Dynamic bookmarking of primary response genes by p300 and RNA polymerase II complexes

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Profiling the dynamic interaction of p300 with proximal promoters of human T cells identified a class of genes that rapidly coassemble p300 and RNA polymerase II (pol II) following mitogen stimulation. Several of these p300 targets are immediate early genes, including *FOS*, implicating a prominent role for p300 in the control of primary genetic responses. The recruitment of p300 and pol II rapidly transitions to the assembly of several elongation factors, including the positive transcriptional elongation factor (P-TEFb), the bromodomain-containing protein (BRD4), and the elongin-like eleven nineteen lysine-rich leukemia protein (ELL). However, transcription at many of these rapidly induced genes is transient, wherein swift departure of P-TEFb, BRD4, and ELL coincides with termination of transcriptional elongation. Unexpectedly, both p300 and pol II remain accumulated or ''bookmarked'' at the proximal promoter long after transcription has terminated, demarking a clear mechanistic separation between the recruitment and elongation phases of transcription in vivo. The bookmarked pol II is depleted of both serine-2 and serine-5 phosphorylation of its C-terminal domain and remains proximally positioned at the promoter for hours. Surprisingly, these p300/pol II bookmarked genes can be readily reactivated, and elongation factors can be reassembled by subsequent addition of nonmitogenic agents that, alone, have minimal effects on transcription in the absence of prior preconditioning by mitogen stimulation. These findings suggest that p300 is likely to play an important role in biological processes in which transcriptional bookmarking or preconditioning influences cellular growth and development through the dynamic priming of genes for response to rechallenge by secondary stimuli.

gene regulation | histone acetylation | transcription | ELL | epigenetics

The capacity to make rapid adjustments in transcriptional control in response to environmental change is critical to the survival of complex organisms. To accomplish this, an elaborate molecular hierarchy has evolved in metazoan cells to assemble the transcriptional machinery rapidly at genes targeted for activation or repression (1, 2). The histone acetyl-transferase p300 and its paralog, CREB binding protein (CBP), play prominent roles in this hierarchy by modifying chromatin structure through histone acetylation and bridging interactions between DNA binding proteins and components of the RNA polymerase II (pol II) transcriptional machinery (3). This is accomplished via multiple overlapping and nonoverlapping protein interaction domains that distinguish p300 and CBP from other histone acetyl-transferases by enabling them to interact with over 300 different factors that influence transcription (4). p300 is therefore likely to play a prominent role as a molecular hub capable of regulating multiple different genes and gene networks.

Despite their capacity to interact physically with a broad range of different genes, the cellular levels of both p300 and CBP are limiting. Mice null for either p300 or CBP are nonviable, and haplosufficiency of either p300 or CBP causes defects in mice and humans characterized by developmental abnormalities (3). Therefore, p300/CBP must target select sets of genes under specific conditions. How and where this targeting occurs across the genome has yet to be fully investigated. In this study, we have combined quantitative (q) ChIP and microarray-based technologies to show that p300 and pol II complexes coassemble rapidly and dynamically at primary response genes to enhance their induction. We show that this process is controlled in vivo by discernible sequential steps in which p300/pol II recruitment and elongation factor assembly do not proceed through an obligatory transition but act instead through distinct and separable steps in which p300/pol II complexes are deposited and remain ''bookmarked'' at proximal promoters to seed or potentiate subsequent rounds of transcriptional initiation and elongation in response to diverse and distinct stimuli. These provocative findings define an essential role for p300 in primary genetic responses and elucidate a previously undescribed mechanism through which transcriptional activation states can be ''remembered'' and/or propagated within cellular populations via a dynamic bookmarking that preconditions genes for altered or preenabled responses to subsequent molecular events.

Results

p300 and pol II Coassemble at Rapidly Expressed Genes. Prior studies from our group showed that p300 associates rapidly with the promoters of several genes in Jurkat human T cells following mitogen stimulation with phorbol ester and ionomycin (P/I) (5, 6). We expanded this observation using ChIP-chip strategies and qChIP to profile genome-wide changes in promoter occupancy of p300 and pol II 45 min following mitogen stimulation (Fig. 1 *A* and *B*). p300 and pol II ChIP DNA from resting and P/I-treated cells was amplified, labeled, and hybridized to a proximal promoter array representing 37,364 promoters spanning −1,000 to +500 bp relative to the transcription start site (TSS). Promoters showing significant occupancy ($-\log_{10} P \geq 6.8$) by either p300 or pol II were identified. As shown in a graphic representation of these binding data (Fig. 1*A*), p300 undergoes a dramatic redistribution across the genome following mitogen stimulation. By hierarchical clustering, this redistribu-

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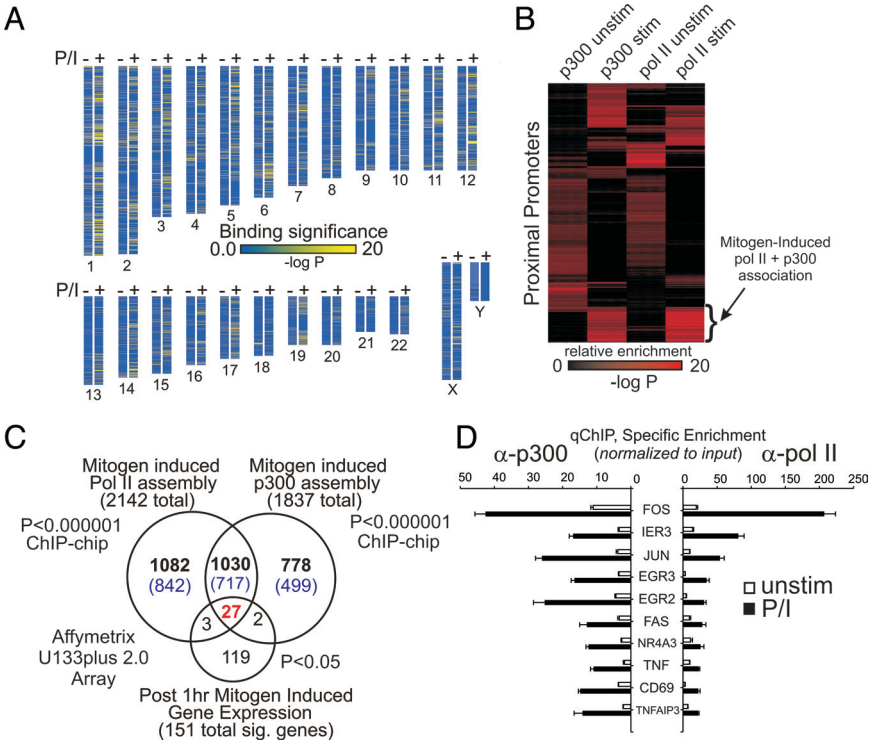

Fig. 1. Genome-wide profiling of p300 promoter occupancy reveals mitogen-induced association with rapidly expressed genes. (*A*) Genome-wide map of mitogen-induced association of p300 with human proximal promoters. Relative enrichment at genomic positions is indicated by yellow intensity in the presence (+) or absence (−) of mitogen. (*B*) Unsupervised hierarchical clustering (using Euclidian distance metric) of p300 and pol II ChIP-chip enrichment significance at the proximal promoters of human RefSeq genes before and after mitogen stimulation for 45 min. Relative enrichment (red) is derived from *P* values calculated for promoter association and expressed as $-\log_{10} P$. Data are the average of 2 biological replicates. (*C*) Venn diagram of overlap of genes that show p300/pol II coassembly following mitogen induction and significantly ($P < 0.05$) increased mRNA expression within 1 h after stimulation. Blue numbers in parentheses indicate the number of annotated genes. (*D*) ChIP-qPCR validation analysis of the association of 10 rapidly inducible genes, identified by ChIP-chip and gene expression data, with pol II and p300, respectively. Error bars represent SEM from 2 independent biological replicates, each measured in triplicate.

tion correlates with dynamic changes in the patterns of both pol II and p300 promoter co-occupancy (Fig. 1*B*).

Five major classes of promoter co-occupancy [supporting information (SI) Table S1*A*] were identified: (*i*) genes that show significant p300 occupancy in resting cells (p300-enriched, total = 2,188); (*ii*) genes that show p300 occupancy that increases in significance following mitogen induction (p300 inducibly enriched, total = 1,837); (*iii*) genes that show significant pol II occupancy in resting cells (pol II-enriched, total = 3,031); (*iv*) genes that show pol II occupancy that increases in significance following mitogen induction (pol II inducibly enriched, total = 2,142); and (*v*) genes that show both p300 and pol II occupancy that increases in significance following mitogen stimulation (p300 and pol II inducibly enriched, total = 1,057).

To define a gene set that had the strongest correlation between p300/pol II assembly and gene expression, we determined how many genes (744 annotated of the total 1,057) also showed rapidly increased RNA expression. Gene expression analysis of Jurkat T cells following mitogen (P/I) stimulation identified 151 annotated genes that increase significantly ($P \leq 0.05$) following 1 h of mitogen stimulation (Fig. 1*C* and Fig. S1). Twenty-seven of these genes are among the 744 annotated genes that rapidly coassembled p300 and pol II (Fig. 1*C*, Table S1*B*, Fig. S1 and S2). Interestingly, many of these rapidly expressed genes are classic primary response or "immediate early" genes, including *FOS*, *JUN*, and *EGR2*, suggesting a prominent role for p300 in rapid genetic responses (Fig. 1*D*, Table S1*B*, Fig. S1 and S2). Members of this class of 27 rapidly induced p300 targets were therefore selected for further study.

Bookmarking of Immediate Early Genes by p300/pol II Complexes. A common feature of many primary response genes, including *FOS,* is their rapid yet transient expression or "spike" kinetics following mitogen induction (7, 8). This is readily demonstrated in the 0–8 h RNA expression profiles of several p300 and pol II targeted genes following mitogen stimulation (Fig. 2*A*). Prior observation of the kinetics of mitogen-stimulated p300 assembly at rapidly induced genes using semiquantitative PCR showed dynamic changes as the promoter evolved over 15-min intervals within 1 h following mitogen activation, where p300 appears to leave the template shortly after transcriptional stimulation in some cases (6). To assess these dynamic changes over a longer time frame and at higher resolution, similar but more sensitive measurements were done using ChIP followed by quantitative real-time PCR. *FOS* was chosen as the prototype gene for study, and primers were designed to detect both mature (spliced) and nascent (unspliced) *FOS* RNA transcript profiles by qRT-PCR. For comparison, factor occupancy was profiled at locations upstream, near the promoter, and through the body of the gene (Fig. 2*B*). This allowed us to profile the in vivo coupling of active transcription with specific factor assembly events at the *FOS* locus. Unexpectedly, although ChIP analysis showed rapid recruitment of pol II to the promoter regions (−0.2 kB) within 30 min following mitogen stimulation, this assembly persisted for up to 8 h. In contrast, pol II transcriptional elongation, as reflected by its appearance at the 3′ end of the gene (+3.0 kB), was very transient, with a kinetic profile similar to that of mature and nascent *FOS* transcript production (Fig. 2 *A* and *B* and Fig. S3*A*). A kinetic overlay of factor assembly and transcription at the *FOS* locus (Fig. 2*C*) illustrates that although pol II assembly and elongation occur rapidly, this phase of transcription attenuates quickly within 2 h, leaving a large population of cells with polymerase accumulated at the proximal promoter in a potentiated or bookmarked state, possibly "primed" for response to subsequent signaling events (Fig. 2 *B* and *C*).

One major role of p300 is to increase the DNA accessibility for pol II and other factors by histone acetylation (3). To correlate changes in *FOS* expression with histone acetylation, the time- and location-dependent changes in the appearance of acetylated histone H3 (K9, K14) and histone H4 (K5, K8, K12, and K16) were profiled at the *FOS* gene. Although both histone H3 and histone H4 acetylation were rapidly induced at the proximal promoter following mitogen stimulation, these levels remained high for 8 h (Fig. 2 *D* and *E*). In a similar manner to pol II (Fig. 2 *B* and *C*), we found that the population of resting cells containing proximally accumulated p300 increases dramatically

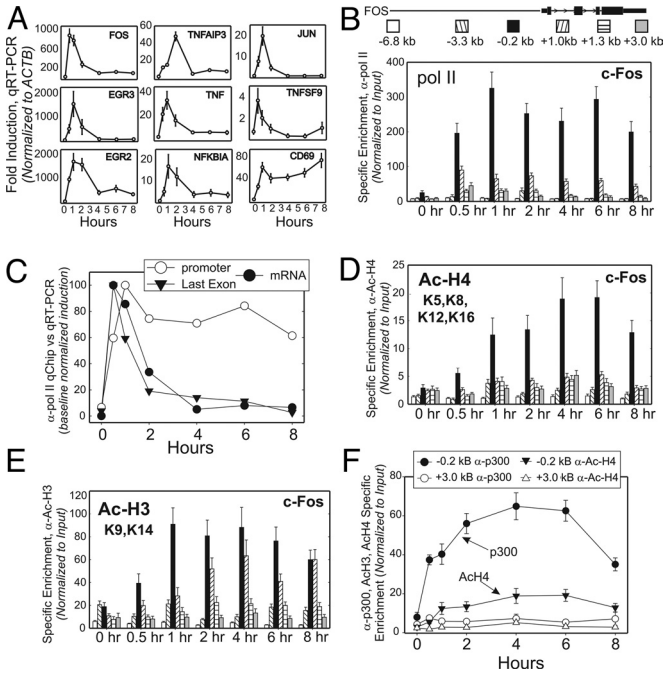

Fig. 2. p300 and pol II recruitment at the *FOS* promoter and pol II elongation are distinct and separate events. (*A*) qRT-PCR profile of p300-associated immediate early gene expression at 0–8 h following mitogen stimulation. Error bars represent SEM from 2–3 biological replicates, each determined in triplicate. (*B*) Time- and position-dependent profile of pol II enrichment at the *FOS* locus at the indicated position (relative to TSS) and time (0–8 h) following stimulation. (*C*) Overlay of *FOS* gene expression with pol II recruitment (promoter) and elongation (last exon) at the *FOS* gene following induction. Shown is the average of 3 independent biological replicates measured in triplicate at least. (*D*–*E*) Histone acetylation profile (H4-K5, K8, K12, K16 and H3-K9, K14, respectively) at the *FOS* locus following mitogen stimulation. (*F*) Overlay of time- and position-dependent profiles of p300 recruitment and H4 acetylation at the *FOS* gene proximal promoter and last exon following mitogen stimulation. H4 acetylation is derived from data in Fig. 1*D*. Error bars in *A*, *B*, *D*, *E*, and *F* represent SEM of 2 biological replicates, each measured in triplicate.

and persists for several hours following stimulation (Fig. S3*B* and Fig. 2*F*). Similar bookmarking by p300 and pol II is observed at the proximal promoters of *EGR2* and *NFKBIA* (Fig. S4 *A* and *B*).

p300 Is Required for Immediate Early Gene Bookmarking by pol II and Transcriptional Induction. Several in vitro protein interaction studies have implicated a role for p300 in the recruitment of pol II and the basal machinery to form the preinitiation complex (PIC) at target genes (3). To define the requirement for p300 in the placement and recruitment of a proximally positioned PIC during immediate early gene expression, we used different gene depletion strategies (Fig. 3 *A*–*D* and Fig. S4*C*). As shown in Fig. 3*A*, depletion of either p300 or CBP by RNAi results in loss of basal *FOS* gene expression in resting cells. Moreover, clonal cell lines in which endogenous p300 expression has been deleted (9) show significantly reduced immediate early gene expression (Fig. 3*B*) that is reduced further on CBP depletion by RNAi (Fig. S4*C*). Finally, cells depleted of p300 show both a dramatic reduction in the level of promoter proximal polymerase and transcriptional responsiveness of the immediate early genes *FOS*, *EGR2*, and *NFKBIA* against a broad array of extracellular stimuli known to activate RAS/MAP kinase and protein kinase C pathways (10) (Fig. 3 *C* and *D* and Fig. S4*D*). These agents include anisomycin, okadaic acid, Trichostatin A (TSA), EGF, insulin-like growth factor 1 (IGF-1), cycloheximide, and sodium butyrate (Fig. 3*D*).

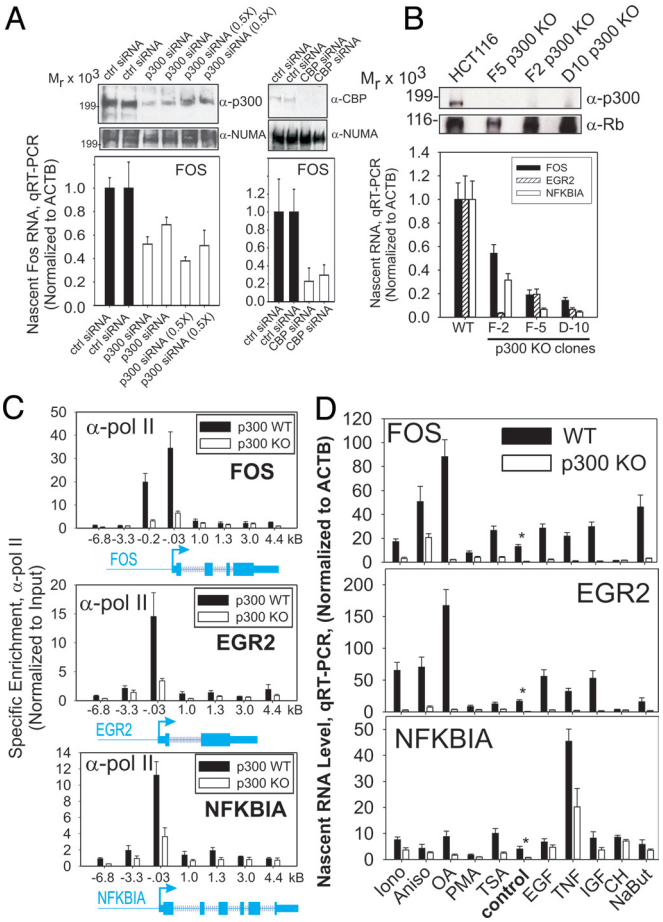

Fig. 3. p300 is required for immediate early gene pol II bookmarking and transcriptional responsiveness. (*A*) RNAi depletion of p300 and CBP in HEK293 cells decreases *FOS* expression (qRT-PCR). Shown are replicates of siRNA depletion of p300 and CBP, including replicates of p300 siRNA depletion performed at a 0.5 lower dose. (*Upper*) p300 and CBP immunoblots with NuMA loading control. (*B*) *FOS*, *EGR2*, and *NFKBIA* expression is depleted in p300-deficient HCT116 clones (p300 KO clones F-2, F-5, and D-10). (*Upper*) p300 immunoblot using Rb as a loading control. (*C*) qChIP analysis of pol II distribution at the *FOS*, *EGR2*, and *NFKBIA* genes in resting cells shows reduced promoter proximal pol II in p300-depleted HCT116 cells. (*D*) Parental (WT) and p300-depleted (p300 KO) cells were stimulated for 30 min with ionomycin (Iono, 1 μM), anisomycin (Aniso, 10 μg/mL), okadaic acid (OA, 500 nM), PMA (1 ng/mL), TSA (25 ng/mL), EGF (50 ng/mL), TNF (5 ng/mL), IGF-1 (50 ng/mL), cycloheximide (CH, 10 μg/mL), and sodium butyrate (NaBut, 5 mM). RNA was isolated and analyzed for nascent transcripts for *FOS*, *EGR2*, and *NFKBIA* by qRT-PCR. Error bars represent SEM of 2 biological replicates, each measured in triplicate. *, untreated control.

Dynamically Bookmarked Pol II Can Be "Restarted" by Sequential Restimulation. The use of the term *dynamic bookmarking* implies a transiently "poised" or "potentiated" state that could facilitate a secondary response to subsequent stimuli that, alone, may have little effect in the absence of preconditioning. To test this idea, P/I-stimulated Jurkat T cells were treated, once the initial spike of transcription had subsided, at 2 h with TSA, a histone deacetylase inhibitor that produces minimal stimulation when added to cells that have not been preconditioned by prior mitogen stimulation with P/I (Figs. 3*D* and 4*B*). As shown in Fig. 4, the transcriptionally arrested polymerase can be reinduced to elongate (Fig. 4*A*) and transcription can be returned to maximal mitogen-induced levels (Fig. 4 *B* and *C*) by sequential addition of TSA at 2 h after a mitogen stimulus, whereas TSA added alone to untreated cells has an undetectable influence on transcription (Fig. 4*B*, open symbols).

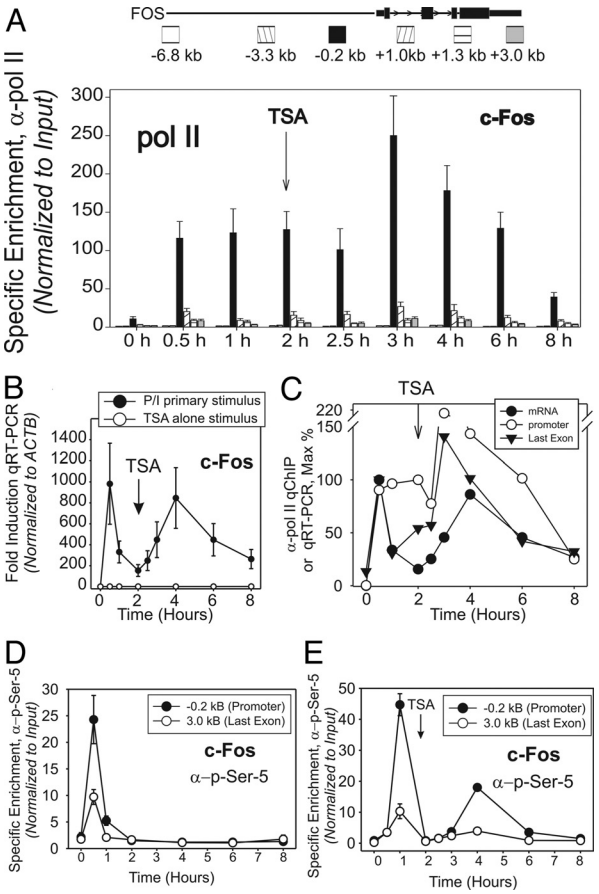

Fig. 4. Pol II elongation and transcription can be restarted by sequential treatment with TSA. Jurkat T cells were treated with P/I and then rechallenged after 2 h with either no treatment or TSA (25 ng/mL, arrow). (*A*) ChIP-qPCR time course of mitogen-induced pol II recruitment to the *FOS* gene with sequential addition of TSA at 2 h following mitogen stimulation (arrow). (*B*) qRT-PCR profile of *FOS* unspliced RNA from Jurkat T cells treated with P/I (closed symbols) and then rechallenged after 2 h with TSA (25 ng/mL, arrow) or treated at 0 h with 25 ng/mL TSA alone (open symbols). (*C*) Line graph overlaying the mitogen-induced ChIP-qPCR profile of pol II recruitment (promoter), elongation (last exon), and gene expression (mRNA) with sequential 2-h addition of TSA (arrow), as shown in *A* and *B*. (*D–E*) ChIP-qPCR profile of pol II CTD Ser-5 phosphorylation at the *FOS* promoter (−0.2 kB) and last exon (+3.0 kB) following mitogen stimulation without (*D*) and with (*E*) the addition of TSA at 2 h, respectively. Error bars represent SEM from at least 2 independent biological replicates, each measured in triplicate.

Similar to the profile of *FOS* expression, there is a rapid spike of transcription initiation-associated pol II C-terminal domain (CTD) Ser-5 phosphorylation (11, 12) that peaks at the *FOS* proximal promoter within 1 h after stimulation (Fig. 4*D*). The transient nature of this peak indicates that the polymerase, accumulated at the proximal promoter following elongation arrest, is relatively hypophosphorylated. As expected, this modification of pol II is transiently reinduced after the addition of TSA (Fig. 4*E*). Consistent with this observation, the proximal promoter recruitment profile of the TFIIH complex (containing the CDK7 kinase responsible for CTD Ser-5 phosphorylation) correlates well with the profile of CTD Ser-5 phosphorylation (Fig. S5 *A* and *B*).

The transient nature of stimulated and restimulated transcription implies that the assembly and activity of factors that control elongation should be equally transient. To interrogate this possibility, we focused on elongation factors that have been previously implicated in the control of immediate early gene expression. One factor is ELL, which had been initially identified as a factor that associates with pol II to increase its elongation efficiency (13, 14). Earlier studies showed that ELL is required for *FOS*-dependent transformation, and recent studies indicate that ELL may be recruited to stalled polymerases at heat shock genes in *Drosophila* (15, 16). Another factor is the positive transcriptional elongation factor (P-TEFb), which is composed of cyclin T1 or T2 and CDK9 active subunits and is responsible for the Ser-2 phosphorylation of the polymerase CTD that promotes elongation and other subsequent steps in the transcription cycle (17). Yet another factor is the bromodomain-containing protein (BRD4), which has been shown to associate with P-TEFb and possibly assist in targeting it to active genes (18, 19). ChIP analysis of all 3 factors shows that they are rapidly recruited to the *FOS* locus following mitogen stimulation. ELL shows higher enrichment at the 3′ end, which is consistent with its association with the elongating polymerase. BRD4 shows significantly higher association with the 5′ end of the gene, suggesting that it does not travel with the polymerase. P-TEFb shows rapid recruitment and significant enrichment at the 3′ end of *FOS*, indicating that it travels with the elongating polymerase. Notably, all 3 factors show rapid return to the *FOS* loci following the addition of TSA (Fig. 5). Consistent with the dynamic recruitment of P-TEFb, Ser-2 phosphorylation of the pol II CTD also shows transient 3′ enrichment and return following TSA treatment (Fig. 5). Interestingly, this striking reactivation of transcription does not appear to be attributable directly to HDAC inhibition, because multiple other agents, distinct from HDAC inhibitors, including anisomycin and okadaic acid, also reactivate transcription to levels that are significantly higher in mitogen-preconditioned cells compared with cells treated in the absence of prior stimulation (Fig. S5*C*).

Dynamic Bookmarking in Primary Human T Cells and Memory T-Cell Subsets. The concept of dynamic bookmarking raises the possibility that this process may play a more general role in biological systems, where preconditioning by environment stimuli is an essential component of the physiological response. The process of T-cell activation is a robust model system for the study of potential mechanisms of transcriptional memory, and examples of preconditioned transcriptional status have been reported in prior studies (20, 21). To pursue this hypothesis, we assessed whether dynamic bookmarking could be observed in primary T cells (Fig. 6 *A* and *B*). P/I stimulation of primary human CD3 cells induces *FOS* expression with spike kinetics nearly identical to those observed in Jurkat T cells (Fig. 6*A*). In contrast, profiles of the pol II distribution at the *FOS* locus show persistent accumulation of pol II at the proximal promoter, which is consistent with dynamic bookmarking (Fig. 6*B*). This memory-like activity of preconditioned cells has been compared by analogy with true immune memory within the T-lymphocyte compartment (20). True T-cell memory is a process in which naive T cells develop into populations of memory cells capable of more rapid "recall" or enhanced response (22) and is an example of how preconditioning events play a physiological role. Several recent studies aimed at defining genetic signatures specific for memory T-cell function have identified multiple different primary and secondary response genes, including classic immediate early genes like *FOS*, that show higher expression in resting memory compared with unstimulated naive T cells (23–25). A reasonable speculation would be that prior conditioning by antigen exposure may result in bookmarking of genes like *FOS* by pol II. As predicted, resting populations of both human donor memory CD4 and CD8 T cells show higher levels of both pol II and p300 at the *FOS* proximal promoter than their naive controls (Fig. 6 *C* and *D*), and basal *FOS* is significantly higher in memory compared with naive CD4 and CD8 T cells (Fig. 6*E*). These observations clearly suggest a role for dynamic

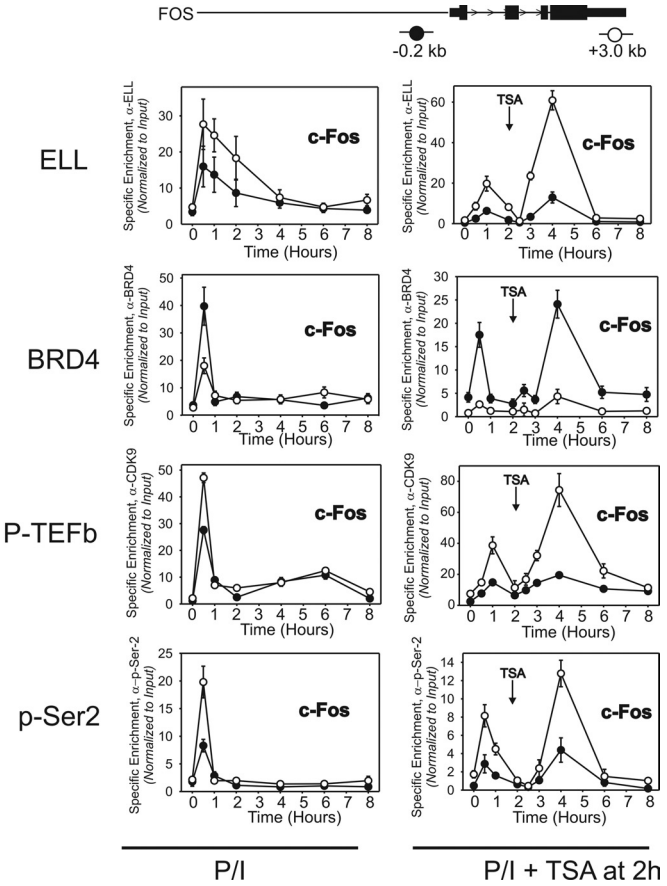

Fig. 5. Mitogen-induced elongation factor recruitment and Ser-2 CTD phosphorylation are dynamic and can be restarted following mitogen stimulation by sequential TSA treatment. (*Left*) ChIP-qPCR time course of ELL, BRD4, P-TEFb (CDK9), and Ser-2 phosphorylated pol II association with the *FOS* promoter (−0.2 kB) and last exon (+3.0 kB) following 0–8 h mitogen stimulation. (*Right*) ChIP-qPCR time course of the return of ELL, BRD4, P-TEFb (CDK9), and Ser-2 phosphorylated pol II at the *FOS* promoter (−0.2 kB) and last exon (+3.0 kB) after TSA treatment at 2 h following mitogen stimulation. Arrow indicates point of addition of TSA (25 ng/mL). Error bars represent SEM from 2 independent biological replicates, each determined from triplicate measurements.

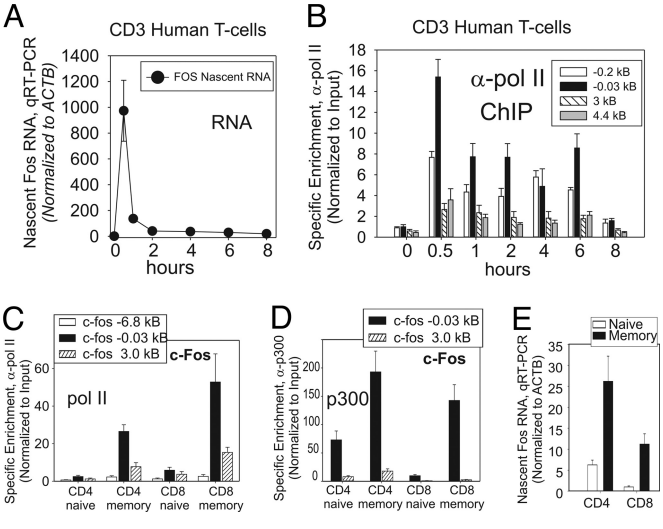

Fig. 6. Conditional accumulation of the prepositioned polymerase occurs at *FOS* in primary human T cells and is found at significantly higher levels in human memory compared with naive T cells. (*A*) Mitogen stimulation of primary human T cells shows spike-like kinetics for *FOS* expression similar to Jurkat T cells. Error bars represent SEM from 2 independent biological replicates. (*B*) Pol II shows persistent accumulation (bookmarking) at the *FOS* promoter of primary human T cells following mitogen stimulation. Error bars represent SEM from 2 different donors, each measured in triplicate. (*C–D*) Purified memory CD4 and CD8 T cells show higher levels of bookmarked pol II (*C*) and p300 (*D*) and higher basal *FOS* expression (*E*) than purified naive CD4 and CD8 T cells. Error bars represent SEM from 2 different donors, each measured in triplicate.

bookmarking in selective genomic "priming" for specialized responses.

Discussion

Exactly how many genes may be controlled by dynamic bookmarking remains to be determined. Recent genome-wide studies have described the widespread occurrence of a constitutively "stalled" or poised polymerase at genes in human and *Drosophila* cells, implying subsequent regulation by postinitiation events (26–30). In addition, polymerases paused at rapidly induced genes like *FOS* have been documented in vitro in several prior studies (31, 32). The preinitiation and reinitiation complexes are highly stable assemblies that make extensive contacts with DNA (33), and p300-containing PIC assemblies have recently been constitutively shown to mark both proximal promoters and enhancers throughout the genome of mammalian cells (34, 35). Although our findings are not inconsistent with the phenomena of transcriptional pausing, the observation that the bookmarked polymerase is depleted of TFIIH and both Ser-5 CTD and Ser-2 CTD phosphorylation suggests that these complexes represent steady-state forms of the PIC in the majority of cells. Detectable stalled or poised polymerase could possibly reflect a stochastically determined percentage of PICs that have undergone promoter clearance and remain transcriptionally engaged.

There are multiple protein associations that could target and stabilize p300 within the PIC at proximal promoters. Pol II and p300 interactions have been well established by in vitro studies that show more avid binding of p300 to hypophosphorylated pol II (36). At immediate early genes like *FOS*, sequence-specific targeting is provided by CREB and ELK through interactions with p300 and PIC components that are stabilized by phosphorylation during mitogen stimulation (3, 37, 38). Understanding the true nature of the bookmarked polymerase will require further investigation. Depending on both promoter context and the type of stimulus, there is likely to be considerable heterogeneity in the complexes and components that assemble over time within the PIC. Although the patterns of factor assembly measured at short time points immediately after stimulation by semiquantitative analysis are complex (6), they are superimposed by the overall pattern created by the steady-state detection of the PIC as it transitions from initiation to elongation and reinitiation (33). This complexity will reflect different fine-tuned aspects of promoter-specific regulation.

Determining whether transcriptional initiation or transcriptional elongation is the rate-limiting step in vivo will continue to be a challenge. Defining whether the polymerase is engaged at a position 30 bp or more downstream of the TSS or "parked" in a promoter proximal PIC within 100 bp upstream of the TSS is beyond the resolution of ChIP technology. The lack of Ser-5 CTD phosphorylation and TFIIH at the bookmarked polymerase, combined with the robust nature of transcriptional reinitiation at genes like *FOS*, suggests that more attention must be given to early events before promoter clearance as possible rate-limiting steps in *FOS* transcription. What it is that accounts for the rapid attenuation of transcription is a central question. Although the negative elongation factor (NELF) could have a role (39), the stimulus dependence of its action (40) and the

observation that cells depleted of NELF still show rapid attenuation of *FOS* transcription following mitogen stimulation (41) suggest involvement by other regulatory factors and events. The mediator complex has an established role in the formation of the PIC and the control of subsequent rounds of transcriptional reinitiation (33); therefore, much more focus will now have to be placed on the possible functional interplay between the mediator, p300, histone/protein acetylation, and elongation factor assembly during the transcription cycle. A clearer elucidation of these multiple events is certain to provide needed insights into the molecular mechanisms that govern how gene regulatory memory is formed and propagated during cellular differentiation, development, and other physiological states in which preconditioning plays a critical role. These insights will not only have an impact on our understanding of immunological memory but may begin to shed light on other complex physiological responses, including long-term cognitive memory, drug tolerance, and addiction.

Materials and Methods

Cell Culture and ChIP. Complete methods for cell culture propagation; cell sorting; and transfection of Jurkat, HEK293, and HCT116 cells are provided in *SI Materials and Methods*. Cells were stimulated with 1 μM ionomycin (Calbiochem) and 50 ng/mL PMA (Sigma-Aldrich) at indicated time points as previously described (5). Cells were then fixed in 1% formaldehyde for 5 min and subjected to ChIP analysis as previously described (6). The complete protocol is provided in *SI Materials and Methods*. Primary human T cells were obtained by apheresis from the National Institutes of Health (NIH) Clinical Center Division of Transfusion Medicine and expanded by 3 cycles of phytohemagglutinin (PHA)/IL-2 stimulation before resting for 24 to 48 h before use. Human CD4 and CD8 T cells were sorted from healthy donors according to their memory differentiation under sterile conditions using a FACS Aria system (Becton Dickinson) in a BSL-3 facility (complete methods are described in *SI Materials and Methods*).

p300 and pol II Genome-Wide Promoter Location Analysis. To identify p300/pol II binding sites, Jurkat T cells were treated with P/I for 0 or 45 min before cross-linking with 1% formaldehyde and were subjected to ChIP. ChIP-chip, DNA purification, and ligation mediated PCR (LMPCR) were performed as previously described (42) using antibodies against p300 and the CTD of pol II. ChIP-chip samples were labeled and hybridized to NimbleGen HG17 proximal promoter microarrays (Roche). Details of the procedure and data analysis are provided in *SI Materials and Methods*.

Gene Expression Analysis. Gene expression analysis was performed using U133 plus 2.0 Genechips (Affymetrix) and is described in *SI Materials and Methods*.

Real-Time PCR. PCR reactions were performed using SYBR Green Mix (Invitrogen) and 7300 Thermal Cycler (Applied Biosystems) instructions and were analyzed for ChIP and RT-PCR according to the manufacturer's instructions. Primer sequences for ChIP, qRT-PCR for mRNA, and qRT-PCR for nascent RNA (amplicon-containing intronic sequences) are provided in *SI Text*.

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