tion in *hsp70* RNA when FACT or Spt6 protein levels are depleted a few fold by RNA interference (RNAi) treatments of Kc cells, these proteins are abundant and may be in excess, especially during heat shock when the general reduction of transcription of most genes presumably increases the availability of elongation factors (*16*).

Another factor implicated in the control of transcription through chromatin, Spt5, is also recruited to *hsp70* upon heat shock and tracks along the gene with kinetics similar to Pol II, FACT, and Spt6. In contrast to FACT and Spt6, the level of Spt5 associated with *hsp70* is less at the 3′ end of the gene than at the leader region after a 5-min heat shock (Fig. 3E and Fig. 2). As we have shown previously (*14*), even before heat shock we detect a strong signal for Spt5 in the region of the paused polymerase (leader region). Spt5 is known to have a role with the NELF complex (*22*) in restricting Pol II's elongation early in the transcription cycle and in stimulating the mRNA 5′-capping machinery (*23*, *24*), activities that require its association with the leader region. Thus, in addition to a positive role in elongation (*14*, *15*, *25*), Spt5 appears to have a role that is both spatially and temporally separate from that of FACT and Spt6.

Coimmunoprecipitation in *Drosophila* nuclear extracts provides support for physical associations of Spt5, Spt6, FACT, and elongationally active Pol II (fig. S1). These results are consistent with those from yeast that support the idea that multiple Spt5 complexes exist, one of which is an elongation complex that includes Spt5, Spt6, and FACT (*26*). Other elongation factors, the Paf1 complex and the chromodomain adenosine triphosphatase (ATPase), Chd1, also show physical and genetic interactions with FACT (*27*, *28*), indicating that transcription elongation through chromatin in vivo involves a sophisticated molecular machine.

Nucleosome reassembly after transcription-induced disassembly is essential for the integrity of chromatin structure. A link between Spt6 and nucleosome reassembly is known (*13*), and recent genetic evidence suggests a similar role for FACT (*29*). Our data demonstrate that upon *hsp70* induction, FACT and Spt6 are strongly recruited to regions of *hsp70* occupied by nucleosomes. Spt6 has been shown to interact with H3 and H4 (*13*), and FACT with H2A and H2B (*11*). In the accompanying paper (*30*), it is shown that the SSRP1 subunit contacts the H3·H4 tetramer of the disassembled nucleosome, whilst Spt16 maintains interaction with the displaced H2A·H2B dimer. It is appealing to speculate that chromatin reassembly is facilitated by Spt6 stabilizing the nucleosomes via interaction with H3 and H4 and by FACT maintaining a stable interaction with both the remodeled nucleosome and the displaced H2A·H2B dimer (*29*, *31*). Whether disassembly or reassembly is the most critical function of FACT in vivo remains an intriguing question.

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33. Materials and Methods are available as supporting online material on *Science* Online.


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**Supporting Online Material**

www.sciencemag.org/cgi/content/full/301/5636/1094/DC1

Materials and Methods

Fig. S1

15 April 2003; accepted 3 July 2003

# Transcription Elongation Factors Repress Transcription Initiation from Cryptic Sites

**Craig D. Kaplan, Lisa Laprade, Fred Winston***

**Previous studies have suggested that transcription elongation results in changes in chromatin structure. Here we present studies of *Saccharomyces cerevisiae* Spt6, a conserved protein implicated in both transcription elongation and chromatin structure. Our results show that, surprisingly, an *spt6* mutant permits aberrant transcription initiation from within coding regions. Furthermore, transcribed chromatin in the *spt6* mutant is hypersensitive to micrococcal nuclease, and this hypersensitivity is suppressed by mutational inactivation of RNA polymerase II. These results suggest that Spt6 plays a critical role in maintaining normal chromatin structure during transcription elongation, thereby repressing transcription initiation from cryptic promoters. Other elongation and chromatin factors, including Spt16 and histone H3, appear to contribute to this control.**

Although the molecular function of Spt6 in vivo is not understood, evidence suggests that it is involved in transcription elongation, mRNA processing, and interaction with nucleosomes [reviewed in (*1*)]. The function of Spt6 seems to be closely related to that of Spt4 and Spt5 (*2*–*6*), and to Spt16, Spt10, Bur1, Bur2, and core histones (*1*, *7*–*14*).

To characterize the requirement for Spt6 in RNA polymerase (Pol) II transcription in *S. cerevisiae*, we performed microarray analysis of a temperature-sensitive *spt6* mutant, *spt6-1004*, and an isogenic wild-type strain [Materials and Methods, Supporting Online Material]. These experiments revealed a large number of mRNAs whose levels were altered in the *spt6-1004* mutant (*15*). For many of the genes whose expression appeared to be increased by the *spt6-1004* mutation, Northern analysis detected new transcripts of smaller size (Fig. 1, A and B). Using a combination of 5′ and 3′ specific probes, we determined that the shorter transcripts observed for at least two of these genes, *RAD18* and *FLO8*, correspond to the 3′ portions of the genes (Fig. 1C). Single-stranded probes showed that these shorter transcripts are on the same strand as the full-length transcripts (*15*).

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To examine this effect in greater detail, we focused on the short *FLO8* transcript. To map the 5′ end of the short *FLO8* transcript, we used primer extension and RACE (rapid amplification of cDNA ends) analysis [Fig. 2A and (*15*)]. By primer-extension analysis, we detected a cluster of 5′ ends for *FLO8* at +1679, +1684, and +1685 with respect to the *FLO8* ATG (Fig. 2A). These 5′ ends were the only ones observed by primer extension between positions +1500 and +1820. RACE analysis also identified 5′ ends in this region (*15*).

Several lines of evidence suggest that the *FLO8* short transcript arises from initiation by RNA Pol II rather than by processing of the wild-type *FLO8* mRNA. First, examination of the *FLO8* DNA sequence identified a consensus TATA sequence starting 54 base pairs 5′ of the +1679 site (Fig. 2A). In addition, our microarray analysis was performed with poly(A)$^+$ selected mRNA, and the RACE protocol relies on the presence of a 5′ methylated guanosine (5′-meG-cap) and 3′ poly(A) tail. Finally, we performed chromatin immunoprecipitation (ChIP) to examine the association of RNA Pol II and the TATA box–binding protein (TBP) near the *FLO8* putative internal initiation sites. The results showed a modest, but statistically significant, increase in the levels of both RNA Pol II and TBP association with the internal *FLO8* initiation region specifically in the *spt6-1004* mutant after incubation at nonpermissive temperature (Fig. 2B). In addition, we tested whether the production of the short *FLO8* transcript requires the internal TATAAA sequence at position +1626. Mutation of this TATAAA to CCTAGG abolishes production of the short transcript in an *spt6-1004* mutant (Fig. 2C). These data suggest that in *spt6-1004*, TBP binding to the TATAAA at +1626 allows aberrant transcription initiation within *FLO8*.

To determine whether these aberrant transcripts were specific to *spt6* mutants, we tested other mutants for production of the short *FLO8* transcript. We examined several mutants that have been shown to allow activation of the *S. cerevisiae SUC2* promoter lacking its upstream activation sequence (UAS) region (*SUC2ΔUAS*) (*11*), as both production of the *FLO8* short transcript and expression of *SUC2ΔUAS* appear to be cases of expression from minimal promoters. Northern analysis shows that mutations in *SPT10*, *SPT21*, *SPT16*, *BUR1*, *BUR2*, and the histone H3-encoding gene *HHT1* cause production of the short *FLO8* transcript (Fig. 3). In contrast, mutations in *SPT4*, *SPT5*, *MOT1*, and *BUR6* do not [Fig. 3 and (*15*)].

Because Spt6 has been shown to interact with histones (*16*, *17*) and *spt6* mutations have been shown to affect chromatin structure (*16*), we examined whether *spt6-1004* causes an alteration in chromatin structure at the nonpermissive temperature, coincident with cryptic promoter usage. To do this, we assayed chromatin from an *spt6-1004* strain for sensitivity to micrococcal nuclease (MNase) when incubated at nonpermissive temperature. The results show that *spt6-1004* chromatin exhibits an increased sensitivity to MNase compared with *SPT6* chromatin (Fig. 4A). This increased MNase sensitivity is similar to that observed in previous studies when histones were depleted (*18*–*20*).

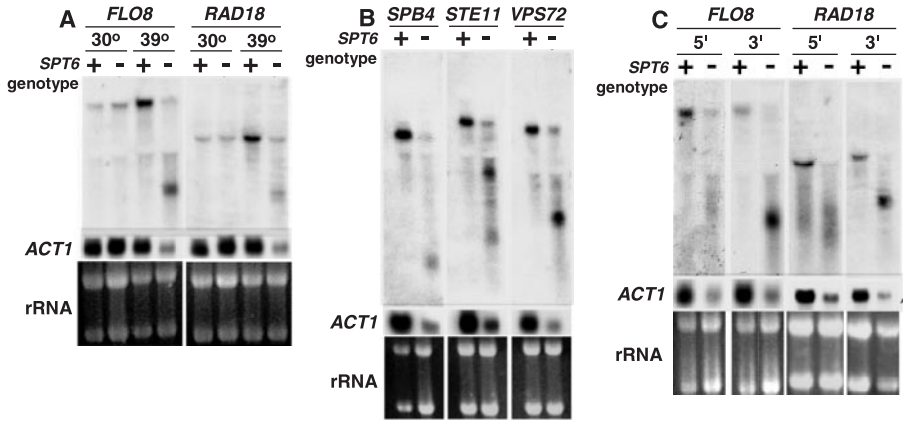


**Fig. 1.** Production of aberrant transcripts in *spt6-1004* at 39°C. (**A**) Total RNA from wild-type or *spt6-1004* strains, grown at 30°C or after an 80-min shift to 39°C, was analyzed by Northern blot for *FLO8* and *RAD18* RNAs. Ribosomal RNA (rRNA) is shown as a control for the amount of RNA in each lane. *ACT1* mRNA levels are reproducibly decreased in *spt6-1004*, but *ACT1* does not exhibit shorter transcripts (*15*); therefore, *ACT1* serves as an internal control for an *spt6-1004* mutant phenotype. (**B**) Northern analysis for *SPB4*, *STE11*, and *VPS72* in strains grown at the nonpermissive temperature (39°C). (**C**) Northern analysis of *FLO8* and *RAD18* with probes specific for the 5′ and 3′ coding regions of each gene.

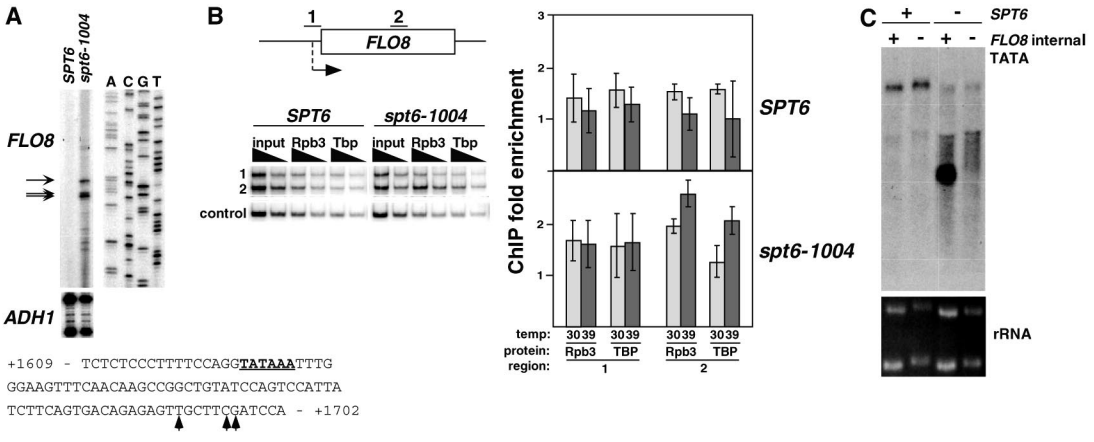


**Fig. 2.** Characterization of the *FLO8* short transcript. (**A**) Primer extension of the *FLO8* short transcript was performed to detect *FLO8* transcripts with 5′ ends in the coding region. The level of *ADH1* mRNA was measured as a control for the amounts of RNA used for the *SPT6* and *spt6-1004* strains. The *FLO8* coding sequence that contains the internal initiation site is shown below the gels. The 5′ ends found by primer-extension analysis are indicated by the arrows. The consensus TATA box is underlined. (**B**) ChIP analysis of the *FLO8* internal promoter. ChIP analysis was performed for TBP and RNA Pol II. A schematic of *FLO8* is shown, with the black bars representing the regions amplified by polymerase chain reaction (PCR). The control region is on chromosome V, in a region lacking open reading frames (*32*). The graphs represent the ratio [(ChIP region *n*/ChIP control) ±SD of three independent experiments (30°C) or six independent experiments (39°C)]. Student's *t* test (two-tailed, equal variance) *P* values for *spt6-1004* ChIPs for TBP and Rpb3 at 39°C, relative to ChIP values in *SPT6* at 30°C or 39°C or *spt6-1004* at 30°C, are all <0.006. (**C**) Northern analysis of a *flo8* internal TATA mutation. The *flo8-100* mutation changes the internal *FLO8* TATA sequence at position +1626 from TATAAA to CCTAGG.

Spt6 has been shown to be physically associated with transcribed regions (*3*–*5*, *13*, *15*). Furthermore, it has been proposed that Spt6 facilitates RNA Pol II passage through nucleosomes or restores normal chromatin structure in the wake of RNA Pol II transcription (*2*). Therefore, we tested whether transcription is required to cause the increased MNase sensitivity observed in *spt6-1004* mutants. First, we examined the MNase sensitivity of *FLO8* and an untranscribed gene, *GAL1*. Second, we constructed double mutants containing *spt6-1004* and *rpb1-1*, a temperature-sensitive mutation in the gene encoding the largest subunit of RNA Pol II. After a shift to the nonpermissive temperature, the *rpb1-1* mutation causes a rapid shutoff of transcription (*21*). Our results (Fig. 4B) show that the *spt6-1004*–dependent MNase hypersensitivity is severe over *FLO8*, but it does not significantly occur over the repressed *GAL1* gene. Furthermore, the hypersensitivity is suppressed by the *rpb1-1* mutation, evident both in total chromatin (Fig. 4B, right panel) and at *FLO8* (Fig. 4B, left panel). The *rpb1-1* mutation alone causes no detectable effect on MNase sensitivity (Fig. 4A). In contrast, inhibition of translation by cycloheximide has no effect on MNase sensitivity in an *spt6-1004* background (Materials and Methods; fig. S1). These results are consistent with the hypothesis that the increased MNase sensitivity observed in the *spt6-1004* mutant is dependent on RNA Pol II transcription.

The MNase hypersensitivity in an *spt6-1004* mutant could be caused by either loss of nucleosomes or an altered nucleosome structure. To distinguish between these possibilities, we measured the level of histone H4 at *FLO8* by ChIP in wild-type and *spt6-1004* strains. Our results demonstrate that the level of H4 is significantly reduced over *FLO8* specifically in the *spt6-1004* mutant (Fig. 4C, compare lanes 3 and 4 to 7 and 8). Furthermore, this loss does not occur at *GAL1*, a sequence that is not transcribed.

Our results show that the elongation factor Spt6 is required to repress transcription initiation from a cryptic promoter within *FLO8* and that such repression likely occurs at cryptic promoters throughout the genome. These findings support a model in which Spt6 promotes the restoration of normal chromatin structure in the wake of RNA Pol II elongation (*2*, *22*). By this model, in an *spt6* mutant, RNA Pol II elongation causes a global disruption of chromatin structure, creating a permissive chromatin environment for transcription initiation from cryptic sites that fortuitously contain a TATA element and proximal initiation site. Other investigators have also proposed that the passage of RNA Pol II alters chromatin structure [reviewed in (*1*, *23*–*26*)]. Our model is also analogous to that in studies demonstrating that intergenic transcription in the human β-globin locus remodels chromatin to allow gene expression (*27*).

Our model for transcription from cryptic initiation sites can be applied to previously observed phenotypes for *spt6* mutants. First, the initiation from cryptic promoters within coding regions is similar to the previous demonstration that mutations in *SPT6* and several other genes

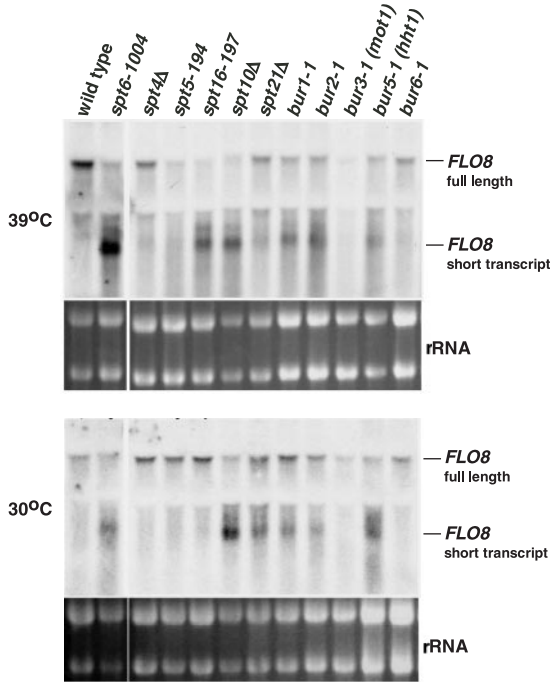


**Fig. 3.** Northern analysis of *FLO8* transcripts in mutant strains related to *spt6*. The top panel shows RNA prepared from strains incubated at 39°C for 80 min after growth at 30°C and the bottom panel is RNA from strains grown at 30°C. For the *spt6-1004* and *spt16-197* temperature-sensitive mutants, the level of the short *FLO8* transcript is greater at 39°C.

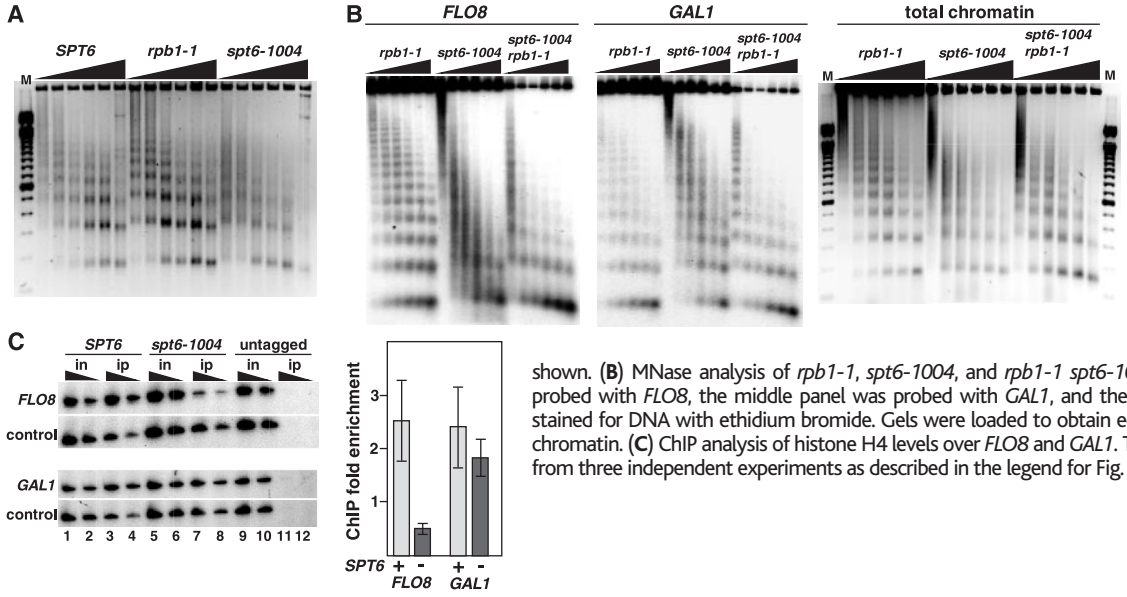


**Fig. 4.** MNase and ChIP analysis. Cells were grown at 30°C, shifted to 39°C, and samples were prepared as described in Materials and Methods and table S1. The undigested DNA is present owing to unspheroplasted cells that are inaccessible to MNase. (**A**) MNase analysis of total chromatin from *SPT6*, *rpb1-1*, and *spt6-1004* strains. Inverse images of agarose gels stained for DNA with ethidium bromide are shown. (**B**) MNase analysis of *rpb1-1*, *spt6-1004*, and *rpb1-1 spt6-1004* strains. The left panel was probed with *FLO8*, the middle panel was probed with *GAL1*, and the right panel is total chromatin, stained for DNA with ethidium bromide. Gels were loaded to obtain equal levels of MNase-accessible chromatin. (**C**) ChIP analysis of histone H4 levels over *FLO8* and *GAL1*. The values shown are calculated from three independent experiments as described in the legend for Fig. 2B. M, molecular mass markers.

allow transcription initiation from a yeast promoter lacking its UAS (*6*, *11*, *28*, *29*). Our model may also explain the isolation of mutations that impair transcription elongation factors, including Spt6, as suppressors of a transcription initiation defect of *his4-912*δ, an insertion of the long terminal repeat of a Ty retrotransposon in the *HIS4* promoter (*30*, *31*). Similar to transcription initiation from within coding regions, suppression of *his4-912*δ may also be a case of promoter activation by transcription elongation–mediated chromatin changes that occur in *spt6* mutants.

Our results indicate that chromatin-mediated repression of promoter usage operates on a scale larger than previously thought and that RNA Pol II and transcription elongation factors play important roles in determining chromatin structure within transcribed regions. By maintaining a specific chromatin structure over transcribed regions, RNA Pol II, Spt6, and other factors prevent improper initiation that would be harmful to normal gene expression. Although our study was performed exclusively in *S. cerevisiae*, prevention of such aberrant transcription is likely to be at least as important in larger eukaryotes with more complex genomes.


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Materials and Methods
Fig. S1
References

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# *Helicobacter pylori* Vacuolating Cytotoxin Inhibits T Lymphocyte Activation


**Bettina Gebert, Wolfgang Fischer, Evelyn Weiss, Reinhard Hoffmann, Rainer Haas***



***Helicobacter pylori* (*Hp*) vacuolating cytotoxin VacA induces cellular vacuolation in epithelial cells. We found that VacA could efficiently block proliferation of T cells by inducing a $G_1$/S cell cycle arrest. It interfered with the T cell receptor/interleukin-2 (IL-2) signaling pathway at the level of the $Ca^{2+}$-calmodulin–dependent phosphatase calcineurin. Nuclear translocation of nuclear factor of activated T cells (NFAT), a transcription factor acting as a global regulator of immune response genes, was abrogated, resulting in down-regulation of IL-2 transcription. VacA partially mimicked the activity of the immunosuppressive drug FK506 by possibly inducing a local immune suppression, explaining the extraordinary chronicity of *Hp* infections.**


*Helicobacter pylori* (*Hp*) causes chronic gastritis, peptic ulcer disease, and is an early risk factor for gastric cancer (*1*). To establish a chronic infection, *Hp* possibly evades host responses through the inhibition of antigen-specific T cell proliferation (*2*). Major histocompatibility complex (MHC) class II–restricted, cell-mediated mechanisms control *Hp* infection (*3*), and adoptive transfer of $CD4^+$ T cells demonstrated a protective role of T cells (*4*). Gastroduodenal disease depends on the expression of major bacterial virulence factors, such as the vacuolating cytotoxin (*vacA*) or the *cag* pathogenicity island (*cag*-PAI). The *cag*-PAI encodes a type IV secretion system, injecting the bacterial protein CagA into gastric epithelial cells, or professional phagocytes (*5*, *6*). CagA eventually becomes tyrosine-phosphorylated and activates Ras signaling via the Raf/Mek/Erk stress kinase pathway to cause epithelial cell proliferation and scattering (*7*). VacA, a secreted 95 kD protein, varies in the signal sequence (s1a, s1b, s1c, s2) and/or its middle region (m1, m2) between different *Hp* strains (*8*). Among other functions, VacA selectively inhibits the invariant chain (Ii)–dependent pathway of antigen presentation mediated by the MHC class II (*9*) and might induce apoptosis in epithelial cells (*10*).

To investigate *Hp*–T cell interactions, Jurkat T cells were infected with different *Hp* wild-type (wt) strains and isogenic mutants lacking *cagA*, *vacA*, or both genes (table S1). A stimulation of bacteria-infected T cells [multiplicity of infection (MOI) of 30] with phytohemagglutinin/phorbol myristate acetate (PHA/PMA) resulted in a strong reduction of Jurkat T cell proliferation (40 to 60%), compared with the stimulated but uninfected control (Fig. 1A). Also *Campylobacter jejuni* C64, producing the cytolethal distending toxin (CDT) known to inhibit cell cycle progression in epithelial cells (*11*), caused a pronounced proliferation inhibition. But neither *C. jejuni* C63, with a defective *cdt* operon, nor *Escherichia coli* HB101 showed comparable effects (Fig. 1A). Fresh human peripheral blood lymphocytes (PBLCs) reacted even more dramatically. Activated *Hp*-infected PBLCs were reduced in their proliferation to the level of resting (nonstimulated) PBLCs (Fig. 1B). Use of $CD4^+$ T cell subpopulations purified from PBLC resulted in similar data.

Preparation of supernatants [concentrated bacterial culture supernatant (CCS)] from genetically defined isogenic *Hp* strains producing ($CCS^+$) or not producing VacA ($CCS^-$) (Fig. 1,


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