gosaccharides would be necessary. However, the acid lability of the linkages between the KDO molecules makes this difficult. The chemical identity of the chlamydial molecule bearing the cross-reactive antigen is still unknown. In the PCP isolation procedure, the CT-GL behaved like rough LPS but other glycolipids could show similar solubility properties. In SDS-PAGE, it also behaved like Re-LPS (*10*). Schramek *et al.* (*4*) compared a PCP extract of *C. psittaci* with the LPS of a rickettsia (*Coxiella burneti*) and concluded that it contained lipid A; unfortunately, the rickettsial LPS is not well characterized. Endotoxic properties of *Chlamydia* characteristic of lipid A are pyrogenicity in rabbits and the ability to gelate a *Limulus* amoebocyte lysate (*3, 4*). We tested our CT-GL preparation for the latter property and obtained a positive gelation reaction with concentrations of ≥ 0.5 ng/ml (*17*). Together these data suggest that the chlamydial glycolipid is structurally and functionally related to Re-type LPS. We believe that the demonstration of LPS as a component of *Chlamydia* will facilitate studies of the changes believed to take place in the chlamydial cell envelope during its intracellular growth cycle (*18*). It is probable that the biologically active LPS also has a role in the pathogenesis of chlamydial infections.

Note added in proof: The structure of the KDO-region of Re-LPS has recently been reexamined and may contain only a KDO-disaccharide (*19*). This does not alter the conclusions in this report.

MARJATTA NURMINEN
MAIJA LEINONEN
PEKKA SAIKKU
P. HELENA MÄKELÄ

National Public Health Institute, 00280 Helsinki 28, Finland, and *Department of Virology, University of Helsinki, 00290 Helsinki 29*

References and Notes

1. T. Grayston and S. Wang, *J. Infect. Dis.* **132**, 87 (1975).
2. A. Tamura, A. Matsumoto, G. P. Manire, N. Higashi, *J. Bacteriol.* **105**, 355 (1971).
3. V. J. Lewis, W. L. Thacker, S. H. Mitchell, *J. Gen. Microbiol.* **114**, 215 (1979).
4. S. Schramek, J. Kazar, E. Sadecky, *Acta Virol. (Engl. Ed.)* **24**, 224 (1980).
5. C. Galanos, O. Lüderitz, E. T. Rietschel, O. Westphal, in *Biochemistry of Lipids II*, T. W. Goodwin, Ed. (University Park Press, Baltimore, 1977), vol. 14, p. 239.
6. S. P. Dhir *et al.*, *J. Immunol.* **109**, 116 (1972); A. A. Benedict and E. O'Brien, *ibid.* **76**, 293 (1956); P. Reeve and J. Taverne, *J. Gen. Microbiol.* **27**, 501 (1962).
7. O. Lüderitz *et al.*, *Ann. N.Y. Acad. Sci.* **133**, 349 (1966).
8. B. A. D. Stocker and P. H. Mäkelä, in *Microbial Toxins*, S. Ajl, Ed. (Academic Press, New York, 1971), vol. 4, p. 369; R. G. Wilkinson, P. Gemski, B. A. D. Stocker, *J. Gen. Microbiol.* **70**, 527 (1972).
9. C. Galanos, O. Lüderitz, O. Westphal, *Eur. J. Biochem.* **9**, 245 (1969).
10. Either CT-GL or Re-LPS, 5 μg in 10 μl of H_2O, were mixed with equal amounts of the Laemmli sample buffer and applied to a 15 percent polyacrylamide gel [U. K. Laemmli, *Nature (London)* **227**, 680 (1970)]. After electrophoresis the gel was stained by silver nitrate [C.-M. Tsau and C. E. Frasch, *Anal. Biochem.* **119**, 115 (1982)].
11. V. S. Waravdekar and L. D. Saslaw, *J. Biol. Chem.* **234**, 1945 (1959).
12. M. Leinonen, J. Luotonen, E. Herva, K. Valkonen, P. H. Mäkelä, *J. Infect. Dis.* **144**, 570 (1981).
13. P. Prehm, S. Stirm, B. Jann, K. Jann. *Eur. J. Biochem.* **66**, 369 (1976); K. Kotelko, W. Gromska, M. Papierz, Z. Sidorczyk, D. Krajewska, K. Szer, *J. Hyg. Epidemiol. Microbiol. Immunol.* **21**, 271 (1977).
14. M. Volkert and P. Muller-Christensen, *Acta Pathol. Microbiol. Scand.* **31**, 21 (1955).
15. M. R. Rosner, J. Y. Tang, I. Barzilay, H. G. Khorana, *J. Biol. Chem.* **254**, 5906 (1979).
16. W. Dröge, V. Lehmann, O. Lüderitz, O. Westphal, *Eur. J. Biochem.* **14**, 175 (1970); P. F. Mühlradt, V. Wray, V. Lehmann, *ibid.* **81**, 193 (1977).
17. The CT-GL was dissolved in pyrogen-free water and tested in the *Limulus* lysate assay with the method and controls provided by the supplier of the test (E-Toxate, Sigma). The detection limit of the test was 0.5 ng of the endotoxin reference per milliliter of solution.
18. A. Matsumoto and G. P. Manire, *J. Bacteriol.* **104**, 1332 (1970).
19. S. M. Strain, S. W. Fesik, I. M. Armitage, *J. Biol. Chem.* **258**, 2906 (1983).
20. J. Schachter and K. F. Mayer, *J. Bacteriol.* **99**, 636 (1969).
21. N. Kuusi, M. Nurminen, H. Saxén, P. H. Mäkelä, *Infect. Immun.* **34**, 328 (1981).
22. P. Saikku, J. Paavonen, P. Väänänen, A. Vaheri, *J. Clin. Microbiol.*, in press.
23. We thank E. Neuvonen, National Veterinary Institute, Helsinki, for the rabbit antiserum to *Chlamydia psittaci*; C. Mordhorst, State Serum Institute, Copenhagen, Denmark, for the complement fixation antigen; K. Kotelko, Lodz University, Lodz, Poland; O. Lüderitz and K. Jann, Max-Planck Institute for Immunobiology, Freiburg, FRG; and B. A. D. Stocker, Stanford University, Stanford, Calif., for bacterial strains. Supported in part by SITRA, the Finnish National Fund for Research and Development.

11 January 1983; revised 7 March 1983

Transcriptional Regulation of Globin Gene Expression in the Human Erythroid Cell Line K562

Abstract. *The effect of hemin on the rate of synthesis and the level of globin messenger RNA's in the human erythroid cell line K562 was examined by means of cloned hybridization probes specific for each of the human embryonic, fetal, and adult globin genes. Hemin increases both the rate of transcription and the level of accumulation of ζ-, ε-, γ-, and α-globin messenger RNA's by a factor of 3 to 5. Thus, hemin induction of globin gene expression in K562 cells is at the level of transcription.*

Erythroid cell lines that are inducible for globin gene expression provide useful models for studying eukaryotic gene regulation (*1–3*). The most thoroughly studied of these lines are mouse erythroleukemia (MEL) cells, which are virally transformed erythroid precursor cells thought to be arrested on or before the proerythroblast stage (*1*). Treatment of MEL cells with dimethyl sulfoxide (DMSO) or various other chemical agents results in terminal erythroid cell differentiation and a 10- to 50-fold increase in the accumulation of adult α- and β-globin proteins (*4*).

An inducible human erythroid cell line, K562, was established from a patient with chronic myelogenous leukemia (*2*). Although K562 was derived from a pregranulocytic cell (*2*), it was subsequently shown to display erythroid cell specific markers, suggesting that the line is a multipotent hematopoietic precursor (*5*). Treatment of these cells with sodium butyrate, hydroxyurea, or hemin resulted in an increase in red cell–specific proteins including hemoglobin (*5–7*). The globin chains synthesized are primarily the embryonic α- and β-like globins, ζ and ε, respectively, and the fetal γ-globin. Low amounts of adult α-globin are also produced, but no adult β-globin is observed (*6, 7*).

In the case of MEL cells the accumulation of globin messenger RNA (mRNA) and hemoglobin after induction by DMSO results at least in part from transcriptional activation of adult globin genes (*8*). In order to investigate the level at which globin gene expression is controlled in K562 cells, we have examined the effect of hemin treatment on the level of globin mRNA's and on the rate of globin gene transcription.

We estimated the levels of specific globin mRNA's by RNA blotting (*9*), and the rates of globin gene transcription by an in vitro nuclear transcription assay in combination with a "dot blot" procedure (*8, 10, 11*). In the latter assay, labeled nascent RNA is prepared from isolated nuclei and hybridized with recombinant plasmids carrying specific DNA sequences bound to nitrocellulose filters. Such a nuclear transcription assay supports elongation of initiated RNA polymerase II transcripts, but does not permit reinitiation (*10*). Thus, the amount of radioactivity incorporated into transcripts that hybridize to a specific DNA fragment reflects the number of RNA polymerase molecules engaged in the transcription of the corresponding fragment of nuclear DNA.

The hydridization probes used are indicated on the map of the human α- and β-globin gene clusters (Fig. 1). The β-globin gene cluster contains an embryon-

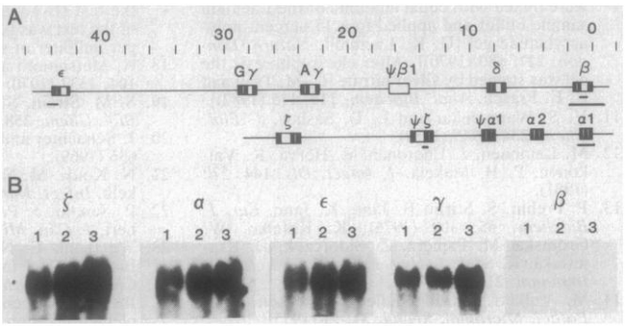

Fig. 1. (A) Maps of the human α- and β-globin gene clusters and positions of the hybridization probes. The positions of globin probes used for RNA blotting experiments or dot blot hybridizations is indicated on the maps. Detailed description of the subclones carrying these sequences is provided in the legend to Fig. 2. For RNA blotting experiments, the restriction fragments from the relevant recombinant plasmids were purified by agarose gel electrophoresis. (B) RNA blotting analysis of globin mRNA from K562 cells. A variant K562 line which grows as a monolayer (*19*) was maintained in Dulbecco's modified Eagle's medium (DME) supplemented with 10 percent fetal calf serum. Cytoplasmic RNA was extracted as described (*20*) from uninduced cells (lanes 1), cells grown for 4 days in hydroxyurea (12 μg/ml) (lanes 2), or cells grown for 4 days in hemin (50 μ*M*) (lanes 3). RNA samples (15 μg) were subjected to electrophoresis on 1.5 percent agarose-formaldehyde gels, blotted onto nitrocellulose paper (*9*), and hybridized with ^{32}P-labeled DNA probes specific for human ζ-, α-, ε-, γ-, or β-globin sequences [as for (A), above]. Only the portions of the autoradiograms corresponding to the globin mRNA migration position are shown. The intensities of the signals were estimated by scanning the autoradiograms with a densitometer.

ic (ε) globin gene, two nonallelic fetal (^{G}γ and ^{A}γ) globin genes and two adult (δ and β) globin genes (*12*). The α-globin gene cluster consists of an embryonic (ζ) globin gene, an embryonic pseudogene (ψζ), an adult globin pseudogene (ψα1), and two functional adult (α1 and α2) globin genes (Fig. 1) (*13*).

Treatment of K562 cells with hemin results in the production of ζ-, ε-, α-, and γ-globin polypeptides, but no β-globin is detected (*6, 7*). Exposure to hydroxyurea also results in accumulation of globin protein (*6*). An RNA blotting analysis of cytoplasmic RNA prepared from uninduced K562 cells and from cells treated with hemin or hydroxyurea is shown in Fig. 1B. A band corresponding to RNA of 600 to 700 nucleotides in length is observed with all of the hybridization probes except β-globin. Treatment with hemin results approximately in a fivefold increase in ζ-, ε-, and γ-globin mRNA's and a threefold increase in α-globin mRNA. The extent of hemin stimulation of globin mRNA accumulation in K562 corresponds roughly to the increase in globin polypeptide synthesis induced by hemin (*6, 7*). Thus, it appears that the hemin stimulation of globin synthesis in K562 is likely to be a consequence of increased globin mRNA levels. Exposure of K562 cells to hydroxyurea results in only a threefold increase in ζ- and ε-globin mRNA. Little, if any, increase in α and γ gene mRNA levels is observed.

To determine whether the increase in globin mRNA's in K562 cells after treatment with hemin or hydroxyurea is due to an increase in the rates of transcription, labeled nascent RNA was isolated from nuclei prepared from K562 cells before or after exposure to inducer and hybridized with recombinant plasmids carrying specific globin sequences bound to nitrocellulose filters (Fig. 2). The intensities of the spots, normalized to the size of the globin specific sequences contained within the cloned DNA fragment, provide an estimate of the rate of transcription of each globin gene. We detected transcription of the ζ-, α-, ε-, and γ-globin genes but not of the β-globin gene. Upon exposure to hemin, there was a fivefold increase in the rate of transcription of the ζ-, ε-, and γ-globin genes and a threefold increase for the α-globin gene. After treatment with hydroxyurea there was only a three- to fourfold increase of the rate of transcription of the ζ- and ε-globin genes, and no increase in the rate of α- and γ-globin transcription was observed. In control experiments no transcripts hybridizing to pBR322 sequences and no variation in the rates of transcription of actin genes upon exposure to hemin or hydroxyurea were observed (Fig. 2).

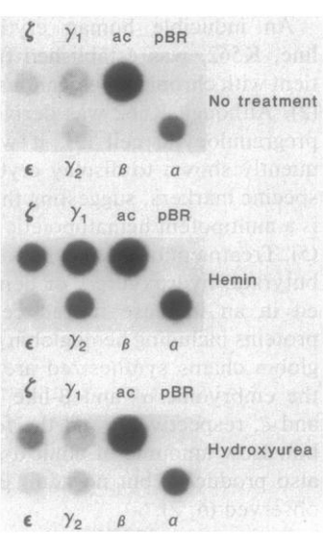

Fig. 2. Dot hybridization of RNA labeled in isolated nuclei from K562 cells. Nuclei were isolated and incubated in presence of α-^{32}P-labeled uridine triphosphate, and RNA was purified and annealed to plasmid DNA's spotted onto nitrocellulose filters as described (*20*). The volume of hybridization was 1.5 ml and the same amount of label (approximately 15 × 10^6 cpm) was used for uninduced and induced cells. Nuclei were from uninduced K562 cells or K562 cells grown for 3 days in hemin (50 μ*M*) or hydroxyurea (12 μg/ml). The DNA fragments were as follows: ζ, plasmid pHPζ carrying the 399-bp Pst I–Hinc II restriction fragment from the human ψζ-globin gene (*13*); γ1, plasmid pR^{A}γ carrying the entire human ^{A}γ globin gene; ac, plasmid LK 215 carrying a human γ-actin cDNA; pBR, plasmid pBR322; ε, plasmid pε 0.7 carrying the 0.7-kb Bam HI restriction fragment from the human ε-globin gene (*21*); γ2, plasmid JW151 carrying a human γ-globin cDNA (*22*); β, plasmid pHβIS carrying the Bam HI–Eco RI restriction fragment containing the large intervening sequence of the human β-globin gene (*12*); α, plasmid pα1PstI carrying the human α1-globin gene on a 1.5-kb Pst I fragment (*20*). The intensities of the signals were estimated by scanning the autoradiograms with a densitometer.

The correspondence between the levels of globin polypeptides and mRNA's and the rates of transcription of the corresponding genes in K562 cells before and after hemin induction suggests that the increase in globin polypeptides in hemin-induced cells results primarily from an increase in the rate of globin gene transcription. Our data also indicate that the absence of β-globin polypeptide in K562 cells is due to the lack of transcription of the gene.

Hemin has various effects on erythroid cells; it controls a translational repressor of protein synthesis in rabbit reticulocytes (*14*), it is required in conjunction with butyric acid for induction of heme-depleted MEL cells (*15*), and it induces accumulation of globin polypeptides in K562, human erythroleukemia, and MEL cells (*2, 3, 16*). In MEL cells, nuclear run-off transcription studies have shown that, in contrast to DMSO-induction, hemin-induction of α- and β-globin gene expression is not at the level of transcription (*8, 17*). Our results therefore suggest that the mechanism of hemin induction of globin gene expression is fundamentally different in MEL and K562 cells. In contrast to DMSO induction of MEL cells, hemin treatment of K562 cells does not lead to terminal differentiation (*18*), an indication that transcriptional activation of globin genes in K562 cells can be uncoupled from commitment to terminal differentiation.

Although the initial finding that K562

cells are inducible for globin polypeptide synthesis suggested that these cells would be suitable for studies of globin gene expression (*6*), the level at which hemin acts was not previously examined. Our studies indicate that the effect of hemin is at the level of transcription and that it should be possible to use K562 cells in DNA-mediated gene transfer experiments to identify sequences necessary for the regulation of human embryonic globin gene transcription.

PATRICK CHARNAY
TOM MANIATIS

Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts 02138

References and Notes

1. C. Friend, W. Scher, J. G. Holland, T. Soto, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 378 (1975).
2. C. B. Lozzio and B. B. Lozzio, *Blood* **45**, 321 (1975).
3. P. Martin and T. Papayannopoulou, *Science* **216**, 1233 (1982).
4. P. A. Marks and R. A. Rifkind, *Annu. Rev. Biochem.* **47**, 419 (1978).
5. L. C. Anderson, M. Jokinen, C. G. Gahmberg, *Nature (London)* **278**, 364 (1979).
6. T. R. Rutherford, J. B. Clegg, D. J. Weatherall, *ibid.* **280**, 164 (1979); T. R. Rutherford, J. B. Clegg, D. R. Higgs, R. W. Jones, J. Thompson, D. J. Weatherall, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 348 (1981).
7. E. J. Benz *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 3509 (1980).
8. E. Hofer, R. Hofer-Warbinek, J. Darnell, *Cell* **29**, 887 (1982).
9. D. Goldberg, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5794 (1980); B. Seed and D. Goldberg, unpublished.
10. M. Groudine, M. Peretz, H. Weintraub, *Mol. Cell. Biol.* **1**, 281 (1981).
11. F. C. Kafatos, C. W. Jones, A. Efstratiadis, *Nucleic Acids Res.* **7**, 1541 (1979).
12. E. F. Fritsch, R. M. Lawn, T. Maniatis, *Cell* **19**, 959 (1980).
13. J. Lauer, C.-K. J. Shen, T. Maniatis, *ibid.* **20**, 119 (1980); N. J. Proudfoot and T. Maniatis, *ibid.* **21**, 537 (1980); N. J. Proudfoot, A. Gil, T. Maniatis, *ibid.* **31**, 653 (1982).
14. M. Gross and M. Rabinovitz, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1565 (1972).
15. T. R. Rutherford and D. J. Weatherall, *Cell* **16**, 415 (1979).
16. B. P. Alter and S. C. Goff, *Blood* **50**, 867 (1977).
17. H. L. Profous-Juchelka *et al.*, *Mol. Cell. Biol.* **3**, 229 (1982).
18. A. Dean, F. Erard, A. B. Schneider, A. N. Schechter, *Science* **212**, 459 (1981).
19. M. V. Chao, unpublished results.
20. ———, P. Mellon, P. Charnay, T. Maniatis, R. Axel, *Cell* **32**, 483 (1983).
21. F. E. Baralle, C. C. Shoulders, N. J. Proudfoot, *ibid.* **21**, 621 (1980).
22. J. T. Wilson, L. B. Wilson, J. K. Deriel, L. Villa-Komaroff, A. Efstratiadis, B. G. Forget, S. M. Weissman, *Nucleic Acids Res.* **5**, 563 (1978).
23. Supported by NIH grant 5 RO1 HL278989 (T.M.) and grants from the Fogarty International Center of the NIH and EMBO (P.C.).

9 February 1983

Pulsing Electromagnetic Fields Induce Cellular Transcription

Abstract. *Weak, pulsing electromagnetic fields can modify biological processes. The hypothesis that responses to such induced currents depend on pulse characteristics was evaluated by using transcription as the target process. Two pulses in clinical use, the repetitive single pulse and the repetitive pulse train, were tested. These pulses produced different results from each other and from controls when transcription in dipteran salivary gland cells was monitored with tritiated uridine in transcription autoradiography, cytological nick translation, and analysis of isolated RNA fractions. The single pulse increased the specific activity of messenger RNA after 15 and 45 minutes of exposure. The pulse train increased specific activity only after 45 minutes of exposure.*

Basic cellular phenomena such as growth, differentiation, dedifferentiation, and repair have been modified by weak direct current (*1*). Electrical currents induced in tissues by weak, pulsing electromagnetic fields (PEMF's) are also biologically active in regeneration and repair (*2*). Recently, the repetitive pulse train (PT) has been shown to have a significant clinical impact in repairing recalcitrant bone fractures (*3*), while the repetitive single pulse (SP) has had beneficial effects in avascular necrosis and in osteoporosis (*4*). These different effects appear to depend on specific waveform parameters in the driving pulse and in the asymmetrical induced pulsing current, parameters that may be determined in part by passive electrical characteristics of the target tissue.

We tested the hypothesis that exogenous PEMF's trigger selected cellular responses (*5*) by studying alterations in normal RNA transcription patterns in the salivary gland chromosomes of the dipteran *Sciara coprophila*. The nuclei of the nondividing cells of the salivary gland contain four polytene chromosomes engaged in interphase synthetic functions, including RNA transcription and cyclical DNA replication (*6*). Normal transcription patterns during cellular differentiation in salivary gland chromosomes of this organism are known from transcription autoradiography. Thus, transcription can be followed at the cytological level and correlated with chromosome structure by studying banding or puff formation in the giant chromosomes (*6*).

Salivary glands of late fourth-instar female larvae were dissected in Schneider's *Drosophila* medium containing [^{3}H]uridine (250 μCi/ml; 40.8 Ci/mmole) (New England Nuclear). Whole glands (attached to the larval bodies) in medium were placed in PEMF's for various periods (5 to 90 minutes). Controls for these experiments were established under conditions that were identical except for the absence of the fields. The waveform and other characteristics of the fields are shown in Fig. 1.

Transcription was measured in three ways. First, nascent RNA chains attached to specific chromosome regions were identified by conventional autoradiography. Second, regions of the chromosomes sensitive to deoxyribonuclease I were examined by nick translation (with [^{125}I]deoxycytidine triphosphate) directly on the DNA of the cytological preparations (*7*). Deoxyribonuclease I preferentially attacks transcriptively active regions of chromatin (*8*). Autoradiographic patterns resulting from either approach are similar, but the use of nick translation produces greater specificity in the labeling pattern. Third, RNA's of various size classes were isolated by sucrose density gradient centrifugation and analyzed for changes in the pattern of [^{3}H]uridine incorporation. These tests showed characteristic alterations of transcription by each of the pulse patterns. Various size classes of RNA were influenced by both waveforms in a different manner. Furthermore, the responses to different periods of exposure were different for the two pulse types.

The transcription autoradiogram of a cell incubated in [^{3}H]uridine in the pres-

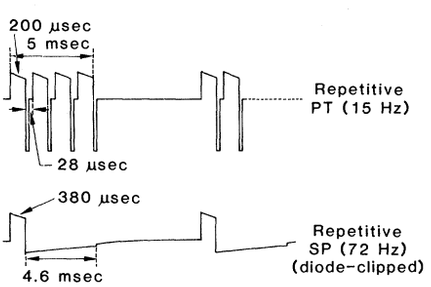

Fig. 1. Major waveform characteristics of the SP and PT stimuli (Biosteogen system 204, Electro-Biology, Inc.). Pulse amplitude, on a calibrated coil probe of 15 mV (coupled to a Tectronix 5103N oscilloscope), was equal to 1.5 mV per centimeter of cortical bone (*2*). The glands were exposed to PEMF's in 0.5 ml of Schneider's *Drosophila* medium in petri dishes (60 by 15 mm) between a pair of 10 by 10 cm Helmholtz aiding coils, delivering an average magnetic field parallel to the floor of the dish. Coil orientation was vertical. The rate of change of the magnetic field (*dB/dt*) was approximately 0.1 G/μsec for PT's and 0.05 G/μsec for SP's (*15*). Frequency content of the two pulses, derived by discrete Fourier transforms, differs significantly (*16*).