of both transcriptional and post-transcriptional control in histone mRNA regulation; perhaps only transcriptional control is conferred by the sequences used here.

Our results show in vivo that the 5′ sequence of a mammalian histone gene is able to confer cell cycle regulation on expression of a heterologous gene to which it is fused in the same transcriptional orientation. This observation is in agreement with a recent report that in in vitro systems, promoter elements in the 5′-flanking sequence of a human histone H4 gene are specifically recognized by cellular factors produced in S-phase nuclear extracts (*13*). A combination of both approaches should yield information on the *trans*-acting regulatory factors and the *cis*-acting elements involved in the regulation. A further investigation into the cell cycle regulation of the histone genes can be achieved by more detailed analysis of the sequence and function of the 1.1-kb fragment. Furthermore, with the isolation of this regulatory-promoter sequence, it may now be possible to direct the expression of any compatible heterologous gene in a cell cycle–regulated manner in mammalian cells.

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Antiprotozoal Activity of Tricyclic Compounds

The observations by Zilberstein and Dwyer (*1*) on the antileishmanial effects of tricyclic antidepressants are intriguing in the context of earlier studies of the antiprotozoal activities of phenothiazines and other closely related compounds. In 1891, Guttman and Ehrlich (*2*) first noted that methylene-blue dye (Fig. 1a) was effective in the treatment of *vivax* malaria and presumably, therefore, lethal to *Plasmodium vivax*. In several studies, phenothiazines have been found to have lethal effects on protozoa. We demonstrated that chlorpromazine (figure 1b) and several of its derivatives killed *Leishmania donovani* promastigotes, extracellular amastigotes, and amastigotes within human macrophages in vitro (*3*). Furthermore, chlorpromazine reduced the parasite burden of hamsters infected with *L. donovani* (*4*). Similar in vitro effects of these compounds on *Trypanosoma brucei brucei* have been described by Seebeck and Gehr (*5*).

The data presented by Zilberstein and Dwyer are fascinating in relation to the prior observations (*3, 4*). Clomipramine (3-chloroimipramine, figure 1c), the prototype compound demonstrated in their study to have antileishmanial activity, is identical to chlorpromazine (figure 1b) with the exception of two carbons substituted for the sulfur at position 5. The concentration of clomipramine required to kill 50 percent (LD_{50}) of promastigotes of *L. donovani* was 24 μ*M* (1), while the LD_{50} for chlorpromazine was 30 μ*M* (*4*). Similarly, imipramine (clomipramine

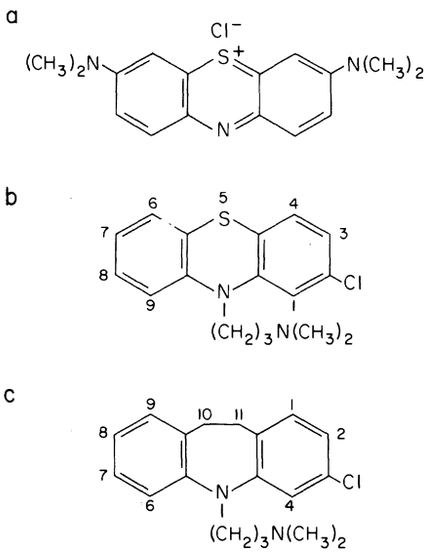

Fig. 1. Structural formulas for (a) methylene blue, (b) chlorpromazine, and (c) clomipramine.

without the 3-chloro substitution) was inactive ($LD_{50} > 50$ μ*M*), as was promazine (chlorpromazine without the 2-chloro group, $LD_{50} > 100$ μ*M*). Further comparisons are limited by the available data; it appears that some, but not just any, substitutions at positions 2, 3, 4, 7, and 8 on the outer rings are essential for lethal activity against promastigotes. In both studies, amastigotes were more sensitive to certain of the analogs tested, such as 7,8-dihydroxychlorpromazine (*3*) and 3-chloroimipramine (*1*).

While we speculated that a membrane event might be responsible for the lethal activity of these drugs against protozoa (*3*), the data demonstrating impairment of l-proline transport and the suggestion of disturbance of parasite transmembrane proton gradient provide further insight into these unusual effects (*1*). Recently, potential relevance of these observations has been illustrated by the studies of Henriksen and Lende (*6*). In an uncontrolled trial of topical chlorpromazine ointment (2 percent) in three patients with diffuse cutaneous leishmaniasis caused by *L. aethiopica,* a condition usually resistant to conventional therapy with pentavalent antimonials, healing was observed. Clearly, all these data taken together provide an exciting incentive to continue the investigation of this family of closely related tricyclic compounds for antiprotozoal activity.

ERIK L. HEWLETT
Divisions of Clinical Pharmacology and *Geographic Medicine, Departments of Medicine* and *Pharmacology, University of Virginia School of Medicine, Charlottesville, Virginia 22908*
RICHARD D. PEARSON
Division of Geographic Medicine, Departments of Medicine and *Pathology, University of Virginia School of Medicine, Charlottesville*

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The mode of action of antiparasitic drugs is generally unknown. We have demonstrated that *Leishmania donovani* promastigotes have active transport systems that are driven by proton motive forces (*1*). Further, we have shown that a plasma membrane proton-translocating adenosinetriphosphatase (ATPase) provides the chemiosmotic energy that drives active transport in these cells (*2*). Such plasma membrane activities have been described in bacteria and fungi (*3*). However, this mechanism appears to be unique to *Leishmania*, as it does not exist in its mammalian host. Previous observations in mammalian cells demonstrated that both antipsychotic and antidepressant tricyclic compounds are able to interact with proton-transporting ATPases. For example, the antipsychotic compound trifluoroperazine was found to inhibit H^+, K^+-ATPase, and H^+ accumulation in Hog gastric cells (*4*). Further, this compound and other phenothiazine and thioxanthene derivatives efficiently inhibited the proton-translocating ATPase of mammalian mitochondria (*5*). Tricyclic antidepressants also have a striking effect on energy-linked reactions in mitochondria; for example, both imipramine and clomipramine inhibited mitochondrial F_0,F_1-ATPase and uncoupled oxidative phosphorylation (*6*). However, unlike classical uncouplers, the tricyclic antidepressants had little effect on the stimulation of proton efflux in intact mitochondria (*7*). The cumulative data suggest that the uncoupling of oxidative phosphorylation is due to specific interactions of the tricyclic compounds with the F_1-ATPase rather than to an ionophoric-like activity of these drugs. As we have suggested previously, the tricyclic antidepressants appear to disrupt the leishmanial plasma membrane ATPase. As a result, membrane potential is reduced, active transport is inhibited, and intracellular *p*H homeostasis is presumptively disrupted, resulting in rapid cell death (*8*). On the basis of their structural similarities, it is anticipated that the tricyclic antipsychotic and antidepressant compounds have a similar mode of action affecting energy-linked reactions in the plasma membrane of *Leishmania*. To date, many investigators have sought to rationally design drugs that act against parasites. Having demonstrated the proton-translocating ATPase in the leishmanial plasma membrane and its inhibition by tricyclic compounds, we believe that a rational target for antiparasitic drugs has been defined.

DAN ZILBERSTEIN
DENNIS M. DWYER
Cell Biology and Immunology Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20205

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