clonal NCM II 274 antibody [N. Ikegaki, J. Minna, R. H. Kennett, *EMBO J.* **8**, 1793 (1989)] was used to detect the c-Myc protein.
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Nucleoid Condensation in *Escherichia coli* That Express a Chlamydial Histone Homolog

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Chlamydial cell types are adapted for either extracellular survival or intracellular growth. In the transcriptionally inert elementary bodies, the chromosome is densely compacted; in metabolically active reticulate bodies, the chromatin is loosely organized. Condensation of the chlamydial nucleoid occurs concomitant with expression of proteins homologous to eukaryotic histone H1. When the *Chlamydia trachomatis* 18-kilodalton histone homolog Hc1 is expressed in *Escherichia coli*, a condensed nucleoid structure similar to that of chlamydiae is observed with both light and electron microscopy. These results support a role for Hc1 in condensation of the chlamydial nucleoid.

Chlamydiae are bacterial, obligate, intracellular parasites of humans and animals that undergo an unusual biphasic developmental cycle (*1*). *Chlamydia trachomatis* is the leading cause of preventable blindness and is the most prevalent sexually transmitted disease in industrialized countries (*2*). Infection is initiated by metabolically inert elementary bodies (EBs) that are approximately 0.3 μm in diameter and have a dense core of condensed chromatin unique among prokaryotes. Within 8 hours after infection, EBs differentiate to larger, more pleomorphic, and metabolically active reticulate bodies (RBs) having chromatin that appears more disperse. The RBs multiply by binary fission until 18 to 48 hours after infection, when they begin to differentiate into EBs. Formation of this dense nucleoid structure is accompanied by oxidative cross-linking of outer membrane proteins to form a rigid cell-wall complex (*3*, *4*).

Chlamydial nucleoid condensation may be mediated by the activities of a family of developmentally regulated, highly basic DNA-binding proteins present in EB chromosome preparations (*5–8*). *Chlamydia trachomatis* serovars have two lysine-rich proteins with primary sequence homology to eukaryotic histone H1 (*5*, *6*, *8*) that are expressed only during the late stages of chlamydial intracellular development. One of these histone homologs, Hc1, has an apparent molecular weight of 18 kD and is conserved among all serovars; the other varies from 23 to 32 kD in size among serovars (*9*). The chlamydial gene encoding Hc1, *hctA*, has been cloned and sequenced, and an immunoreactive partial gene product was conditionally overexpressed (*5*).

Full-length expression of Hc1 was accomplished in a T7 RNA polymerase promoter system (Fig. 1A) (*10*). The recombinant product was reactive with a polyclonal, monospecific antiserum to Hc1 (Fig. 1B). The protein Hc1 is expressed in *Escherichia coli* in quantities similar to those observed in *C. trachomatis* (6.0 ± 0.3% of total soluble protein by densitometry for each). It is unclear why Hc1 expression in the pT7 expression system is not greater, but the basic nature of Hc1 and its presumed DNA-binding abilities may limit transcription of host DNA.

Induced *E. coli* containing either pT763, in which the inserted Hc1 gene is in an expressing orientation, or pT751, in which the direction of transcription is opposite that of the T7 promoter, were examined by light microscopy with acridine orange staining. When visualized with acridine orange staining (*11–13*), the nucleoid of *E. coli* expressing Hc1 is highly condensed in comparison to the nonexpressing control (Fig. 2).

Electron microscopic examination of these strains confirmed the presence of a compact nucleoid structure unique to the Hc1-expressing strain (Fig. 2). The ultrastructural appearance is reminiscent of corresponding structures in intermediate developmental forms of *C. trachomatis* (Fig. 2E). Late (24 to 48 hours after infection) inclusions of chlamydiae are characterized by the presence of numerous typical EBs with electron-dense nucleoids and contracted cell walls. These late inclusions also contain RBs in which neither structure is condensed as well as a substantial number of intermediate forms that have condensed nucleoids but uncontracted cell walls (Fig. 2E). These intermediate forms resemble the recombinant *E. coli* strains expressing Hc1. The nucleoids of *E. coli* expressing Hc1 also display a surrounding region of cytoplasm consisting of electron-transparent material interspersed with fibrillar projections that form a distinct radiative pattern. A similar structure can be observed surrounding chlamydial nucleoids in intermediate forms as the chromatin condenses (Fig. 2E) (*14*). This similarity suggests that the higher order nuclear structure observed in the recombinant organisms resembles that of native chlamydiae and that Hc1 is sufficient to induce compaction of the chromatin.

Thin sections of *E. coli* K38(pT763 pGP1-2) were subjected to immunoelectron microscopy with polyclonal anti-Hc1 antibodies. Although the gold particles appear somewhat dispersed throughout the orga-

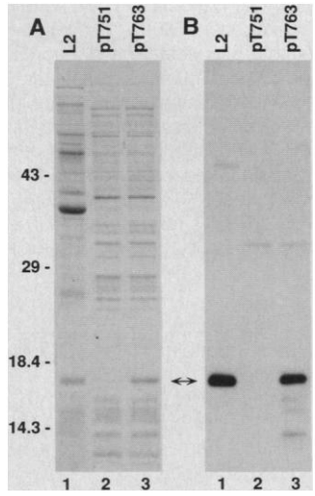

Fig. 1. Full-length expression of chlamydial Hc1 in *E. coli*. (**A**) A 12.5% polyacrylamide gel analysis of the cloned gene, stained with Coomassie brilliant blue. The *C. trachomatis* L2 lysate (lane 1) was prepared from purified EBs (*32*). Lanes 2 and 3 (pT751 and pT763) show nonexpressing and expressing orientations, respectively, of the Bam HI to Eco RI fragment that contains *hctA*, which has been described (*5*). This fragment was cloned into the same sites in pT7-5 and pT7-6 (*10*) and transformed into K38(pGP1-2), which provides a thermoinducible source of the T7 RNA polymerase. Samples were prepared as follows: cultures were grown at 30°C in Luria broth containing carbenicillin (250 μg/ml) and kanamycin (50 μg/ml) to an optical density $OD_{600\ nm}$ of 0.2; they were then heat-shocked at 42°C for 30 min and grown for an additional 90 min at 37°C. (**B**) An identical gel probed with a polyclonal rabbit antiserum that had been raised in response to affinity-purified chlamydial Hc1 (*5*).

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nism, there is a concentration of label over the nuclear material (Fig. 2F). Outside the nuclear material a diffuse region with few or no gold particles associated was usually observed, whereas the remainder of the cytoplasm was labeled, although at a lower density than the nucleoid. Labeling of the cytoplasm may be due to the presence of unbound or plasmid-associated Hc1, as analysis of isolated plasmid DNA by polyacrylamide gel electrophoresis (PAGE) and Western (protein) immunoblotting revealed the presence of Hc1 (*15*).

We isolated intact, condensed *E. coli* nucleoids by lysozyme treatment and nonionic detergent lysis (*16, 17*). The [^{3}H]thymidine-labeled nucleoids were purified by centrifugation through sucrose gradients, and Hc1 was found to co-sediment with these nucleoid particles (Fig. 3). Multiple forms of DNA-containing structures in the Hc1-expressing strain presumably reflect varying levels of organization of the nucleoids (Fig. 3A). In contrast, nucleoids in control *E. coli* not expressing Hc1 appear to be a more homogeneous population (Fig. 3C). The *E. coli* chromosome is known to be only loosely organized into nucleoids and is consequently more buoyant in these gradients (*18*).

Deoxyribonuclease (DNase) I treatment of either preparation shifts the sedimentation pattern. Controls are nearly completely digested, and the radioactivity fails to enter the gradient (Fig. 3D). Preparations containing Hc1 are also largely digested, but there is a proportion of residual DNA dense enough to enter the gradient (Fig. 3B). The majority of Hc1 fails to enter the gradient after DNase I treatment, indicating that its sedimentation is dependent on an association with nucleoid DNA. Thus, the nucleoid structures observed consist of both Hc1 and *E. coli* chromosomal DNA.

The interaction of Hc1 with DNA may be largely nonspecific, as implied by compaction of the *E. coli* chromosome. The densely compacted nucleoid of chlamydial EBs requires that the electrostatic repulsion of the negatively charged phosphate groups of DNA be overcome. Positively charged EB-specific proteins with homology to eukaryotic histone H1 have been identified in EBs and are associated with the condensed nucleoid (*5–7*). These proteins are relatively abundant in EBs and are thought to function as mediators of the nuclear condensation that characterizes the RB to EB transition. A number of prokaryotic histone-like proteins with probable roles in bacterial DNA organization have been identified; of these, Alg P of *Pseudomonas aeruginosa* bears primary sequence homology to eukaryotic H1 (*19, 20*). Histone-like elements may control bacterial virulence in many systems (*21–24*). It has been suggested that these proteins may not function as conventional transcriptional regulators but act instead by modifying DNA structure, thereby providing a mechanism of global regulation of gene expression in bacteria (*21*). Many of these histone-like proteins are thought to interact with DNA in a relatively nonspecific fashion to alter chromatin structure and DNA topology or available supercoils (*25–27*). Changes in DNA supercoiling in response to environmental signals such as osmolarity, temperature, or anaerobiosis have been proposed to function in prokaryotic gene regulation by effecting promoter activity (*28–30*). Although there is no direct evidence regarding the supercoiling state of the chlamydial chromosome, it has been shown that polymorphic plasmid DNAs carried in C. *trachomatis* have distinct levels of supercoiling that vary with the developmental stage, with the more highly supercoiled states occurring in EBs (*31*).

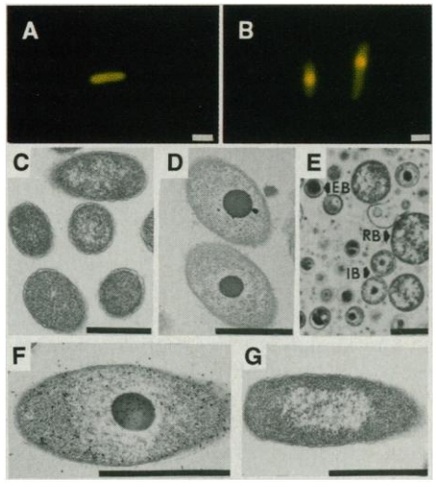

Fig. 2. (**A**) Acridine orange–stained *E. coli* K38(pGP1-2 pT751) containing the *hctA* gene in nonexpressing orientation (*33*). Samples were prepared in liquid media as in Fig. 1. (**B**) Acridine orange–stained *E. coli* K38(pGP1-2 pT763) (expressing Hc1) prepared as in Fig. 1. The bright, strongly fluorescent material in the rods represents the condensed nucleoid structure. (**C** and **D**) Electron microscopy of nonexpressing and expressing orientations of Hc1, respectively. Samples were prepared in liquid media as in Fig. 1; 0.5-ml samples were collected by centrifugation (5000*g*, 10 min), fixed, and stained with uranyl acetate and lead citrate as described (*34*). (**E**) Uranyl acetate–stained *C. trachomatis* L2 inclusion 30 hours after infection of HeLa 229 cells, showing EBs, intermediate bodies (IBs), and RBs, prepared as described (*34*). (**F** and **G**) Immunoelectron microscopy on thin sections of K38(pGP1-2 pT763) and K38(pGP1-2 pT751), respectively, with anti-Hc1 polyclonal antisera. Microscopy was done with the use of direct antibody-gold conjugates as described (*35, 36*). Scale bars, 0.5 μm.

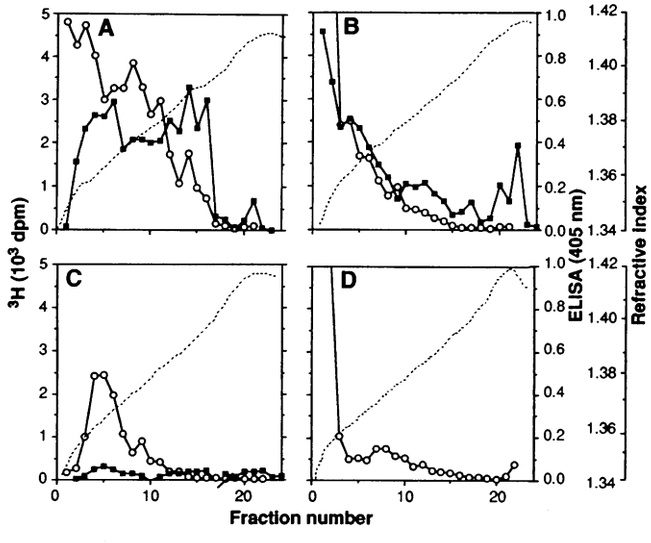

Fig. 3. Sucrose gradient sedimentation patterns of *E. coli* nucleoids containing chlamydial Hc1. (**A**) *Escherichia coli* nucleoids isolated from a strain, K38(pGP1-2 pT763), that expressed chlamydial Hc1. Nucleoids were prepared by gentle lysis after labeling with [^{3}H]thymidine and were spun on 0 to 60% (linear) sucrose gradients (*37*). Fractions were analyzed for their refractive index (broken lines), total ^{3}H disintegrations per minute (○), and enzyme-linked immunosorbent assay (ELISA) reactivity (OD_{405}) (■) with anti-Hc1 sera. (**B**) Nucleoid and Hc1 sedimentation is dependent on DNase I treatment. Although some ELISA reactivity remains in the gradient, the majority is shifted to a position of lower density; the remainder may imply some degree of protection from digestion by association with Hc1 because the control (D) is completely digested. (**C**) Nucleoids isolated from an *E. coli* strain bearing a plasmid (pT751) arranged such that the *hctA* gene is not expressed by the T7 promoter. Thus, these cells do not contain Hc1. (**D**) Nucleoids from (C) without Hc1 are completely digested by DNase I treatment.

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37. The 25-ml cultures were prepared as described, except that at an $OD_{600\ nm}$ of 0.1, 10 μCi of [^{3}H]thymidine were added to each culture. After induction, the cells were lysed by the procedure of Sjåstad *et al.* (*17*), with the exception that hen egg-white lysozyme (500 μg/ml) was used in place of T4 lysozyme. Unincorporated label and soluble protein were removed by repeated centrifugation through 100 mM sucrose cushions as described. The pellet was resuspended in 500 μl of buffer A [20 mM tris, pH 8.1, 50 mM NaCl, 5 mM EDTA, 0.5 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride (PMSF), and 10% glycerol] and loaded onto a 0 to 60% (w/v) linear sucrose gradient in the same buffer. Samples were centrifuged at 1250*g* for 20 min, and 0.5-ml fractions were removed from the top and analyzed as described. For DNase I treatment, the pellet after three pelletings through 100 mM sucrose solutions was resuspended in 500 μl of buffer B (10 mM tris, pH 8.0, 0.5 mM $CaCl_2$, and 5 mM $MgCl_2$), 250 U of DNase I was added, and the suspension was incubated at 37°C for 15 min before loading onto a linear sucrose gradient as above.
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Reciprocal Regulation of Adipogenesis by Myc and C/EBPα

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3T3-L1 adipoblasts that express large amounts of c-Myc cannot terminally differentiate, raising the possibility that Myc inhibits the expression of genes that promote adipogenesis. The CCAAT/enhancer binding protein (C/EBPα) is induced during 3T3-L1 adipogenesis when cells commit to the differentiation pathway. Transfection of 3T3-L1 adipoblasts with the gene that encodes C/EBPα caused overt expression of the adipocyte morphology. Expression of Myc prohibited the normal induction of C/EBPα and prevented adipogenesis. Enforced expression of C/EBPα overcame the Myc-induced block to differentiation. These results provide a molecular basis for the regulation of adipogenesis and implicate Myc and C/EBPα as pivotal controlling elements.

Proliferation and differentiation are often alternative and mutually exclusive pathways for living cells. Because specific genes control these pathways, the decision to either proliferate or differentiate may be governed by the ratio of gene products that promote each pathway. Inappropriate expression of genes that promote proliferation can favor proliferation over differentiation, which can result in neoplasia. Given that proliferation is generally incompatible with differentiation and vice versa, a gene that controls both pathways in a reciprocal manner might provide a molecular basis for this observation.

The decision to either proliferate or differentiate begins at the cell surface with cues received from the environment. These cues initiate events that cause changes in gene expression, a process often controlled at the level of transcription initiation. Because sequence-specific DNA binding proteins regulate transcription initiation (*1*), they are likely to participate in the regulation of cellular proliferation and differentiation.

The sequence-specific DNA binding protein c-Myc controls cellular proliferation and differentiation (*2*). Expression of c-Myc increases when quiescent cells are induced to proliferate (*3*) and decreases when actively growing cells enter either a quiescent or replicative senescent state (*4, 5*). Deregulated expression of c-Myc promotes cellular transformation and inhibits terminal differentiation both in vitro and in vivo (*2*). These and other observations suggest that c-Myc activates genes that promote proliferation (*2, 6*). However, it is also possible that Myc suppresses genes that restrict growth (*7*). One such candidate gene codes for the sequence-specific DNA binding protein C/EBPα (*8–11*), which promotes 3T3-L1 adipoblast differentiation (*11*). Expression of C/EBPα increases during adipogenesis (*9, 10*), and its premature expression in proliferating adipoblasts causes cessation of mitotic growth (*11*). That quiescent 3T3-L1 adipoblasts do not express C/EBPα suggests that C/EBPα is not a general growth suppressor. Enforced expression of c-Myc prevents 3T3-L1 adipogenesis by inhibiting the ability of cells to commit to the differentiation pathway (*12, 13*). Because C/EBPα has been implicated in the promotion of 3T3-L1 adipogenesis, we investigated whether expression of C/EBPα was sufficient for 3T3-L1 adipogenesis and whether Myc prevented adipogenesis by inhibiting induction of C/EBPα.

3T3-L1 adipoblasts were transfected (*14*) with pZip-NeoSV(X) (*15*) and a plasmid that contains the rat C/EBPα gene (pMSV-C/EBP) (*8*) under the control of the murine sarcoma virus (MSV) promoter. As controls, cells were transfected with a plasmid that contained the MSV promoter but lacked the C/EBPα gene (pEMSV) (*16*). After transfection, cells were selected in G418 (Boehringer Mannheim) for 2 to 3 weeks, and the number of G418-resistant colonies was recorded. Dishes of cells that received pMSV-C/EBP produced fewer colonies (~20% of the controls) than those that received pEMSV (Table 1). The yield of G418-resistant colonies, and the percentage of adipocyte colonies, was a func-

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