

Regulation of the *Chlamydia trachomatis* Histone H1-Like Protein Hc2 Is IspE Dependent and IhtA Independent

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The chlamydial histone-like proteins, Hc1 and Hc2, function as global regulators of chromatin structure and gene expression. Hc1 and Hc2 expression and activity are developmentally regulated. A small metabolite that disrupts Hc1 interaction with DNA also disrupts Hc2 interactions; however, the small regulatory RNA that inhibits Hc1 translation is specific to Hc1.

The obligate intracellular bacterium *Chlamydia trachomatis* is the leading cause of infectious blindness worldwide (19) and the most prevalent bacterial agent of sexually transmitted disease in industrialized and developing countries (20). Chlamydiae undergo a unique biphasic developmental cycle, alternating between two developmental forms. Infection of the host cell is initiated by the metabolically inert elementary body (EB), which possesses a characteristic core of condensed chromatin. Following internalization, the chromatin becomes dispersed, transcription and translation are initiated, and EBs differentiate to the metabolically active reticulate bodies (RBs). RBs replicate for 18 to 48 h within a membrane-bound vacuole called an inclusion until they asynchronously differentiate to EBs, a process characterized by compaction of the chromatin. The EBs are released from the host cell, and a new round of infection begins (9, 15).

Two DNA binding proteins, Hc1 and Hc2, are believed to be responsible for the compaction of the chlamydial genome (4, 8, 12, 17). Hc1 and Hc2 (encoded by *hctA* and *hctB*, respectively) bear primary amino acid similarity to eukaryotic histone H1 and are expressed late in the developmental cycle concomitantly with RB-to-EB conversion and chromatin condensation (3, 15). Hc1 is conserved among the chlamydiae, while Hc2 displays variable molecular weight depending on the species (7, 13, 14, 16, 18). Overexpression of either of these two proteins in *Escherichia coli* results in compaction of the bacterial chromatin, although the ultrastructure of the DNA-protein complex is distinct (2, 4). In particular, overexpression of Hc1 in *E. coli* causes chromatin condensation ultrastructurally similar in appearance to the spherical condensed nucleoids of EBs (2), while overexpression of Hc2 induces a condensed nucleoid with a more toroidal appearance. Expression of Hc1 in *E. coli* leads to a down regulation of transcription, translation, and bacterial replication in conjunction with condensation of the chromatin (1). Hc1 and Hc2 have both been found to strongly

repress transcription and translation in vitro by modulating DNA topology and/or binding to DNA and RNA (1, 10, 11).

The observed effects on chromatin structure and activity in *E. coli* are believed to reflect the native function of the histones in chlamydiae as they are considered to act as global regulators of chlamydial gene expression during the terminal differentiation of RBs to EBs at the end of the developmental cycle. Expression of the chlamydial histones in *E. coli* is effectively lethal, as *E. coli* apparently lacks the ability to release its chromatin from the constraints imposed by Hc1 or Hc2 binding.

Surprisingly, dispersal of the nucleoid requires chlamydial transcription and translation with negligible loss of Hc1. Because chlamydial dissociation of the histone-DNA complex requires transcription and translation, and as chlamydia lack a genetically tractable system, we designed a heterologous genetic screen to identify proteins involved in the regulation of Hc1 by selecting for chlamydial genes that relieved the lethal phenotype of Hc1 overexpression in *E. coli*. This selection identified two separate loci on the chlamydial genome that rescue *E. coli* from the growth restrictions imposed by Hc1 (5). The first locus, CT804, encodes IspE, an intermediate enzyme

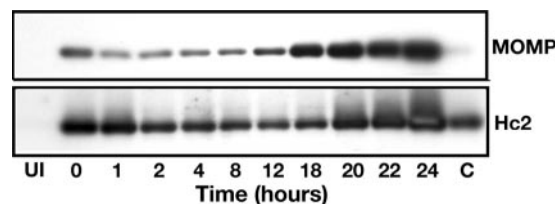


FIG. 1. Expression of Hc2 during infection. The figure shows the results of Western blot analysis of *C. trachomatis* L2 Hc2 and major outer membrane protein (MOMP) over a 24-h infection time period. HeLa cells in 24-well plates were infected at a multiplicity of infection of 500 for 30 min at 4°C to allow attachment, the inoculum was removed, and the cultures were fed with prewarmed medium and incubated at 37°C. The time of temperature upshift was considered time zero. The cultures were solubilized at the times indicated, and equal amounts were loaded for sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting. “UI” indicates uninfected sample, and “C” (control) represents samples collected from a 24-h infection in the continuous presence of chloramphenicol. Results shown are representative of three separate experiments.

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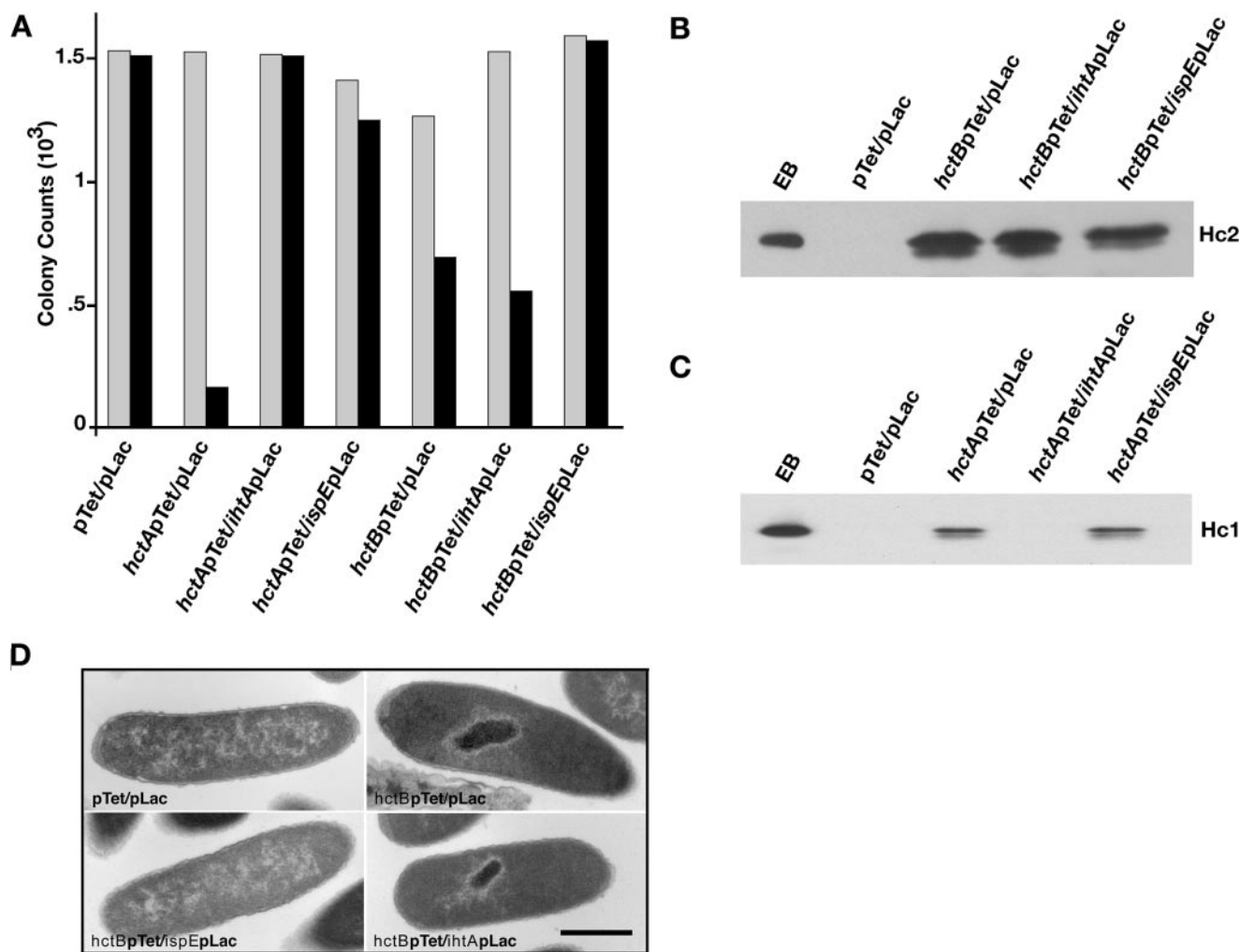


FIG. 2. IspE but not IhtA rescues *E. coli* from Hc2-induced growth repression. (A) Viability assay of *E. coli* expressing Hc2 and rescuing clones. Cultures expressing Hc1 and rescuing clones served as a control for IspE and IhtA activity. Samples not induced for Hc1 or Hc2 expression are represented by the gray bars, and samples induced for Hc1 or Hc2 are represented by the black bars. (B) Western blot analysis of Hc2 content in *E. coli* coexpression. The indicated strains were induced to express IhtA (*hctBpTet/ihtApLac*) or IspE (*hctBpTet/ispEpLac*) for 2 hours. Hc2 expression was then induced for an additional 5 h. Cultures were normalized for OD₅₅₀ prior to loading. *C. trachomatis* L2 EBs served as a reference for Hc2 position, and *E. coli* (pTet/pLac) cultures grown under the same conditions as above served as a negative control. (C) Western blot analysis of Hc1 content in *E. coli* coexpressing Hc1 and the constructs indicated above. Cultures were treated as above, and EBs served as a reference for Hc1 migration. (D) Ultrastructural analysis of *E. coli* induced to express vector controls (pTet/pLac), Hc2 (*hctBpTet/pLac*), Hc2 plus IspE (*hctBpTet/ispEpLac*), or Hc2 plus IhtA (*hctBpTet/ihtApLac*). Bar, 0.5 μ m.

of the nonmevalonate, 2-C-methyl-D-erythritol-2,4-cyclodiphosphate (MEC) pathway of isoprenoid biosynthesis. *E. coli* coexpressing IspE and Hc1 grew normally, although it expressed Hc1 to a level equivalent to that which condensed the chromatin of parental Hc1-expressing controls. This situation is equivalent to that of germinating EBs in which the histone remains present but no longer functions in condensation of the chromatin. Deproteinized extract from IspE-expressing bacteria caused dispersal of purified chlamydial nucleoids, suggesting that chlamydial histone-DNA interactions are disrupted by a small metabolite in the methylerythritol phosphate pathway of isoprenoid biosynthesis rather than by direct action of IspE (5). The second locus encodes a small regulatory RNA (sRNA) that inhibits Hc1 translation but did not affect *hctA* mRNA transcription or stability (6). This sRNA is thought to act as an additional checkpoint to negatively

regulate Hc1 synthesis. The chlamydial sRNA, IhtA (inhibitor of *hctA* translation), inhibits Hc1 translation during the exponential phase of chlamydial growth and is down regulated late in the chlamydial life cycle, concurrently with Hc1 translation and RB-to-EB differentiation (6). Because of the dramatic effects of Hc1 on chromatin structure and activity, Hc1 expression and activity thus appear to be tightly regulated transcriptionally as well as posttranscriptionally. Here we investigate whether these mechanisms of Hc1 regulation are specific for Hc1 or more general, including the other DNA binding protein, Hc2.

The condensed nucleoid of the EB disperses rapidly upon infection, and the EB is transcriptionally active within 2 hours of entry (15). Hc1 and Hc2 are late gene products whose transcription occurs only late in the developmental cycle (3, 8, 12, 15, 17). Hc1 levels remain essentially constant through the

first 8 h of infection, decrease significantly by 12 h, and then increase between 18 and 20 h as RBs begin to differentiate to EBs (5). We analyzed *C. trachomatis* Hc2 during a 24-h infection by immunoblotting with a rabbit anti-Hc2 serum and densitometry. HeLa cells in 24-well plates were infected at a multiplicity of infection of approximately 500 to facilitate detection of Hc2 during the initial hours of infection. The chlamydial major outer membrane protein was included as a control for the level of infection. Hc2 levels remained constant during the first hour of infection and decreased slightly by 2 and 4 h. Thus, like Hc1, Hc2 is present during the initial nucleoid decondensation although presumably unable to bind to and condense the chromatin. Hc2 content decreased slowly until late gene expression was initiated between 18 and 20 h postinfection, and Hc2 levels increased with differentiation of RBs to EBs (Fig. 1) (3, 15).

Hc2 binds to and condenses DNA in vitro and condenses the chromatin upon expression in *E. coli* (4, 11). *hctB*, the gene encoding Hc2, was cloned into the pTet vector described previously (5) and expressed in *E. coli* DH5 α PRO to verify condensation of the *E. coli* chromatin and determine the effects on viability. Expression of Hc2 (*hctB*pTet/pLac) in this system resulted in a 50% reduction in cell viability compared to an approximately 90% reduction in viability by Hc1 (Fig. 2A). Because expression of Hc2 in *E. coli* resulted in decreased viability, we were able to examine the ability of *ispE* and *ihtA* to restore viability when coexpressed with *hctB*. *E. coli* constructs expressing *hctA* (*hctA*pTet/pLac), *hctA* and *ispE* (*hctA*pTet/*ispE*pLac), and *hctA* and *ihtA* (*hctA*pTet/*ihtA*pLac) were included to control for IspE and IhtA activity. *E. coli* DH5 α PRO, transformed with the indicated constructs, was grown in Luria-Bertani broth (LB) containing 100 μ g ml⁻¹ carbenicillin, 34 μ g ml⁻¹ chloramphenicol, and 50 μ g ml⁻¹ spectinomycin at 30°C. Overnight cultures were diluted to an optical density at 550 nm (OD₅₅₀) of 0.2 and grown in the presence of 1 mM isopropyl-1-thio- β -D-galactopyranoside (IPTG) for 2 hours to induce IhtA and IspE production. Cultures were then split, and Hc1 or Hc2 protein expression was induced in one half by the addition of 100 ng ml⁻¹ anhydrotetracycline for 5 h, at which point samples were diluted and plated on agar to assay cell viability (Fig. 2A). In agreement with previous results, expression of either *ihtA* or *ispE* with *hctA* rescued *E. coli* from the lethal effects of Hc1 expression. Coexpression of *ispE* with *hctB* (*hctB*pTet/*ispE*pLac) rescued *E. coli* from the repressive phenotype of Hc2 protein expression, suggesting that the same small molecule derived from the MEC pathway of isoprenoid biosynthesis also released Hc2 from the chromatin. Coexpression of *ihtA* with *hctB* (*hctB*pTet/*ihtA*pLac) did not, however, rescue the repressed growth phenotype induced by Hc2 production (Fig. 2A). The effect of expression of both *ispE* and *ihtA* on Hc2 protein levels was assessed by immunoblotting (Fig. 2B). Cultures were normalized for OD₅₅₀ prior to loading. Hc2 protein levels of *ihtA*-coexpressing cells (*hctB*pTet/*ihtA*pLac) were similar to levels present in control samples that expressed *hctB* only (*hctB*pTet/pLac). This result is in agreement with the inability of *ihtA* expression to rescue the repressive phenotype of Hc2. In contrast, coexpression of *ihtA* with *hctA* (*hctA*pTet/*ihtA*pLac) resulted in Hc1 protein levels below the limits of detection (Fig. 2C) (6). Thus, the sRNA IhtA appears to specifically

regulate *hctA* and has no effect on the regulation of *hctB*. In *E. coli* coexpressing *hctB* and *ispE*, Hc2 was present at levels similar to those in controls expressing Hc2 alone. Thus, although Hc2 was present at levels equivalent to those required to inhibit growth of the control (*hctB*pTet/pLac), it was unable to restrict growth when coexpressed with *ispE*. These results are similar to those previously reported (5) in which Hc1 protein levels of *ispE*-coexpressing *E. coli* (*hctA*pTet/*ispE*pLac) approximate levels present in *hctA*-only-expressing cells (*hctA*pTet/pLac) (Fig. 2C). Examination of the nucleoid structure by transmission electron microscopy confirmed the ability of Hc2 to condense the chromatin in cells expressing *hctB* only (Fig. 2D). *E. coli* coexpressing *hctB* and *ispE* had a chromatin structure identical to that of the vector-only control (pTet/pLac) despite the presence of Hc2 at levels sufficient to condense the chromatin of Hc2-only controls. *E. coli* coexpressing *hctB* and *ihtA* displayed a condensed chromatin structure identical to that of *E. coli* expressing *hctB* only (Fig. 2D).

The data suggest that the disruption of chlamydial histone-DNA interactions by the small molecule MEC upon initial infection is a general mechanism of histone-DNA release and functions to release both Hc1 and Hc2 from chromatin. The sRNA IhtA, on the other hand, regulates Hc1 synthesis specifically and has no regulatory effect on Hc2. Whether sRNA regulation of Hc2 or other chlamydial differentially expressed genes is a more general mechanism of developmental regulation in chlamydiae awaits additional experimentation.

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