**Epigenetic Reprogramming by Adenovirus e1a**
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# Epigenetic Reprogramming by Adenovirus e1a

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Adenovirus e1a induces quiescent human cells to replicate. We found that e1a causes global relocalization of the RB (retinoblastoma) proteins (RB, p130, and p107) and p300/CBP histone acetyltransferases on promoters, the effect of which is to restrict the acetylation of histone 3 lysine-18 (H3K18ac) to a limited set of genes, thereby stimulating cell cycling and inhibiting antiviral responses and cellular differentiation. Soon after expression, e1a binds transiently to promoters of cell cycle and growth genes, causing enrichment of p300/CBP, PCAF (p300/CBP-associated factor), and H3K18ac; depletion of RB proteins; and transcriptional activation. e1a also associates transiently with promoters of antiviral genes, causing enrichment for RB, p130, and H4K16ac; increased nucleosome density; and transcriptional repression. At later times, e1a and p107 bind mainly to promoters of development and differentiation genes, repressing transcription. The temporal order of e1a binding requires its interactions with p300/CBP and RB proteins. Our data uncover a defined epigenetic reprogramming leading to cellular transformation.

The adenovirus small e1a oncoprotein interacts with multiple cellular factors to induce cell cycling in $G_0$-arrested cells to favor viral replication. Mutations of e1a regions that interact with the RB proteins or p300/CBP [cyclic adenosine monophosphate response element–binding protein (CREB)–binding protein] result in loss of e1a-transforming and mitogenic activities (*1*–*3*) (figs. S1 and S2). Binding of e1a to p300/CBP inhibits transcriptional activation by certain enhancers (*4*); however, it is unclear how this interaction promotes cell cycling and why it is required for e1a oncogenicity. The e1a-p300/CBP interaction causes a factor of ~3 reduction in total cellular histone 3 $Lys^{18}$ acetylation (H3K18ac) specifically (*5*). Therefore, we sought to determine how e1a affects the genome-wide distributions of its interacting cellular factors as well as histone modifications (including H3K18ac) to establish an oncogenic gene expression program.

Using chromatin immunoprecipitation (ChIP) combined with microarrays (*6*), we examined the genome-wide binding of e1a at 2, 6, 12, and 24 hours (here and below, all times are post-infection) of confluent, contact-inhibited human IMR90 primary fibroblasts (ATCC CCL-186) in which e1a induces entry into S phase between 18 and 24 hours (fig. S2). We used an Agilent microarray containing probes for ~17,000 promoters, tiling an 8-kb region, which we divided computationally into 16 fragments of 500 base pairs (bp) each, spanning –5.5 to +2.5 kb of the transcription start site (TSS). Cells were infected with Ad5 mutant *dl*1500, which expresses only the small e1a protein (*7*). Using unbiased partitional clustering, we grouped the genes primarily into three clusters that captured the main trends in the data. We calculated a $Z$ score to indicate the degree of enrichment for a given factor in each cluster.

During the 24-hour period after expression, at a cutoff of $Z \geq 2$, e1a bound to ~70% (9753) of the examined promoters in a temporal manner (Fig. 1A and fig. S3). Both cluster 1 (2414 genes) and cluster 2 (4052 genes) were enriched for e1a binding at 2 and 6 hours but became progressively depleted for e1a by 24 hours. Such transient e1a binding was observed earlier for the *Cdc6* gene (cluster 2) (*8*). Cluster 3 (7326 genes) showed relative lack of e1a binding at 2 and 6 hours but significant enrichment at 24 hours. The e1a-binding patterns at 6 and 24 hours were essentially opposite to each other (Pearson correlation $r$ for average e1a binding = –0.46). Small e1a binding at 12 hours exhibited a transition-like state between early and late times, correlating at –0.28 and +0.21 with binding at 6 and 24 hours, respectively. Cluster 1 was enriched for genes involved in responses to pathogens and inflammation; cluster 2 for genes involved in cell growth, division, and DNA synthesis; and cluster 3 for development/differentiation, including homeobox domain–containing genes and cell-cell signaling (figs. S3 and S4).

Because e1a directly displaces the RB proteins from E2F transcription factors (*1*), we determined the average levels of e1a binding across promoter regions bound by E2Fs as determined in other cell lines (*9*). E2F-target genes were greatly enriched in cluster 2 (fig. S5), as were consensus E2F binding sites (*10*) (fig. S6), and were bound by e1a predominantly within 2 kb of the TSS at 6 hours but not at 24 hours (fig. S5, A to C). Thus, E2F–RB protein complexes may help e1a target the promoters of cell cycle genes early after infection.

To determine how e1a affects gene expression, we compared the expression profile of *dl*1500-infected to mock-infected confluent cells at 6, 12, and 24 hours. Cluster 1 genes were activated at 6 hours, consistent with a cellular response to viral infection, but were considerably

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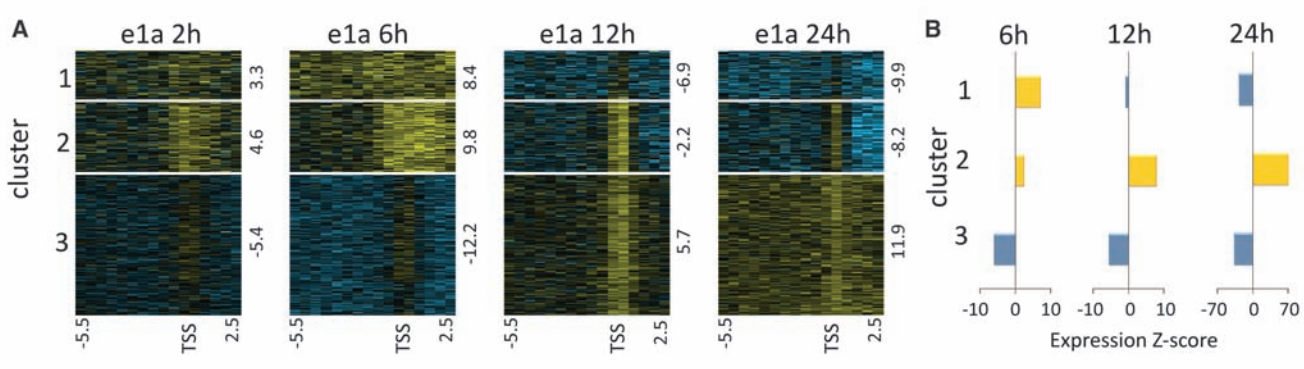


**Fig. 1.** Temporally ordered pattern of e1a binding reprograms host cell gene expression profile. (**A**) Time course of e1a genome-wide localization in IMR90 fibroblasts. Each row represents the promoter of a gene in 500-bp intervals from –5.5 to +2.5 kb of the transcription start site (TSS). Enrichment $Z$ scores are indicated to the right of each cluster. (**B**) Relative gene expression changes of the three clusters at 6, 12, and 24 hours after e1a expression (note the scale).

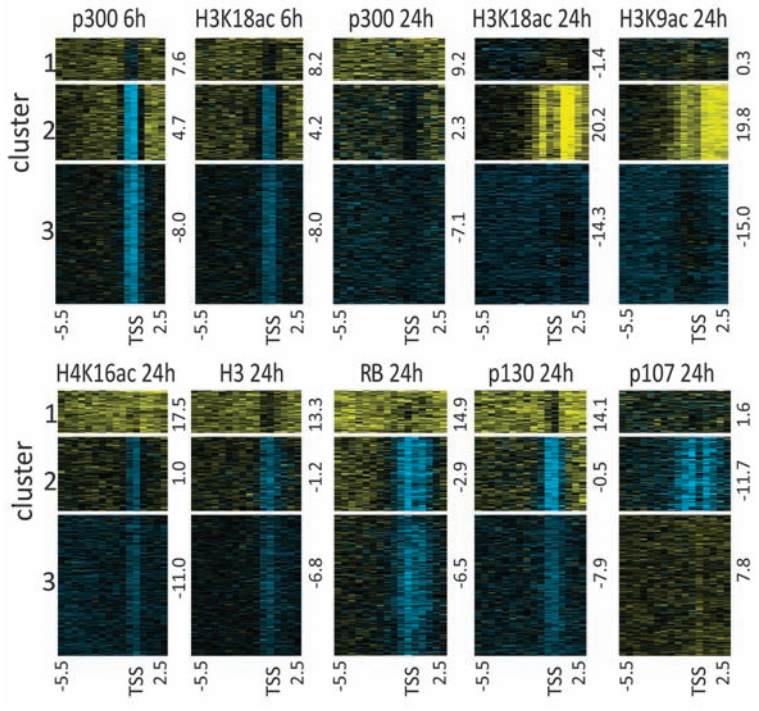


**Fig. 2.** Redistribution of transcriptional co-regulators and epigenetic reprogramming by e1a. The genome-wide distributions of p300, H3K18ac, H3K9ac, H4K16ac, histone H3, RB, p130, and p107 in the three e1a-binding clusters at the indicated times after e1a expression are shown.

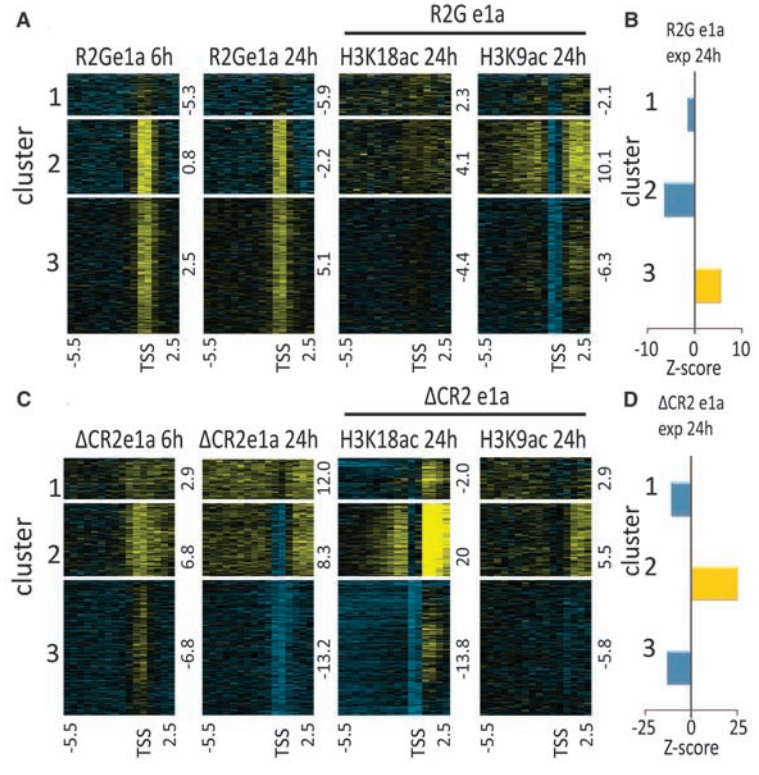


**Fig. 3.** Temporally ordered target gene selection and proper binding by e1a requires interactions with p300/CBP and the RB proteins. (**A** and **C**) Time course of R2Ge1a and ΔCR2e1a genome-wide localization in IMR90 fibroblasts in the three WTe1a-binding clusters (Fig. 1A). Also shown are the distribution and enrichment *Z* scores of H3K9ac and H3K18ac in R2Ge1a- or ΔCR2e1a-infected versus mock-infected cells. (**B** and **D**) Relative expression levels of the three clusters in each mutant 24 hours after e1a expression.

repressed by 24 hours (Fig. 1B; note the scales). Cluster 2 and 3 genes were progressively induced and repressed by 24 hours, respectively. The 24-hour expression profile was very similar to that induced by wild-type Ad5 (*11*), which suggests that small e1a induces most of the changes in host cell gene expression. Therefore, our data indicate that early e1a binding leads to activation of genes involved in cell cycling and proliferation and repression of antiviral response genes by 24 hours. Late e1a binding results in repression of development, differentiation, and cell-cell signaling genes.

We next analyzed H3K18ac. Relative to results from mock-infected cells, H3K18ac antibody ChIP yielded about one-third as much DNA from *dl*1500-infected cells, consistent with e1a-induced global H3K18 hypoacetylation (*5*) (fig. S7); we used equal amounts of ChIPed DNA for microarray analyses. Clusters 1 and 2 were enriched for H3K18ac at 6 hours mainly in regions away from the TSS, but only cluster 2 genes retained significant H3K18ac by 24 hours (Fig. 2). Cluster 3 genes were depleted or showed little enrichment for H3K18ac at 6 and 24 hours (Fig. 2). Therefore, e1a induces global H3K18 hypoacetylation, whereas the remaining H3K18ac at the molecular level is associated with a limited but biologically related set of genes with subsequent activation of their expression. The distribution of H3K9ac was similar to that of H3K18ac; by contrast, H4K16ac and histone H3 (irrespective of modifications) were enriched in cluster 1 at 24 hours and were specifically depleted around the TSS of cluster 2 and 3 genes (Fig. 2).

The e1a N terminus and conserved region 1 (CR1) directly bind p300/CBP (*2*); therefore, we asked whether these histone acetyltransferases and PCAF (p300/CBP-associated factor) were present at the e1a-target genes. p300 associated with genes in clusters 1 and 2, but at 6 and 24 hours p300 was significantly depleted from cluster 3 (Fig. 2). PCAF, and to lesser extent CBP, were also enriched in clusters 1 and 2 at 24 hours (fig. S8). The binding of p300 showed significant overlaps with H3K18ac at 6 hours in clusters 1 and 2, but only cluster 2 genes maintained high levels of H3K18ac at 24 hours (Fig. 2). In cluster 1, despite binding of p300/CBP/PCAF, H3K18ac was reduced and the genes were repressed at 24 hours. Repression of cluster 1 may be due to association of RB and p130 repressive complexes (see below) and/or e1a inhibition of p300/CBP activity at these genes (*4*). Note that both e1a and p300 bound to cluster 1 and 2 genes at 6 hours, whereas p300 remained at these promoters at 24 hours when e1a was depleted from them. This may involve continued recruitment of p300 by other transcription factors and/or direct binding to acetylated histones through the p300/CBP bromodomains (*12*).

In wild-type e1a (WTe1a)–expressing cells, p107 mRNA and protein increased at 24 hours, whereas expression of RB and p130 remained

unchanged (fig. S9). To determine whether the RB proteins contribute to e1a-mediated gene repression, we mapped genome-wide binding patterns of the three RB proteins at 24 hours in *dl*1500-infected versus mock-infected cells. RB and p130 were significantly enriched in cluster 1, consistent with repression of these genes at 24 hours (Fig. 2). RB, p130, and especially p107 were significantly depleted from the TSS regions of genes in cluster 2, perhaps because of WTe1a's ability to displace the RB proteins from E2Fs (*1*, *13*). p107 bound significantly to promoter regions of cluster 3 genes that were also bound by e1a at 24 hours and repressed. Thus, e1a probably uses RB and p130 to repress transcription of antiviral response genes, and uses p107 for repression of genes that would otherwise promote cellular differentiation and inhibit cell cycling.

Next, we analyzed gene expression, histone modifications, and e1a binding after expression of two e1a mutants. The $Arg^2 \rightarrow$ Gly (R2Ge1a) mutation disrupts the e1a-p300/CBP interaction, whereas deletion of CR2 (ΔCR2e1a) abolishes the high-affinity e1a interaction with RB proteins, but not the weaker e1a CR1–RB interactions (*1*). The R2G mutation greatly reduced binding of e1a to cluster 1 and to regions of cluster 2 genes away from the TSS (Fig. 3A), regions bound by p300 at 6 hours in WTe1a-expressing cells. In contrast to WTe1a, R2Ge1a also bound to development and differentiation genes at 6 hours (mainly around the TSS), and the binding patterns at 6 and 24 hours were similar to each other ($r = 0.44$; Fig. 3A). At E2F-target genes, R2Ge1a binding also remained unchanged between the time points (fig. S5). R2Ge1a induced only very slight enrichment of p300 or H3K18ac at the cluster 1 and 2 genes (Fig. 3A and fig. S10), but the H3K9ac pattern was similar to that induced by WTe1a, except that there was deacetylation at the TSS (Fig. 3A). R2Ge1a did not induce the gene expression changes observed with WTe1a and could not significantly suppress expression of antiviral genes (cluster 1, Fig. 3B and fig. S11). Thus, the e1a-p300/CBP interaction is required for the proper targeting and temporal order of e1a binding, the redistribution of p300 and H3K18ac, and e1a-regulated gene induction and repression.

ΔCR2e1a bound significantly to cluster 1 and 2 genes, including E2F-target genes at 6 hours, despite the CR2 deletion (Fig. 3C and fig. S5). However, in contrast to WTe1a, at 24 hours, ΔCR2e1a remained broadly associated with the cluster 1 and 2 promoter regions, except around the TSS of cluster 2 and 3 genes. ΔCR2e1a did not exhibit a temporally ordered pattern of binding; 6-hour and 24-hour binding patterns were similar ($r = 0.63$). These results suggest that e1a regions other than CR2 contribute to initial targeting of e1a to cell cycle and growth genes, but that CR2 is required for retention of WTe1a at the TSS of cluster 2 and 3 genes and its redistribution from clusters 1 and 2 to cluster 3 genes. In ΔCR2e1a-expressing cells, H3K18ac distribution was similar to that induced by WTe1a, but H3K9 showed much less hyperacetylation in cluster 2 and less hypoacetylation in cluster 1 relative to WTe1a or R2Ge1a (Fig. 3C). Because ΔCR2e1a cannot displace RB proteins from E2Fs (*1*), these results suggest that displacement of RB proteins and their associated histone deacetylases (*2*) from cluster 2 genes, and their transfer to cluster 1 genes, may contribute to changes in H3K9ac. The expression profile induced by ΔCR2e1a at 24 hours was similar to WTe1a ($r = 0.69$; fig. S11), except that the amplitude of gene induction and repression in the three clusters was decreased (Fig. 3D), particularly for certain critical genes (fig. S12); this probably explains ΔCR2e1a's lack of mitogenic activity. The data from the R2G and ΔCR2 mutants indicate that hyperacetylation of H3K18ac is required for transcriptional induction and relies on e1a regions necessary for interaction with p300/CBP, whereas changes in H3K9ac induced by both WTe1a and R2Ge1a are insufficient to induce transcriptional changes and depend on the high-affinity e1a–RB protein interactions.

By binding to the promoters of a large number of genes in a precise, time-dependent manner, e1a orchestrates redistribution of specific transcriptional co-regulators with associated epigenetic activities to promote S-phase entry and active repression of differentiation (fig. S13). Transcriptional reprogramming through use of epigenetic modifiers may have parallels in nonviral mechanisms of oncogenesis (*3*).


**References and Notes**

1. X. Liu, R. Marmorstein, *Genes Dev.* **21**, 2711 (2007).
2. A. J. Berk, *Oncogene* **24**, 7673 (2005).
3. See supporting material on *Science* Online.
4. D. Chakravarti *et al.*, *Cell* **96**, 393 (1999).
5. G. A. Horwitz *et al.*, *Science* **321**, 1084 (2008).
6. B. Ren *et al.*, *Science* **290**, 2306 (2000).
7. C. Montell, G. Courtois, C. Eng, A. Berk, *Cell* **36**, 951 (1984).
8. M. K. Ghosh, M. L. Harter, *Mol. Cell* **12**, 255 (2003).
9. X. Xu *et al.*, *Genome Res.* **17**, 1550 (2007).
10. O. Elemento, N. Slonim, S. Tavazoie, *Mol. Cell* **28**, 337 (2007).
11. D. L. Miller, C. L. Myers, B. Rickards, H. A. Coller, S. J. Flint, *Genome Biol.* **8**, R58 (2007).
12. S. Mujtaba, L. Zeng, M. M. Zhou, *Oncogene* **26**, 5521 (2007).
13. J. R. Nevins, *Curr. Opin. Genet. Dev.* **4**, 130 (1994).



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**Supporting Online Material**

www.sciencemag.org/cgi/content/full/321/5892/1086/DC1
Materials and Methods
SOM Text
Figs. S1 to S13
Table S1
References


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# Heterochromatin Integrity Affects Chromosome Reorganization After Centromere Dysfunction


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The centromere is essential for the inheritance of genetic information on eukaryotic chromosomes. Epigenetic regulation of centromere identity has been implicated in genome stability, karyotype evolution, and speciation. However, little is known regarding the manner in which centromere dysfunction affects the chromosomal architectures. Here we show that in the fission yeast *Schizosaccharomyces pombe*, the conditional deletion of the centromere produces survivors that carry either a neocentromere-acquired chromosome at the subtelomeric region or an acentric chromosome rescued by intertelomere fusion with either of the remaining chromosomes. The ratio of neocentromere formation to telomere fusion is considerably decreased by the inactivation of genes involved in RNA interference–dependent heterochromatin formation. By affecting the modes of chromosomal reorganization, the genomic distribution of heterochromatin may influence the fate of karyotype evolution.


The stable maintenance and propagation of linear eukaryotic chromosomes during cell division requires two specialized chromosomal structures: telomeres and centromeres. Telomeres protect the ends of linear chromosomes and prevent their fusion (*1*), whereas centromeres are essential for equal chromosome separation during M phase (*2*). The centromeric DNA sequence by itself cannot specify centromere identity, and instead epigenetic regulation plays a dominant role in most eukaryotes (*2*–*5*). However, when an epigenetically marked authentic centromere becomes unavailable, the type of molecular components that contribute to recruit kinetochore proteins such as CENP-A (a centromeric histone H3 variant) onto a new position (the neocentromere locus) remains