DEK is a mammalian protein that has been implicated in the pathogenesis of autoimmune diseases and cancer, including acute myeloid leukemia, melanoma, glioblastoma, hepatocellular carcinoma, and bladder cancer. In addition, DEK appears to participate in multiple cellular processes, including transcriptional repression, mRNA processing, and chromatin remodeling. Sub-nuclear distribution of this protein, with the attendant functional ramifications, has remained a controversial topic. Here we report that DEK undergoes acetylation in vitro at lysine residues within the first 70 N-terminal amino acids. Acetylation of DEK decreases its affinity for DNA elements within the promoter, which is consistent with the involvement of DEK in transcriptional repression. Furthermore, deacetylase inhibition results in accumulation of DEK within interchromatin granule clusters (IGCs), sub-nuclear structures that contain RNA processing factors. Overexpression of P/CAF acetylase drives DEK into IGCs, and addition of a newly developed, synthetic, cell-permeable P/CAF inhibitor blocks this movement. To our knowledge, this is the first reported example of acetylation playing a direct role in relocation of a protein to IGCs, and this may explain how DEK can function in multiple pathways that take place in distinct sub-nuclear compartments. These findings also suggest that DEK-associated malignancies and autoimmune diseases might be amenable to treatment with agents that alter acetylation.
function in pre-mRNA splicing through protein-protein interactions (20).

There is a growing body of evidence suggesting that separate steps along the pathway of gene expression are integrated (24, 25). As DEK has defined categorization as a single function protein, it is likely poised at the interface of multiple components of the gene expression pathway; however, this poses the question of how DEK participates in processes known to occur in separate sub-nuclear compartments. The majority of endogenous DEK is associated with chromatin and DNA, whereas only 10% of DEK is released with RNase treatment (26). Nonetheless, DEK has been found in association with RNA and RNA-processing factors by separate groups using multiple experimental approaches.

One mechanism by which a single polypeptide can exhibit different properties is through post-translational modifications. Acetylation can modify protein function in a number of ways. The most well known mechanism is that of histone acetylation, which by altering the charge and size of particular lysine residues results in a loosened association with DNA and a subsequent increase in local gene expression (27). It has recently become clear that many transcription factors are acetylated, which often results in an enhancement of function (27, 28). Mechanisms mediating this increase in transcriptional potential include alterations in DNA-binding affinity (p53, GATA-1, GATA-2, E2F, and c-Myb) (28–33), affinity for negative regulators (NF-κB and B-Myb) (34, 35) or positive cofactors (p53) (36), and localization (CIITA and HNF-4) (37, 38). Here we report that DEK is acetylated in the cell, and in vitro it is a substrate for the acetyltransferases CBP (CREB-binding protein), p300, and P/CAF (p300/CBP-associated factor). Differential reactivity of full-length versus N-terminally truncated DEK to an antibody that specifically recognizes acetylated lysine residues suggests that DEK is acetylated within the first 70 amino acids and that these modifications may have important functional consequences. Indeed, we show that increased acetylation of DEK results in a significantly decreased affinity for DNA. In addition, we find that acetylation markedly alters the localization of DEK inhibition of deacetylase activity triggers redistribution of DEK from a diffusely nuclear to a punctate pattern within the nuclear space. We show that this pattern results from the accumulation of DEK in structures known as nuclear speckles or interchromatin granule clusters (IGCs), which are well characterized sub-nuclear domains containing RNA-processing and transcription factors. Importantly, DEK can be driven into the IGCs by overexpression of P/CAF, but not CBP/p300, and this movement can be prevented by specifically blocking the activity of P/CAF with a newly developed, synthetic, cell-permeable inhibitor. Another group of investigators has observed that, in a small percentage of cells, DEK is found at a higher concentration in IGCs than in the nucleus at large (20). Our finding both strengthens and explains their observation by revealing the particular condition that favors localization of DEK to the IGC, namely its acetylation by P/CAF. Although there have been several examples of post-translational modifications controlling the movement of proteins within the cell, this is the first reported example of acetylation playing a direct role in the relocation of a protein to the IGC. It seems that the degree of acetylation, and its regulation, allow DEK to function in multiple pathways that take place in distinct sub-nuclear compartments.

MATERIALS AND METHODS

Cell Culture and Transfection—T98G cells were purchased from ATCC and passaged in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum and antibiotics. GFP-DEK was constructed as a fusion protein between DEK and an enhanced variant of Aequorea victoria green fluorescent protein. Primers with EcoRI (5’) and EcoRI (3’) restriction sites were designed for PCR-based subcloning of the dek coding region into the pGNVL3 mammalian GFP vector (gift of T. Glaser, University of Michigan). Cells were transfected with 2.5 μg of GFP-DEK, P/CAF, or CBP plasmid using Lipofectamine 2000 (Invitrogen). After transfection, cells were treated overnight with 1 μM TSA or 5 μM sodium butyrate, or left untreated, and fixed for immunocytochemistry on the following day. Alternatively, untransfected cells were treated overnight with 330 nM TSA or left untreated and fixed the following day.

Construction of DEK-encoding Adenovirus—FLAG-tagged DEK was excised from pCMV-Tag1 (Stratagene) using HindIII and BamHI and introduced via those sites into the adenoviral shuttle plasmid pACCMVpLPa(–)loxP-SSP. This vector was linearized with SfiI, and the transgene was recombined into E1A/E1B-deficient adenovirus by the University of Michigan Vector Core. Virus was harvested from E1A/E1B-positive producer cells, purified by cesium chloride density gradient centrifugation, and adsorbed for 1 h with poly-L-lysine-coated unit concentration. T98G cells were transduced at a multiplicity of infection of 100 by incubation for 48 h followed by cell harvesting for immunoprecipitation.

Immunoprecipitation and Western Blotting—Immunoprecipitation of FLAG-DEK from transduced T98G cells was performed using agarose-conjugated anti-FLAG M2 monoclonal antibodies (Sigma) by following the manufacturer’s instructions. Cells were washed with PBS, lysis with regards to cell lysis, incubation with antibodies and washing, and elution by competition with 3xFLAG peptide. For Western blotting, proteins were transferred from the gel to polyvinylidene difluoride membrane, blocked in 5% powdered milk, and probed with either horseradish peroxidase-conjugated anti-FLAG antibody at a dilution of 1:1000 (Sigma), or monoclonal anti-acetylysine antibody at a dilution of 1:1000 (Cell Signaling Technologies) or monoclonal anti-DEK antibody (Biosciences), followed by horseradish peroxidase-conjugated anti-mouse IgG (Molecular Probes) for chemiluminescent detection.

Production of Recombinant His-DEK in Baculovirus—DEK coding sequence was subcloned into the pBacPAK-His6 vector for recombinase into the baculovirus genome. Recombination, virus production, cell transduction and harvesting, and protein purification were performed as indicated in the manufacturers’ instructions for the BacPAK Baculovirus Expression System (Clontech) and HIS-trap nickel-chelating columns (Amersham Biosciences).

In Vitro Protein Acetylation Assay—Recombinant His-DEK was dialyzed overnight at 4 °C into acetylation buffer (10 mM Tris, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, and 5% glycerol). DEK was then incubated at 30 °C for 1 h with 50 μM [3H]Acetyl-CoA (Amersham Biosciences), 10 mM sodium butyrate, and 50 μM of either CBP, p300, or P/CAF, which had been purified as previously described (39). Reactions were resolved by SDS-PAGE; gels were stained with Coomassie Blue reagent and dried. The 14C signal was detected using a phosphorimaging screen and FX phosphorimaging device (Bio-Rad).

Dissociation Constant Measurements Using Electrophoretic Mobility Shift Assay—Constant-Dissociation Constant—Dissociation titrations were conducted by incubating immunoprecipitated FLAG-DEK (10-fold on either side of the dissociation constant) with a 32P-end-labeled DNA probe (5 μM) (sense, 5’TAT ACT TGG TCA GGG CCA ATT GCA TCT AGG AGA-3’T) containing the pets site (bold letters (15)) at 22 °C in 1 h in binding buffer (20 mM Tris, pH 7.4, 150 mM NaCl, 5% sucrose w/v; 1 μl of total volume). The dependence of binding affinity on buffer ionic strength was assessed by performing a series of binding titrations as a function of NaCl concentration (85 mM to 350 mM). Bound and free DNA were separated by electrophoresis in a non-denaturing polyacrylamide gel (4%) at 170 V at 4 °C for 1 h in 1× TBE buffer (100 mM Tris, 90 mM boric acid, 1 mM EDTA, pH 8.4). The gels were dried and exposed to a phosphor screen overnight, and the bands were quantified using a Storm 840 PhosphorImager with ImageQuant software (Amer sham Biosciences). The data were fit via nonlinear least-squares regression to the single-site binding isotherm: % free DNA = Kd + [protein].

From the above equation, the apparent Kd corresponds to the protein concentration at which half the DNA is bound (40). Errors in Kd are the standard error based on a minimum of three trials.

Immunocytochemistry—PBS washes were performed in between each of the following steps; when indicated, PBS washes contained 0.1% saponin. Cells were washed and fixed for 10 min in 4% paraformaldehyde. Fixed cells were washed, blocked for at least 1 h with 0.2% bovine serum albumin in PBS, rewash with PBS/saponin, and incubated for 1 h with mouse monoclonal anti-SC35 (35 μg/ml; Sigma) in PBS/saponin. If cells were not transfected with GFP-DEK, then the incubation included polyclonal...
anti-DEK serum at a 1:200 dilution (gift of G. Grosvenor). Cells were then rewarshed in PBS/saponin and reblocked with goat serum in PBS/saponin at a 1:50 dilution for 1 h, followed by PBS/saponin washing and a 1-h incubation with Alexa fluor 594-conjugated goat anti-mouse IgG (20 μg/ml; Molecular Probes) in PBS/saponin. If cells were not transfected with GFP-DEK, then incubation included Alexa fluor 488-conjugated goat anti-rabbit IgG (20 μg/ml; Molecular Probes). Cells were washed with PBS/saponin, refixed for 10 min, rewarshed, and incubated with 4',6-diamidino-2-phenylindole for 10 min, rewarshed with PBS, and distilled water then dried overnight. Coverslips were mounted with Antifade A reagent (Molecular Probes), and images were captured with a Zeiss Laser Scanning Microscope (LSM 510, version 2.8 SFI). For staining of PODs, the procedure was identical except for the use of polyclonal rabbit anti-CBP as the primary antibody (10 μg/ml, Upstate Biotechnology), and Alexa fluor 594-conjugated goat anti-rabbit IgG as the secondary antibody (20 μg/ml, Molecular Probes).

Preparation of H3-CoA-20-Tat and H3-(Ac)-20-Tat—H3-CoA-20-Tat (Ac-ARTKQTARKSTGGKCoAAPRKOLGYRKKRRQRRR-OH) is a derivatized version of the synthetic P/CAB inhibitor H3-CoA-20, in which the C-terminal sequence ends in the 12 amino acid residues of the cell permeabilizing Tat sequence. H3-(Ac)-20-Tat, a control compound, differs from H3-CoA-20-Tat in that the CoA moiety is replaced by a hydrogen atom. These compounds were synthesized by the solid phase method on a Rainin PS3 peptide synthesizer using the Fmoc (N-(9-fluorenylmethoxycarbonyl) strategy, analogous to the previously described method for H3-CoA-20 (41, 42). The epsilon amino group of the lysine residue that corresponds to Lys-14 of histone H3 was protected with the 2% hydrazine in dimethylformamide for 3 h at room temperature. The bromoacetylated peptide resin was then reacted with 5 equivalents of bromoacetic acid (thioanisole:ethanedithiol:triisopropylsilane (81.5:5:5:5:2.5:1)) for 4 h at room temperature, lyophilized, and purified initially by passage over the C-terminal sequence ends in the 12 amino acid residues of the cell permeabilizing Tat sequence. H3-(Ac)-20-Tat, a control compound, differs from H3-CoA-20-Tat in that the CoA moiety is replaced by a hydrogen atom. These compounds were synthesized by the solid phase method on a Rainin PS3 peptide synthesizer using the Fmoc (N-(9-fluorenylmethoxycarbonyl) strategy, analogous to the previously described method for H3-CoA-20 (41, 42). The epsilon amino group of the lysine residue that corresponds to Lys-14 of histone H3 was protected with the 2% hydrazine in dimethylformamide for 3 h at room temperature. The bromoacetylated peptide resin was then reacted with 5 equivalents of bromoacetic acid and 5 equivalents of diisopropylcarbodiimide for 16 h at room temperature (or acetic anhydride for 1 h for the control peptide). Peptides were cleaved from the resin with Reagent K (trifluoroacetic acid:phenol:H2O; 81.5:5:5:2.5:5:1) for 4 h at room temperature and subsequently precipitated with ice-cold diethyl ether. Precipitates were collected by centrifugation (3000 rpm, 5 min), the supernatants discarded, and the pellets washed twice with cold diethyl ether (30 ml). Precipitated peptides were dissolved in 5 ml of water, flash-frozen, lyophilized, and purified by preparative reversed phase (C18) high-performance liquid chromatography using a gradient of H2O:CH3CN:0.05% trifluoroacetic acid. The bromoacetylated peptide was conjugated with 2 equivalents of CoASH in a minimal volume of aqueous 0.5 M trimethylammonium bicarbonate (pH 8) for ~16 h at room temperature, lyophilized, and purified initially by passage over anion exchange chromatography (Dowex 1 x 8−100) to remove excess CoASH followed by reversed phase high-performance liquid chromatography in a gradient of H2O:CH3CN:0.05% trifluoroacetic acid. Peptides were confirmed to be >95% pure by high-performance liquid chromatography, and their structural identities were confirmed by mass spectrometry. The inhibitory properties of both peptides against P/CAB and p300 are shown in Table I.

RESULTS

DEK Is an Acetylated Protein—To determine whether DEK is acetylated in vivo, T98G human glioblastoma cells were infected with an adeno viral vector encoding for N-terminally tagged FLAG-DEK. Glioblastoma is one of several tumors that exhibit increased expression of DEK, as compared with its tissue of origin (2). FLAG-DEK was immunoprecipitated with anti-FLAG antibodies and separated by SDS-PAGE. Staining for total protein revealed that one major band is recovered in the immunoprecipitate at 48 kDa and a minor protein band at 35 kDa (data not shown). N-terminal sequencing has previously demonstrated that the 35-kDa band corresponds to a truncated form of DEK (amino acids 70–375) (5). Importantly, immunoprecipitated DEK is reactive with a monoclonal antibody that is specific for acetylated lysine residues (Fig. 1A, lane 4), suggesting that DEK is acetylated in the cell. Although full-length DEK is reactive with both a DEK-specific monoclonal antibody and the acetyllysine-specific antibody, the 35-kDa band is only reactive with the DEK-specific antibody (Fig. 1B, lane 1 versus lane 2). These data indicate that of the 67 potential lysine residues within DEK, the acetylated lysine residue is 1 (or more) of the 7 that are within the first 70 amino acids.

In vitro acetylation assays were used to determine whether DEK could serve as a substrate for the well characterized acetyltransferase proteins CBP, p300, and P/CAB. Fig. 1C indicates that all three enzymes acetylate recombinant DEK purified from baculovirus-infected insect cells. In the absence of an acetyltransferase enzyme, DEK remained unlabeled. Similar in vitro acetylation reactions using FLAG-DEK immunoprecipitated from T98G cells gave identical results (data not shown). These data suggest that acetylation is not occurring solely on the tag, because one recombinant protein is FLAG-tagged while the other is polyhistidine-tagged, and the His tag contains no lysines. These results also indicate that in mammalian cells there are available CBP/p300 and P/CAB acetylation sites within native DEK, which validates the use of deacetylase inhibitor treatment to shift the equilibrium toward more highly acetylated forms of the protein. To confirm that treatment with a deacetylase inhibitor alters the acetylation...
state of DEK in vivo, T98G glioblastoma cells were treated or mock-treated overnight with the deacetylase inhibitor trichostatin A (TSA). Indeed, FLAG-DEK immunoprecipitated from TSA-treated T98G cells demonstrates considerably more reactivity toward the monoclonal anti-acetyllysine antibody than similar amounts of FLAG-DEK isolated from untreated cells (Fig. 1A, lane 3 versus lane 4). These data demonstrate that treatment of DEK-infected cells with the deacetylase inhibitor TSA directly affects the acetylation state of DEK.

Acetylation of DEK Decreases Affinity for DNA—We have previously demonstrated that DEK binds to the TG-rich pets site from the HIV-2 promoter and that dephosphorylation of endogenous DEK results in release of DEK bound to this site (14, 15). These data indicate that, similar to many other proteins, post-translational modifications may play a significant role in the function of DEK within cells. Waldmann et al. (19) have also demonstrated that DEK binds to alternative forms of DNA such as four-way junctions and positive supercoils. Therefore, to investigate the effect of acetylation on the DNA-binding properties of DEK, the binding affinity of several differentially acetylated forms of FLAG-DEK for the pets site was determined using the gel shift assay (Fig. 2). For these experiments, FLAG-DEK was isolated from untreated and TSA-treated T98G cells, as well as from cells treated with cell-permeable inhibitors of P/CAF and CBP (see Table I and discussion of these inhibitors below). The apparent $K_d$ of DEK isolated from untreated cells for the pets site is 350 nM, versus 850 nM for DEK isolated from TSA-treated cells and 210 nM for DEK purified from acetylase inhibitor-treated cells. These data indicate that the more acetylated form of DEK has a 3- to 4-fold decrease in affinity for DNA, as compared with less acetylated forms of DEK. These data are consistent with the general observation that TSA activates transcription, and the finding that DEK likely plays a role in transcriptional repression (15, 16).

A significant driving force for many DNA-binding proteins is the release of cations from the negatively charged phosphate backbone of DNA upon complexation (i.e. the polyelectrolyte effect) (43, 44). Cation release generally results from salt bridge formation between positively charged protein residues and the DNA backbone, and can be interpreted in terms of the number of ionic interactions present in the complex (43). Therefore, to investigate the contribution of ion pairs to the stability of the DEK-pets complex, the binding affinity of DEK for the pets site was determined as a function of $[\text{Na}^+]$ (Fig. 2B), where the slope of the plot of $\ln K_d$ versus $\ln [\text{NaCl}]$ represents the stoichiometry of cation release (43, 45). These data suggest that binding of DEK to the pets site is accompanied by significant cation release from the phosphate backbone and that ionic interactions likely play an important role in complex stability. In contrast, the binding affinity of DEK isolated from TSA-treated cells for the pets site demonstrates a considerably lower dependence on buffer salt concentration (Fig. 2C). These data indicate that fewer ionic interactions are made in the DEK-pets complex following treatment with TSA, presumably due to the neutralization of the positively charged lysine residues within the binding site. Together, these data suggest that lysine residues of DEK play a direct and important role in DNA binding through recognition of the DNA backbone.

Deacetylase Inhibitors Alter the Localization of DEK—Recent reports have demonstrated that acetylation alters the localization of certain transcription factors (37, 38). Therefore, TSA treatment was used to investigate whether acetylation would change the location of DEK. T98G glioblastoma cells were treated or mock-treated with TSA overnight and fixed for immunocytochemistry. Comparison with 4,6-diamino-2-phenylindole staining confirmed that, in untreated T98G cells, DEK is distributed diffusely throughout the cytoplasm as reported previously (Fig. 3A). However, the pattern of DEK localization is dramatically altered in TSA-treated cells (Fig. 3D): in particular, DEK staining adopts a punctate pattern, suggesting that significant amounts of protein have accumulated in specific sub-nuclear structures in response to deacetylase inhibition. These data also indicate that DEK acetylation is dynamic; in other words, DEK is
These assays were carried out with recombinant human P/CAF and p300 as described previously (41, 42) and contained 10 μM acetyl-CoA and 10 μM peptide substrate (H3-20) for P/CAF, and 20 μM acetyl-CoA and 50 μM peptide substrate (H4-20) for p300. The measurements showed standard error < 20%. H3-CoA-20-Tat was shown to be a linear competitive inhibitor of P/CAF versus acetyl-CoA (150 μM H3-20 substrate) with Kᵢ 2.1 ± 0.7 nM (data not shown).

| Compound        | IC₅₀ for p300 | IC₅₀ for P/CAF |
|-----------------|---------------|---------------|
| H3-CoA-20-Tat   | 12 μM         | 0.04 μM       |
| H3-(Ac)-20-Tat  | NA*           | 3.2 μM        |

* NA, not assayed.

Fig. 3. Treatment of T98G cells with TSA results in the concentration of DEK in punctate bodies within the nuclear space, which are IGCs. A–C show an untreated cell, while panels D–I depict cells treated with TSA. A, D, and G show staining with monoclonal antibodies to DEK, B and E show staining with polyclonal antibodies to the splicing factor SC35 (a protein known to be concentrated in IGCs), and H shows staining with anti-CBP (a protein known to localize to PODs). C, F, and I show the merged image, with yellow indicating colocalization. Images were captured with a confocal microscope; bar, 5 μm.

normally a substrate for deacetylase enzymes as well as acetylases.

As the antibody used for the detection of DEK is polyclonal, a vector encoding for DEK fused with an enhanced variant of A. victoria GFP was constructed to independently confirm that the staining corresponded to DEK itself. T98G cells were transfected with this vector, treated overnight with TSA or left untreated, and fixed. As expected, GFP-DEK is diffusely distributed within the nucleus of untreated cells (Fig. 4A). In contrast, GFP-DEK in TSA-treated cells is concentrated in punctate bodies within the nuclear space (Fig. 4D). To more specifically correlate this phenotype with inhibition of deacetylase activity rather than an unpredicted effect of TSA treatment, cells were next treated with the deacetylase inhibitor sodium butyrate. As seen in Fig. 4G, sodium butyrate treatment also results in the relocalization of GFP-DEK to distinct sub-nuclear structures. Identical results were seen with sodium butyrate treatment and endogenous DEK (data not shown). At the concentrations and incubation times used, neither the TSA nor the sodium butyrate treatments caused cells to undergo apoptosis, as determined by visual inspection of cell nuclei with 4’,6-diamidino-2-phenylindole staining.

DEK relocates to the interchromatin granule cluster—There are many distinct structures within the nuclear space, a number of which appear as collections of small, round dots when stained (46). McGarvey et al. (20) have previously demonstrated that in ~15% of cells, DEK appears to be enriched in the nuclear speckles, also termed interchromatin granule clusters (IGCs), which is consistent with their observation that DEK associates with splicing factors. In contrast, acetylation of the oncoprotein EVI1 results in its relocalization to punctate structures that are enriched in CBP, which identifies them as promyelocytic leukemia protein oncogenic domains (PODs) (47, 48). To determine whether the punctate structures in Figs. 3 and 4 are either IGCs or PODs, cells were stained with antibodies specific for either SC35 or CBP, respectively. Figs. 3 (B and E) and 4 (B, E, and H) depict staining of SC35. Figs. 3F and 4 (F and I) indicate substantial colocalization between the DEK-containing bodies and the IGC, whereas Figs. 3C and 4C show that the colocalization signal does not result simply from the overlap of SC35 staining with diffuse nuclear DEK. Colocalization was not seen with anti-CBP staining of PODs, which revealed a set of nuclear bodies distinct from the DEK-containing structures (Fig. 3, G–I). Thus, by preventing deacetylation, TSA and sodium butyrate shift the equilibrium toward the more acetylated form of DEK, which causes this protein to accumulate in IGCs. Our data suggest that, in normal cells, the more acetylated fraction of DEK interacts with mRNA processing proteins within IGCs, which are thought to be accumulation sites of transcriptional factors and mRNA processing factors (49, 50). Indeed, previous data have supported the association of DEK with spliceosome proteins, most notably in complexes important for the coupling of pre-mRNA splicing and post-splicing events (21, 51).

Acetylation by P/CAF drives DEK into IGCs—To investigate which histone acetyltransferase is responsible for the movement of DEK into IGCs, cells were transfected with a vector encoding GFP-DEK and treated with newly developed, specific inhibitors of P/CAF (Table I) or CBP/p300 (52, 53). These inhibitors have a significant advantage over previously employed synthetic histone acetyltransferase inhibitors in that they are cell-permeable and therefore do not require transfection. Inhibition constants were determined as previously described and are shown in Table I (41).

Treatment of GFP-DEK-transfected cells with the selective cell-permeable inhibitor of CBP/p300, followed by treatment with TSA, does not block the movement of GFP-DEK to IGCs (Fig. 5B). In contrast, treatment of cells with the P/CAF inhibitor H3-CoA-20-Tat, prior to TSA treatment, blocks the movement of GFP-DEK to IGCs (Fig. 5C). However, treatment of cells with the control peptide (lacking only the acetyl-CoA functional group), followed by the addition of TSA, has no effect on the localization of DEK (Fig. 5D). These data suggest that, although both P/CAF and CBP/p300 can acetylate DEK in vitro, it is the specific acetylation by P/CAF that results in the movement of DEK to the IGC.

To further investigate the role of P/CAF and CBP in the sub-nuclear movement of DEK, cells were co-transfected with GFP-DEK and a vector encoding either CBP or P/CAF. DEK remains pan-nuclear with the overexpression of CBP (Fig. 6A), whereas overexpression of P/CAF with DEK results in the movement of DEK to IGCs (Fig. 6B). This relocalization can be blocked with the addition of the P/CAF-specific inhibitor (Fig. 6C), but not the P/CAF inhibitor control molecule (data not shown), following co-transfection of plasmids expressing GFP-
DEK and P/CAF. These data support the hypothesis that it is the specific acetylation of DEK, or an associated protein, by P/CAF that causes DEK to move to an alternative location within the nucleus: the IGC. To our knowledge, this is the first demonstration that a specific acetylase can control the movement of a protein into the IGC.

**DISCUSSION**

Although DEK has been associated with multiple disease states, particularly neoplastic conditions such as acute myeloid leukemia, hepatocellular carcinoma, glioblastoma, melanoma, and T-cell large granular lymphocyte leukemia, its role in disease and normal cellular function remains unclear (1, 2, 8, 9). However, the connection of DEK to diverse nuclear functions suggests that additional mechanisms may exist that confer alternative functional properties to DEK. For example, dephosphorylation results in the release of DEK from the pets site (15). Here we demonstrate for the first time that full-length DEK is reactive to an acetyllysine-specific antibody, indicating that DEK is acetylated in the cell. Interestingly, the N-terminally truncated form of DEK is not reactive with this antibody, suggesting that the acetylation site is within the first 70 amino acids.

Similar to other histone proteins and transcription factors, our data suggest that acetylation of DEK may have significant functional consequences, because TSA treatment results in an almost 4-fold decrease in affinity for DNA. The large dependence of binding affinity on NaCl concentration suggests that a considerable number of ionic interactions are made in the DEK/H18528 pets complex, presumably between positively charged lysine and/or arginine residues and the negatively charged DNA phosphate backbone. The generally nonspecific nature of these interactions may indicate why DEK has also been shown to bind alternative DNA structures, such as four-way junctions and positive supercoils, and to play a role in chromatin remodeling (17–19). The decrease in the overall dependence of binding affinity on NaCl concentration for the more acetylated form of DEK suggests that fewer ionic interactions are made in this complex. These data indicate that acetylation of DEK directly affects its ability to bind DNA, as opposed to altering its affinity for potential positive or negative cofactors, as has been described for other transcription factors (34–36). The recognition of alternative DNA structures may also be affected by the acetylation state of DEK, and indirectly contribute to the role of

![Figure 4](image-url)

**FIG. 4.** Treatment of T98G cells with deacetylase inhibitors results in the concentration of GFP-DEK in IGCs. A–C depict an untreated cell, D–F depict a TSA-treated cell, and G–I depict a sodium butyrate-treated cell. A, D, and G show green fluorescence indicating the presence of GFP-DEK, B, E, and H show staining with monoclonal antibodies to the splicing factor SC35, and C, F, and I show the merged image. Images were captured with a confocal microscope; bar, 5 μm.

![Figure 5](image-url)

**FIG. 5.** Treatment of T98G cells with the P/CAF-specific inhibitor H3-CoA-20-Tat prevents TSA-mediated accumulation of DEK in IGCs. A shows a cell after overnight treatment with TSA. B shows a cell pretreated with the CBP/p300 inhibitor Lys-CoA-Tat at a concentration of 50 μM, followed by overnight treatment with TSA. C shows a cell pretreated with the P/CAF-specific inhibitor H3-CoA-20-Tat (50 μM), followed by overnight treatment with TSA. D depicts a cell pretreated with a control inhibitor, H3-(Ac)-20-Tat (50 μM), followed by overnight treatment with TSA.
DEK in disease. Similar to some HMG proteins, the binding of DEK to distorted DNA structures resulting from DNA damage mechanisms, such as UV irradiation, may block access to the DNA repair machinery (54, 55). Treatment of cancer cells with HDAC inhibitors, such as TSA and butyrate, has been shown to have potentially therapeutic effects in certain malignancies (56, 57). It is interesting to speculate that treatment of DEK-associated malignancies with deacetylase inhibitors might have a beneficial effect by causing the release of acetylated DEK from the damaged DNA, thus allowing access to the DNA repair machinery. HDAC inhibitors might also impact upon DEK-associated cancers by altering gene expression patterns (see discussion below).

We have also demonstrated for the first time that acetylation alters the sub-nuclear localization of DEK, as deacetylase inhibition results in the redistribution of DEK from being diffusely nuclear to being concentrated into IGCs. To our knowledge, this is the first demonstration that movement of any protein into the IGC is under the control of acetylation changes. We have not strictly shown that acetylation of DEK is the cause, rather than the consequence, of its relocation into the IGC.

Attempts to address this issue using protein transfection have proven unrewarding, as we have been unable to transduce DEK into cells. This may be due to the highly charged nature of the individual domains of DEK, or to the propensity of this protein to multimerize (see below). However, it appears that the most straightforward explanation for the translocation of DEK into the IGC is that this movement follows acetylation. There are several reasons to assume that this is the case. First, when DEK is bound to DNA, it is tightly associated with chromatin, and acetylases are also known to act on chromatin, so this would be a logical place for DEK/acetylase interaction. Further, because it is the addition of deacetylase inhibitors or transfection of a vector expressing P/CAF that is the first step in our experiments, the simplest explanation is that an acetylation event drives DEK into IGCs. Finally, as is discussed further below, the acetylation of DEK would favor its accumulation in a chromatin-free compartment such as the IGC.

There are several reasons to assume that this is the case. First, when DEK is bound to DNA, it is tightly associated with chromatin, and acetylases are also known to act on chromatin, so this would be a logical place for DEK/acetylase interaction. Further, because it is the addition of deacetylase inhibitors or transfection of a vector expressing P/CAF that is the first step in our experiments, the simplest explanation is that an acetylation event drives DEK into IGCs. Finally, as is discussed further below, the acetylation of DEK would favor its accumulation in a chromatin-free compartment such as the IGC.

Importantly, DEK is driven into the IGCs by overexpression of P/CAF, but not CBP, even though both histone acetyltransferases can acetylate DEK in vitro. In contrast, the majority of studies to date have demonstrated that it is the acetylation by CBP/p300 that has important functional consequences in vivo, including DNA binding, chromatin remodeling, and protein-protein recognition (29, 30, 32–36). Interestingly, one of the few examples of an in vivo role for acetylation by P/CAF is for the Class II transactivator protein, CIITA: acetylation results in relocalization of the protein from the cytoplasm to the nucleus (37). The sub-nuclear movement of DEK can be blocked with a novel P/CAF-specific small molecule inhibitor, but not by a similar CBP/p300 inhibitor or a control molecule. These compounds and other similar small molecule inhibitors of various acetylases have considerable advantage over previously described acetylase inhibitors, because they are cell-permeable and therefore do not require transfection. We believe these compounds will have significant and broad application in the identification of proteins that are acetylated in vivo and further understanding of the functional consequences of acetylation in controlling gene regulation.

IGCs are dense collections of proteins that generally exclude nucleic acid, although RNA is found at the periphery of these structures (60). In fact, IGCs are adjacent to sites of active transcription, which is consistent with the prevailing theory that mRNA processing occurs co-transcriptionally (60). DEK has previously been found to be associated with mRNA-processing factors, although a proteomic analysis of the spliceosome did not identify DEK (61). It is possible though that association of DEK with the spliceosome is dependent on the acetylation state of the cell and may be quite dynamic and transient (62). The data presented in this report suggest the possibility of a previously unknown connection between protein acetylation and mRNA processing. The movement of DEK to the IGC is also consistent with the role of DEK in chromatin remodeling (18), because acetylation of DEK would be expected to interfere with the formation of compact DEK-DNA structures, and hence favor its accumulation in a chromatin-free compartment such as the IGC. Other chromatin-associated architectural proteins are also acetylated, including histones and high mobility group factors (63, 64), although acetylation of these proteins has not been shown to result in their subsequent movement to IGCs.

To date, there is no consensus motif for acetylation by either P/CAF or CBP, although they appear to have different recognition sites and usually acetylate different lysine residues within a protein (27, 64). As such, it is difficult to predict based on sequence alone which lysine residue within DEK may be acetylated by P/CAF and ultimately responsible for the movement to IGCs. Transfection of GFP-DEK mutants with each lysine residue of interest changed to alanine is one of the most direct methods to identify the site responsible for movement of DEK to IGCs, following treatment with TSA. However, glutathione S-transferase-pull-down experiments, yeast two-hybrid analysis, and native gel electrophoresis demonstrate that DEK can physically associate with itself and exists in several multimeric forms (data not shown). Dimerization would allow mutant forms of DEK to associate with endogenous DEK and “piggyback” to IGCs following TSA treatment (65). This phenomenon therefore masks the identification of the lysine responsible for sub-nuclear movement of DEK using site-directed mutagenesis. As such, small interference RNA experiments are currently underway to identify RNA sequences that can knock

**Fig. 6. Overexpression of P/CAF, but not CBP, drives DEK into IGCs.** A shows a cell co-transfected with vectors encoding GFP-DEK and CBP. B shows a cell co-transfected with vectors encoding GFP-DEK and P/CAF. C shows a cell co-transfected with GFP-DEK and P/CAF-expressing vectors followed by treatment with the P/CAF-specific inhibitor H3-CoA-20-Tat (50 μM).
down the expression of endogenous DEK, thus limiting interference from endogenous protein and facilitating the use of DEK mutants in transfection experiments.

Our results suggest a mechanism by which the amount of DEK in various compartments of the nucleus could be regulated, and an explanation for its appearance in multiple nuclear fractions (16, 26). These findings may also be relevant to the recent characterization of a complex containing both DEK and the histone deacetylase HDAC2 (16, 25). These findings may also be relevant to the recent characterization of a complex containing both DEK and the histone deacetylase HDAC2 (16, 26). These findings may also be relevant to the recent characterization of a complex containing both DEK and the histone deacetylase HDAC2 (16, 26).

It is also notable that SR proteins and other factors in IGCs are often determined by phosphorylation (68). A phosphoprotein, and its acetylation may influence, or be influenced by, other post-translational modifications such as phosphorylation; this has been observed for other proteins such as histone H3 and p53 (66, 67). In fact, trafficking of SR splicing factors in and out of the IGC is often determined by phosphorylation (68).

The acetylation of DEK by P/CAF drives DEK from the transcriptional enhancer through disruption of ionic interactions, and thereby promotes transcriptional activation. DEK then moves to the IGCs, where it could potentially participate in RNA-processing events through association with spliceosome proteins. This model is in support of evidence that multiple steps along the pathway of transcription and gene regulation are coordinated, and that post-translational modifications of proteins like DEK may play a critical role in the integration of these steps.

Acknowledgment—Polyclonal antibodies to DEK were a kind gift from Dr. Gerard Grosved.

REFERENCES
1. Kondoh, N., Wakatsuki, T., Ryo, A., Hada, A., Aihara, T., Horiuchi, S., Goseki, N., Matsubara, K., Takakura, K., Shiishita, M., Tanaka, K., Shuda, M., and Yamamoto, M. (1999) Cancer Res. 59, 4990–4996
2. Kroes, R. A., Justrow, A., McLone, M. G., Yamamoto, H., Colley, P., Kersey, D., Kose, F., and Naka, G. (2002) J. Biol. Chem. 277, 5905–5912
3. Kataoka, N., and Dreyfuss, G. (2004) J. Biol. Chem. 279, 7009–7013
4. Maniatis, T., and Reed, R. (2002) Nature 416, 499–506
5. Proudfoot, N. J., Furger, A., and Dye, M. J. (2002) Cell 106, 501–512
6. Kopciuszek, F., Burger, K., Bach, A., and Fackelmayer, F. O., and Gruss, C. (2002) J. Biol. Chem. 277, 24988–24994
7. Waldmann, T., Baack, M., Richter, N., and Gruss, C. (2003) Nucleic Acids Res. 31, 7003–7010
8. McCarver, T., Rosenoa, E., McCracken, S., Li Q., Arnaout, R., Mienjets, E., Nickerson, J. A., Awrey, D., Greenblatt, J., Grosveld, G., and Blencowe, B. J. (2000) J. Cell Biol. 150, 309–320
9. Le Hir, H., Lauzurique, E., Maquat, L. E., and Moore, M. J. (2000) EMBO J. 19, 6860–6869
10. Reichert, V. L., Le Hir, H., Jurisca, M. S., and Moore, M. J. (2002) Genes Dev. 16, 2778–2791
11. Katozka, N., and Dreyfuss, G. (2004) J. Biol. Chem. 279, 7009–7013
12. Kipp, M., Schwab, B. L., Przybylski, M., Nicotera, P., and Fackelmayer, F. O., and Gruss, C. (2002) J. Biol. Chem. 277, 24988–24994
13. Aravind, L., and Koonin, E. V. (2000) Trends Genet. 16, 275–280
14. Galasinski, S. C., Louie, D. F., Gloor, K. K., Resing, K. A., and Ahn, N. G. (2004) Mol. Cell. Biol. 24, 5552–5566
15. Peoplecker, S., Kabbara, N., and Spector, D. L. (2000) J. Cell Biol. 150, 5561–5570
16. Hollenbach, A. D., McPherson, C. J., Mienjets, E. J., Iyengar, R., and Grosveld, G. (2002) J. Cell Sci. 115, 3319–3330
17. Alessi, T., Waldmann, T., Anderson, J. M., Mann, M., Knippers, R., and Gruss, C. (2000) Genes Dev. 14, 1308–1312
18. Kondoh, N., and Dreyfuss, G. (2004) J. Biol. Chem. 279, 24988–24994
19. Waldmann, T., Eckerle, C., Baack, M., and Gruss, C. (2002) J. Biol. Chem. 277, 24988–24994
20. Schubert, S., Horstmann, S., Bartusel, T., and Klempnauer, K. H., (2004) Oncogene 23, 1392–1404
21. Barlev, N. A., Liu, L., Chelov, N. H., Mannsfield, K., Harris, K. G., Halazonetis, T. D., and Berger, S. L. (2001) Mol. Cell. 8, 1243–1254
22. Spilianiakis, C., Papatheophanis, J., and Kretowski, A. (2000) Mol. Cell. Biol. 20, 8489–8498
23. Seogheo, E., Katrakli, N., and Talalainidis, I. (2000) Mol. Cell 5, 745–751
24. Kataoka, N., and Dreyfuss, G. (2004) Genes Chromosomes Cancer 40, 146–151
25. Kataoka, N., and Dreyfuss, G. (2004) J. Biol. Chem. 279, 5460–5464
26. Kataoka, N., and Dreyfuss, G. (2004) Trends Biochem. Sci. 29, 449–453
27. Kataoka, N., and Dreyfuss, G. (2004) J. Biol. Chem. 279, 1813–1817
28. Kataoka, N., and Dreyfuss, G. (2004) Trends Genet. 20, 8489–8498
29. Kataoka, N., and Dreyfuss, G. (2004) Mol. Cell 5, 745–751
30. Kataoka, N., and Dreyfuss, G. (2004) Trends Biochem. Sci. 29, 449–453
31. Kataoka, N., and Dreyfuss, G. (2004) Trends Biochem. Sci. 29, 449–453
32. Marzio, G., Wagenier, C., Gutierrez, M. L., Cartwright, P., Helin, K., and Giacone, M. (2000) J. Biol. Chem. 275, 10879–10882
33. Takamida, A., Tawatari, M., Hayashi, F., Koai, H., Tamai, K., Miyakazi, T., Kinoshita, T., and Saito, H. (2000) Oncogene 19, 444–451
34. Chen, L. F., Fischle, W., Verdin, E., and Greene, W. C. (2001) Science 293, 1553–1567
35. Mihal, S., and Ladinsky, M., (2000) J. Cell Sci. 113, 1811–1815
36. Waldmann, T., Eckerle, C., Baack, M., and Gruss, C. (2002) J. Biol. Chem. 277, 24988–24994
37. Waldmann, T., Baack, M., Richter, N., and Gruss, C. (2003) Nucleic Acids Res. 31, 7003–7010
38. McCarver, T., Rosenoa, E., McCracken, S., Li Q., Arnaout, R., Mienjets, E., Nickerson, J. A., Awrey, D., Greenblatt, J., Grosveld, G., and Blencowe, B. J. (2000) J. Cell Biol. 150, 309–320
39. Le Hir, H., Lauzurique, E., Maquat, L. E., and Moore, M. J. (2000) EMBO J. 19, 6860–6869
40. Reichert, V. L., Le Hir, H., Jurisca, M. S., and Moore, M. J. (2002) Genes Dev. 16, 2778–2791
41. Katozka, N., and Dreyfuss, G. (2004) J. Biol. Chem. 279, 7009–7013
42. Maniatis, T., and Reed, R. (2002) Nature 416, 499–506
43. Proudfoot, N. J., Furger, A., and Dye, M. J. (2002) Cell 106, 501–512
44. Kopciuszek, F., Burger, K., Bach, A., and Fackelmayer, F. O., and Gruss, C. (2002) J. Biol. Chem. 277, 24988–24994
p300/CBP-associated Factor Drives DEK into Interchromatin Granule Clusters
Joanne Cleary, Kajal V. Sitwala, Michael S. Khodadoust, Roland P. S. Kwok, Nirit Mor-Vaknin, Marek Cebret, Philip A. Cole and David M. Markovitz

J. Biol. Chem. 2005, 280:31760-31767.
doi: 10.1074/jbc.M500884200 originally published online June 28, 2005

Access the most updated version of this article at doi: 10.1074/jbc.M500884200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 71 references, 37 of which can be accessed free at http://www.jbc.org/content/280/36/31760.full.html#ref-list-1