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14. G/C cells were labeled with [^{32}P]orthophosphate or [^{35}S]methionine for 3 hours as described (*13*). Nuclear extracts were prepared from labeled cells in the presence of phosphatase inhibitors [G. G. Capps *et al.*, *J. Cell. Biol.* **108**, 1317 (1989)]. Immunoprecipitations were carried out as described (*13*) at a final antibody dilution of 1:1000 with 0.05% SDS, 0.1% deoxycholate, 0.2% Tween-20, and 0.2% NP-40.
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16. C/EBPα and CREB protein were expressed in the bacterial strain BL21(DE3) with the expression vector pET3a [F. W. Studier and B. A. Moffat, *J. Mol. Biol.* **189**, 113 (1986)] and purified by chromatography over a phosphocellulose column. The C/EBPβ lacking the first 11 amino acids was expressed as a fusion protein with six consecutive histidines at its NH_2-terminus in vector pQE9 and purified to ≥95% homogeneity with the Ni-NTA chromatography system (Quiagen, Chatsworth, CA). CaMK reactions (0.1 ml) were performed with purified brain CaMKII (2.5 μg/ml) and substrate protein (50 μg/ml) for 2 min at 30°C in the presence of 50 mM Pipes (pH 7.5), 10 mM $MgCl_2$, calmodulin (10 μg/ml), 0.5 mM $CaCl_2$, 200 μM [γ-^{32}P]ATP (specific activity ≈5 Ci/mmol). Protein kinase A reactions (50 μl) were carried out for 30 min at 30°C with protein kinase A catalytic subunit (2.5 μg/ml) and substrate protein (50 μg/ml) as described (*13*).
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28. We thank S. L. McKnight and M. S. Kapiloff for providing various reagents and expression plasmids and for discussions. We also thank H. Hidaka for supplying KN-62, S. Taylor for supplying purified protein kinase A catalytic subunit, and C. Nelson for maintenance and aid in tissue culture experiments. M.W. is a recipient of a postdoctoral fellowship from the Deutsche Forschungsgemeinschaft. M.G.R. is an investigator with the Howard Hughes Medical Institution. Supported by a grant from the National Institute of Diabetes and Digestive and Kidney Diseases.

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Alternative Forms of Max as Enhancers or Suppressors of Myc-Ras Cotransformation

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Max is a basic-helix-loop-helix–leucine zipper protein capable of forming sequence-specific DNA binding complexes with Myc proteins. An alternatively spliced messenger RNA has been identified that encodes a form of Max truncated at the COOH-terminus. This ΔMax protein retained the ability to bind to the CACGTG motif in a complex with c-Myc but lacks the nuclear localization signal and the putative regulatory domain of Max. When tested in a *myc-ras* cotransformation assay in rat embryo fibroblasts, Max suppressed, whereas ΔMax enhanced, transformation. Thus, the *max* gene may encode both a negative and a positive regulator of c-Myc function.

Members of the *myc* gene family have been implicated in the control of normal cell proliferation as well as in neoplasia (*1*). A more direct role for *myc* genes in transformation is indicated by their ability to transform primary rat embryo fibroblasts in association with the c-Ha-*ras* oncogene (*2*). Adjacent basic-helix-loop-helix (bHLH) and leucine zipper (Zip) DNA binding and dimerization motifs (*3*) in the COOH-terminus of Myc proteins are similar to motifs found in several E-box–binding transcriptional regulators (*4*). The ability of Myc to bind to an E-box–containing core sequence CACGTG (*5*) is enhanced by heterodimerization with the bHLH-Zip protein Max (*6–8*).

We amplified *max*-specific sequences from human erythroleukemia cell (HEL) cDNA by the polymerase chain reaction (PCR) (*9*). Analysis of the PCR products revealed two separate bands of about 500 and 600 base pairs (bp). Sequence analysis indicated that the larger band contained a 101-bp insert in the middle of the coding sequence (Fig. 1A). Further PCR studies from human, mouse, and rat genomic DNA indicated that the additional sequence was derived from an alternatively spliced exon (Fig. 1B) that is conserved in evolution (*10*).

This alternative exon introduced an in-frame translation termination codon, which predicted the formation of a truncated Max polypeptide (ΔMax) that consisted of 103 amino acids. The 98 NH_2-terminal

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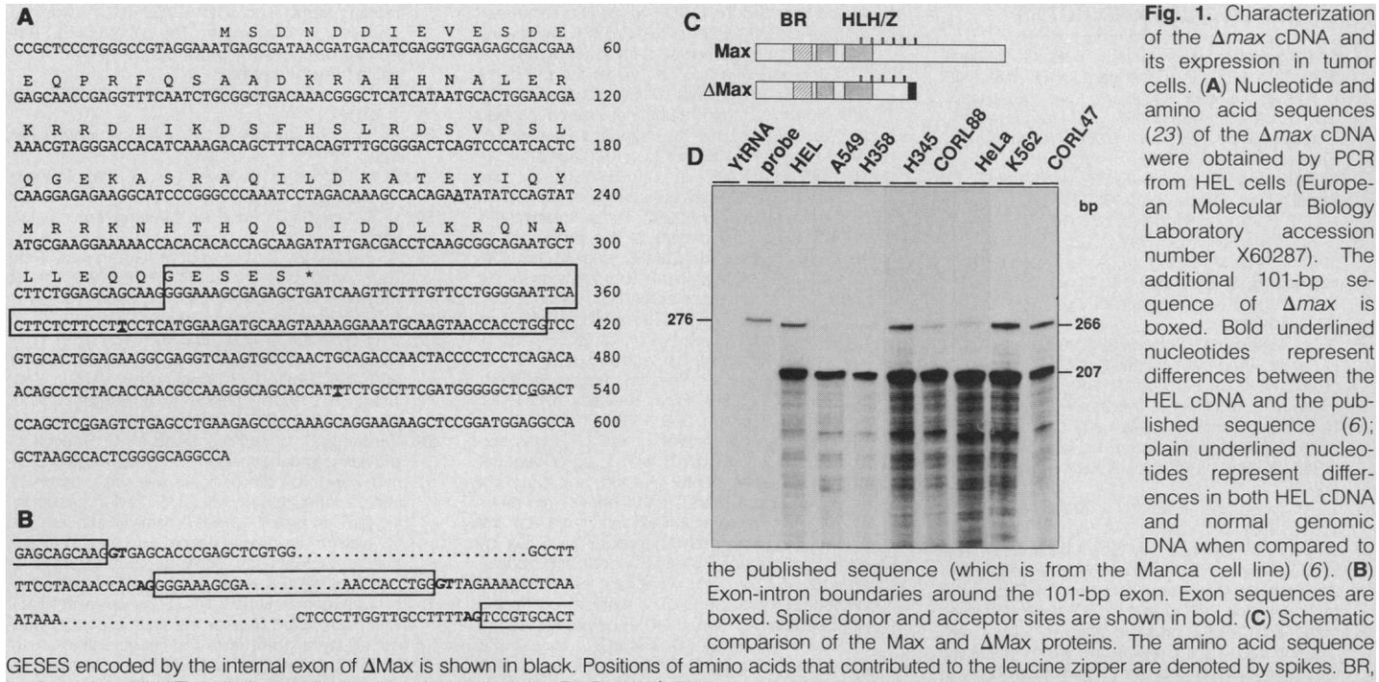

Fig. 1. Characterization of the Δ*max* cDNA and its expression in tumor cells. (**A**) Nucleotide and amino acid sequences (*23*) of the Δ*max* cDNA were obtained by PCR from HEL cells (European Molecular Biology Laboratory accession number X60287). The additional 101-bp sequence of Δ*max* is boxed. Bold underlined nucleotides represent differences between the HEL cDNA and the published sequence (*6*); plain underlined nucleotides represent differences in both HEL cDNA and normal genomic DNA when compared to the published sequence (which is from the Manca cell line) (*6*). (**B**) Exon-intron boundaries around the 101-bp exon. Exon sequences are boxed. Splice donor and acceptor sites are shown in bold. (**C**) Schematic comparison of the Max and ΔMax proteins. The amino acid sequence GESES encoded by the internal exon of ΔMax is shown in black. Positions of amino acids that contributed to the leucine zipper are denoted by spikes. BR, basic region; HLH/Z, helix-loop-helix–leucine zipper domain. (**D**) Poly(A)$^+$ RNA (2 μg) from the indicated cell lines or yeast tRNA (YtRNA) (10 μg) was hybridized to a 276-bp ^{32}P-labeled antisense probe spanning from the alternative exon to the following 3′ exon. The 266- and 207-bp protected bands represent the Δ*max* and *max* mRNAs, respectively. Origins of the human cell lines tested: HEL and K562, erythroleukemia; A549, lung adenocarcinoma; H358, H345, CORL88, and CORL47, small cell lung carcinoma; HeLa, cervix carcinoma.

amino acids of ΔMax were identical with those of Max, while the 5 COOH-terminal amino acids of ΔMax were derived from the alternative exon. Thus, the ΔMax protein was expected to contain an almost intact bHLH-Zip domain but to lack 62 COOH-terminal amino acids (Fig. 1C). Similar to Max (*6*, *7*), ΔMax has two alternative forms with or without an insert of 9 amino acids near the NH_2-terminus. Because this insert also probably results from alternative splicing, the coding region of *max* would be expected to consist of at least five exons.

To confirm that the Δ*max* cDNA clones represented a naturally expressed form of *max*, we conducted ribonuclease (RNase) protection assays as described (*11*). In the various cell lines tested, two major RNase-resistant bands of ~266 and 207 bp were observed (Fig. 1D); minor bands below the latter resulted from partial RNase digestion. The 266- and 207-bp bands corresponded to the expected sizes of bands produced by Δ*max* or *max* mRNAs, respectively, and could also be detected in the normal tissues tested (*12*). In most cases, the ΔMax mRNA was expressed in small amounts, but significant cell line–specific differences in the *max*/Δ*max* ratio were observed in Northern (RNA) blot analysis also, which suggests the possibility of regulation of the two mRNA forms.

Analysis of in vitro–transcribed and translated ΔMax revealed a polypeptide with an apparent molecular size of 16.5 kD in comparison to a similarly analyzed Max protein of 22 kD (Fig. 2A). When mixed with a c-Myc lysate, both ΔMax and Max were detected after immunoprecipitation (*13*) with an anti-Myc antiserum, which indicated that both proteins interacted with c-Myc in vitro (Fig. 2A). To confirm these results, we used a polyclonal antiserum raised against a β-galactosidase–Max fusion protein that contained COOH-terminal sequences of Max (*10*). This antiserum specifically recognized Max, but not ΔMax or c-Myc (Fig. 2B). When lysates that contained c-Myc or ΔMax in addition to Max were analyzed with anti-Max, we observed coprecipitation of both c-Myc and ΔMax. Coprecipitation of ΔMax in the absence of c-Myc indicated that Max and ΔMax also can form heteromeric complexes in vitro.

When a mixture that contained similar amounts of all three proteins was subjected to immunoprecipitation with the anti-Myc

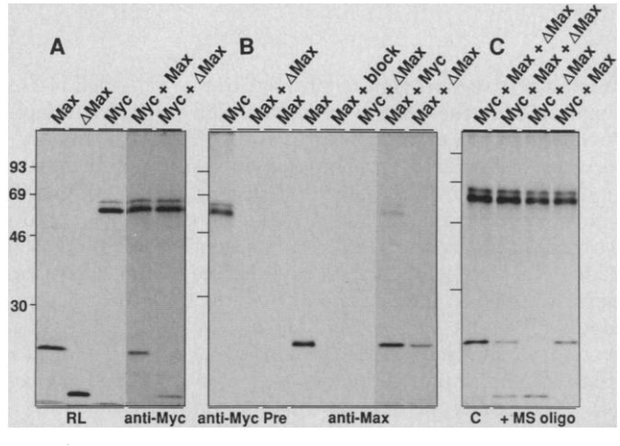

Fig. 2. Formation of heterooligomeric complexes between Max, ΔMax, and c-Myc proteins. (**A**) SDS–polyacrylamide gel electrophoresis of reticulocyte lysates programmed with RNAs (*22*) indicated above the RL lanes. After separate in vitro translations, the indicated lysates were mixed and immunoprecipitated with anti-Myc antiserum (*24*). (**B**) Indicated combinations of c-Myc, Max, and ΔMax containing lysates were mixed and immunoprecipitated with antisera shown at the bottom of the gel. The specificity of the anti-Max antibody was controlled with preimmune serum (Pre) or immune serum blocked with the β-galactosidase-Max fusion protein (Max + block). (**C**) Indicated combinations of c-Myc–, Max-, and ΔMax-containing lysates were mixed and immunoprecipitated with anti-Myc antiserum in the absence (C) or presence (+ MS oligo) of double-stranded E_{MS} oligonucleotide (0.5 μg). Both the Max and ΔMax proteins used contained the insert of nine amino acids except in the first and fourth lanes of (A), where a Max protein that lacked this region was used.

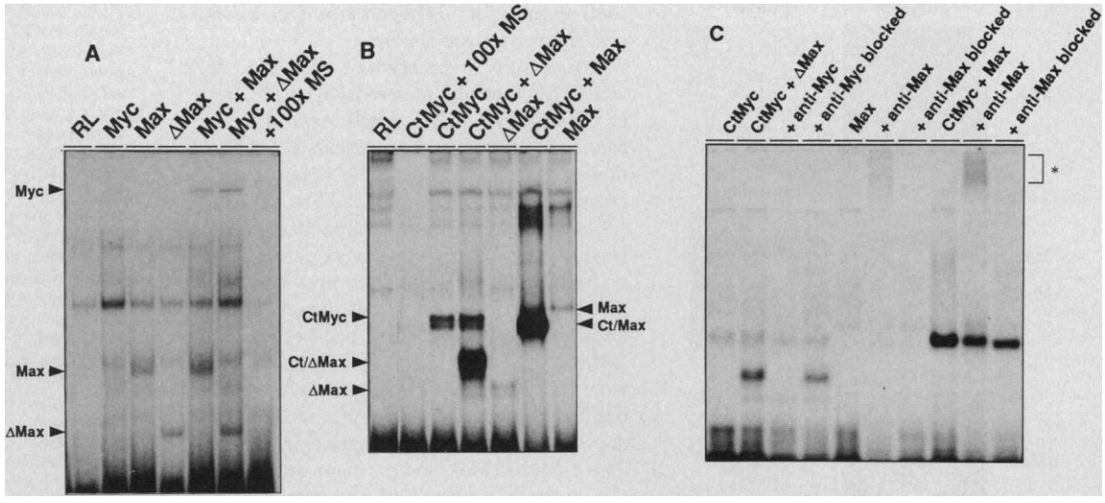

Fig. 3. Electrophoretic mobility-shift assay analysis of c-Myc, Max, and ΔMax proteins. (**A**) The labeled E_{MS} oligonucleotide was incubated with reticulocyte lysates that contained no RNA (RL), with in vitro–translated c-Myc, Max, or ΔMax proteins, or with combinations of these as indicated above the lanes. MS, double-stranded E_{MS} oligonucleotide. In the + 100× MS lane, a 100-fold molar excess of cold oligonucleotide was used as a competitor. Specific shifted complexes are indicated by arrowheads. (**B**) Binding of a COOH-terminal fragment of c-Myc (CtMyc) was analyzed alone or in combination with Max or ΔMax. In addition to the shifted complexes with each protein alone, hetero-oligomeric complexes with intermediate mobilities between CtMyc and ΔMax (Ct/ΔMax) or CtMyc and Max (Ct/Max) are also indicated. (**C**) The presence of CtMyc and Max proteins in the heteromeric complexes was tested with the indicated antibodies. Asterisk, Max-containing complexes supershifted by anti-Max.

antiserum, more abundant amounts of Max than ΔMax were coprecipitated (Fig. 2C). When oligonucleotides that contained the Myc-binding sequence were added to the immunoprecipitates, the relative amount of coprecipitating ΔMax protein increased, while the amount of Max decreased (Fig. 2C), which suggests stabilization of the c-Myc–ΔMax complex in the presence of DNA binding sites.

We next studied the binding of c-Myc, Max, or ΔMax to ^{32}P-labeled E_{MS} oligonucleotide in the electrophoretic mobility-shift assay (*14*). Shifted complexes specific for each protein were observed (Fig. 3A), although the complex obtained by c-Myc alone was weak. When lysates that contained Max or ΔMax were mixed with the c-Myc lysate before the binding reaction (*14*), the weak complex obtained with the c-Myc lysate alone was strengthened in intensity (Fig. 3). As reported (*7*), no complexes of intermediate mobility were observed, perhaps because of the poor solubility of the full-length c-Myc protein in the conditions used. Therefore, we used in vitro–translated COOH-terminal c-Myc protein (CtMyc) that contained only the bHLH-Zip domains (*15, 16*). Incubation of the E_{MS} oligonucleotide with a CtMyc lysate resulted in a complex that migrated slightly slower than the Max complex (Fig. 3B). If either ΔMax or Max was mixed with CtMyc, stronger shifted bands of intermediate mobilities were observed (Fig. 3B), which indicated binding of heteromeric complexes. This result was confirmed by specific antisera against CtMyc or Max (Fig. 3C). An anti-Myc antiserum directed against the COOH-terminal region of c-Myc abolished formation of the Ct/ΔMax heteromeric complexes and diminished the band corresponding to the CtMyc homomeric complex, while anti-Max supershifted the Max-containing complexes (Fig. 3C). Effects of both antisera could be inhibited by blockage with the corresponding antigens. Thus, both Max and ΔMax were able to form heteromeric complexes with the COOH-terminal c-Myc protein and to bind to the E_{MS} oligonucleotide with a stronger affinity than any one protein alone.

To assess the subcellular localization of Max and ΔMax, we transfected COS-7 cells with constructs that expressed tagged Max and ΔMax proteins (*17*). The Max-tag protein was diffusely distributed in nuclei excluding nucleoli (Fig. 4), which resembled the pattern observed for Myc (*16, 18*). Double staining of cells that expressed c-Myc with either Max or Max-tag confirmed the colocalization of these proteins.

For ΔMax-tag, most of the cells displayed a cytoplasmic fluorescence with no detectable signal in the nuclei (Fig. 4). However, when ΔMax-tag was coexpressed with c-Myc, ΔMax-tag–specific fluorescence was observed in the nuclei of cells in colocalization with c-Myc (Fig. 4). By contrast, a mutant c-Myc protein that lacked the HLH domain but retained a nuclear translocation signal (*19*) did not affect the subcellular localization of ΔMax-tag (Fig. 4). These results suggest that Myc and ΔMax proteins associate efficiently in vivo and that this association is required for the nuclear translocation of ΔMax. No significant increase in nuclear fluorescence for ΔMax-tag was observed in cells cotransfected with Max, which suggests that ΔMax and Max do not associate in vivo with each other as efficiently as with Myc.

We next analyzed the ability of Max and ΔMax to affect the functions of c-Myc in vivo by using the rat embryo fibroblast (REF) cotransformation assay (*20*). As expected, cotransfection of c-*myc* and the mutant c-Ha-*ras* oncogene led to formation of transformed

Table 1. Effects of the two forms of Max on the transforming activity of c-Myc in vivo. Rat embryo fibroblast cells were transfected with the indicated plasmids (*20*). Transformed foci were scored 14 days after transfection. Each number represents the sum of transformed foci from five plates. Exp. experiment; ND, not determined.

Plasmids transfected	Transformed foci			Average number of foci
	Exp. 1	Exp. 2	Exp. 3	
pGEJ(6.6)	0	0	0	0
pGEJ(6.6) + LTR-Max	0	0	0	0
pGEJ(6.6) + LTR-ΔMax	0	0	0	0
pGEJ(6.6) + SV-*myc*	25	42	43	37
pGEJ(6.6) + SV-*myc* + LTR-Max	5	18	19	14
pGEJ(6.6) + SV-*myc* + LTR-ΔMax	125	89	90	101
pGEJ(6.6) + SV-*myc* + LTR-Max + LTR-ΔMax	ND	39	39	39
pSV2*neo* + pGEJ(6.6)	0	0	0	0
pSV2*neo* + pGEJ(6.6) + SV-*myc*	47	19	29	32
pSV2*neo* + pGEJ(6.6) + SV-*myc* + LTR-Max	6	0	2	3
pSV2*neo* + pGEJ(6.6) + SV-*myc* + LTR-ΔMax	212	47	58	106

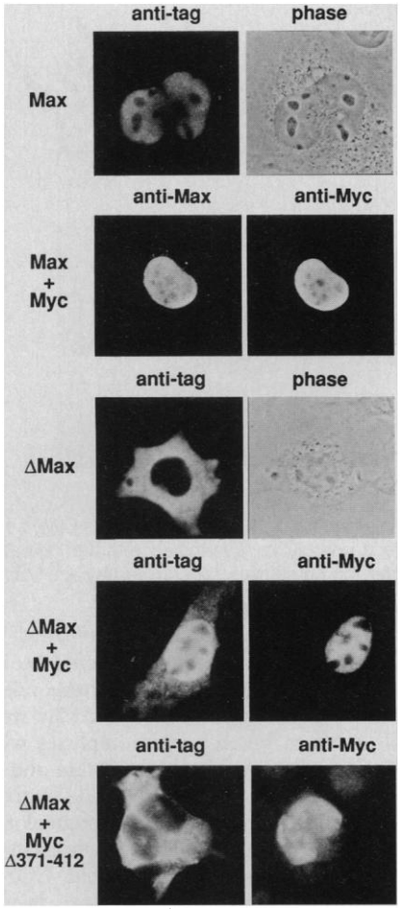

Fig. 4. Subcellular localization of Max, ΔMax, and c-Myc analyzed by indirect immunofluorescence. We transfected COS-7 cells with constructs that expressed tagged Max or ΔMax proteins alone or together with c-*myc* constructs, as indicated on the left side of the micrographs, and stained them with anti-tag, anti-Max, or anti-Myc antisera (*25*), as indicated above the micrographs. Phase, phase-contrast microscopy.

foci (Table 1). Cotransfection of *max* with c-*myc* and c-Ha-*ras* decreased the number of transformed foci. Repression of transformation has also been observed with large amounts of *myn* (the murine homolog of *max*) in a similar cotransformation assay (*21*), although small amounts of *myn* were originally reported to enhance *myc-ras* cotransformation (*7*). By contrast, cotransfection of Δ*max* with c-*myc* and c-Ha-*ras* augmented the number of foci two- to fivefold over that obtained with c-*myc* and c-Ha-*ras* only (Table 1). Introduction of both *max* and Δ*max* together with c-*myc* and c-Ha-*ras* resulted in a number of transformed foci similar to those observed with c-*myc* and c-Ha-*ras* only (Table 1). When pSV2*neo* was used as a selectable marker in the transfections, both the decrease in number of transformed foci on *max-myc-ras* plates and the increase on Δ*max-myc-ras* plates were observed (Table 1), although the total number of G418-resistant foci on all pSV2*neo* plates was similar.

Our results are consistent with a model where Max would preferentially form heterodimers with Myc. High expression of Max would result in an excess of homodimers responsible for the observed suppression of transformation. This situation could occur either by competition for the binding sites of the c-Myc–Max heterodimer or by binding to a different site. High expression of ΔMax might also result in the formation of homodimers, but because the ΔMax homodimers are cytoplasmic, they would be incapable of competing for DNA binding sites. This would explain why ΔMax does not suppress transformation by c-Myc. The c-Myc–ΔMax heterodimers are in turn efficiently transported to the nucleus and enhance the transforming effects of c-Myc.

Alternative splicing, which results in the production of *max* and Δ*max* mRNAs, represents an interesting mechanism for regulation of functionally distinct forms of the Max protein. Because the cell lines studied expressed rather small amounts of Δ*max* mRNA, the physiological significance of the corresponding ΔMax protein in these cells could not be determined. However, the evolutionary conservation of ΔMax strongly suggests that it has a function in some cells, tissues, or developmental phases. Our data on the opposite effects of Max and ΔMax on c-Myc transformation provide clues for the further exploration of the role of Max in c-Myc function.

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9. Polyadenylated [poly(A)$^+$] RNA (1 μg) was reverse-transcribed with oligo(dT) primers and subjected to a 30-cycle PCR (denaturation: 1.5 min at 95°C; annealing: 2 min at 55°C; extension: 3 min at 72°C) with the following primers: *max*-5′, 5′-GCT **CTA GAG** CCG CTC CCT GGG CCG TAG GAA-3′; *max*-3′, 5′-**GGA ATT** CTG GCC TGC CCC GAG TGG CTT AGC-3′ (restriction sites shown in bold). The PCR products were subcloned in pGEM3Zf(+), pSVpoly [A. Stacey and A. Schnieke, *Nucleic Acids Res.* **18**, 2829 (1990)], and pLTRpoly (*10*) expression vectors.
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12. We observed Δ*max* in embryonal brain, lung, heart, kidney, skin, thymus, and muscle, and in adult testis.
13. For immunoprecipitation, the translated proteins were diluted with phosphate-buffered saline [137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, and 1.5 mM KH_2PO_4 (pH 7.4)] that contained NP-40 (1%) and aprotinin (100 U/ml). For coprecipitation, aliquots of the proteins were mixed together and incubated for 30 min at 30°C before dilution. Before three washes in the same buffer, samples were incubated 2 hours with antisera.
14. Reticulocyte lysates that contained in vitro–translated proteins (*22*) were mixed and incubated for 30 min at 30°C. Binding reactions that consisted of 10 mM tris HCl (pH 7.5), 50 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 5% glycerol, 0.5 ng of ^{32}P-labeled double-stranded E_{MS} oligonucleotide [5′-TCA GAC **CAC GTG** GTC GGG TGT TCC TGA-3′ (E-box core sequence in bold)] (*7*), and 0.5 μg of unrelated single-stranded oligonucleotide (5′-GGA ATT CTG GCC TGC CCC GAG TGG CTT AGC-3′) were incubated for 10 min at room temperature. For antibody experiments, polyclonal CT-32 c-Myc antiserum [G. Ramsay, G. I. Evan, J. M. Bishop, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 7742 (1984)] or anti-Max antiserum were added to the lysates 5 min before the labeled oligonucleotide. Protein-DNA complexes were resolved on 4 to 7% polyacrylamide gels in a tris-borate EDTA buffer [22 mM tris base, 22 mM borate, and 0.5 mM EDTA (pH 8.3)] before autoradiography.
15. The c-Myc deletion template (CtMyc), which contained the T7 RNA polymerase binding site as well as sequences for translational initiation linked to sequences that encoded amino acids 343 to 439 of the human c-Myc, was prepared by PCR from the pSV-Tc-*myc* plasmid (*16*).
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17. The *max* and Δ*max* cDNAs were tagged at the COOH-termini with PCR with sequences that encoded human testis–specific lactate dehydrogenase (LDH) as described [J. L. Millan, C. E. Driscoll, K. M. LeVan, E. Goldberg, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 5311 (1987)]. The LDH sequences were detected by a rabbit polyclonal anti-tag antiserum.
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20. For the REF cotransformation assay, ~3 × 10^6 second passage rat embryo cells prepared from 13- to 15-day-old Sprague-Dawley rat embryos were transfected by calcium phosphate coprecipitation with 30 μg of DNA. This DNA included 7.5 μg of pGEJ(6.6), 15 μg of pLTR-*max*, and 15 μg of pLTR-Δ*max* or 7.5 μg of pSV-c-*myc*-1 [American Type Culture Collection (ATCC) 41029] or both, except in cotransfection of both *max* and Δ*max*, where 7.5 μg of both plasmids were used. The pGEJ(6.6) contains the 6.6-kb Bam HI fragment of the human c-Ha-*ras* oncogene subcloned from pEJ6.6 (ATCC 41000) into pGEM7Zf(+). When needed, pSP73 (Promega) was added as a carrier. In a parallel series of experiments pSV2*neo* (1 μg) (ATCC 37149) was cotransfected to allow selection with neomycin analog G418 (Gibco) (0.2 mg/ml).
21. E. B. Ziff, personal communication.
22. The RNAs transcribed in vitro from the pGEM constructs by T7 or SP6 polymerase were translated with pretreated rabbit reticulocyte lysates (Promega).
23. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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25. For immunofluorescence studies, transfected COS-7 cells were stained as described (*16*). In double immunofluorescence, the mouse mono-

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-

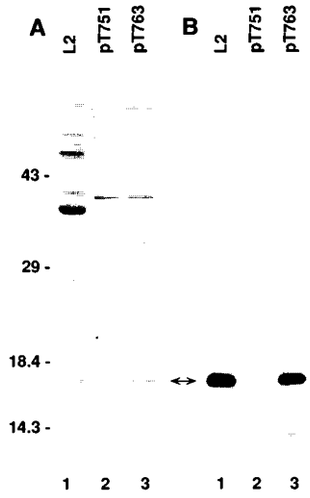

Fig. 1. Full-length expression of chlamydial Hc1 in *E. coli*. (**A**) A 12.5% polyacrylamide gel analysis of the cloned gene, stained with Coomassie brilliant blue. The *C. trachomatis* L2 lysate (lane 1) was prepared from purified EBs (*32*). Lanes 2 and 3 (pT751 and pT763) show nonexpressing and expressing 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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