Subcellular Localization of the Human Proto-oncogene Protein DEK*

Recent data revealed that DEK associates with splicing complexes through interactions mediated by serine/arginine-repeat proteins. However, the DEK protein has also been shown to change the topology of DNA in chromatin in vitro. This could indicate that the DEK protein resides on cellular chromatin. To investigate the in vivo localization of DEK, we performed cell fractionation studies, immunolabeling, and micrococcal nuclease digestion analysis. Most of the DEK protein was found to be released by DNase treatment of nuclei, and only a small amount by treatment with RNase. Furthermore, micrococcal nuclease digestion of nuclei followed by glycerol gradient sedimentation revealed that DEK cosedimentates with oligonucleosomes, clearly demonstrating that DEK is associated with chromatin in vivo. Additional chromatin fractionation studies, based on the different accessibilities to micrococcal nuclease, showed that DEK is associated both with extended, genetically active and more densely organized, inactive chromatin. We found no significant change in the amount and localization of DEK in cells that synchronously traversed the cell cycle. In summary these data demonstrate that the major portion of DEK is associated with chromatin in vivo and suggest that it might play a role in chromatin architecture.

DNA in the nucleus is organized into a hierarchy of structures with the nucleosome as the basic building block. It has become widely accepted that modification of nucleosome structure is an important mechanism that regulates the accessibility of chromatin to DNA binding factors (1, 2). In the search for factors that change the structure of chromatin and the replicational activity of chromatin templates, we recently identified the proto-oncogene protein DEK as a candidate protein that changes the topology of DNA in chromatin in vitro (3). DEK is a 43-kDa phosphoprotein that was first isolated as part of a fusion protein expressed in a subtype of acute myeloid leukemias with (6;9) chromosomal translocations (4). DEK was later identified as an autoimmune antigen in patients with pauciarticular onset juvenile rheumatoid arthritis, systemic lupus erythematosus, and other autoimmune diseases (5–7). In addition, DEK has been reported to be a site-specific DNA binding factor, which recognizes a specific DNA element in the human immunodeficiency virus enhancer (8).

In a recent study, it was demonstrated that DEK associates with splicing complexes through interactions promoted by SR proteins. It was shown that DEK associates with mRNA in a splicing-dependent manner, indicating that it could function to coordinate splicing with subsequent steps in gene expression (9). In addition DEK was found in a ~355-kDa five-component complex at a conserved position 20–24 nucleotides upstream of exon-exon junctions (10).

Our recent experiments have identified DEK as a protein that induces alterations in the superhelical density of DNA in chromatin (3). The change in topology was only observed with chromatin but not with naked DNA and depends on the presence of histone H2A/H2B dimers. In addition we could show that DEK inhibits the replication efficiency of chromatin templates but not of naked DNA in vitro, demonstrating that DEK acts in a chromatin-specific manner. Association with chromatin has already been reported by Fornerod et al. (11), who demonstrated that DEK is associated with condensed chromosomes during metaphase.

Thus, DEK seems to be a factor with dual RNA and DNA binding properties. In order to elucidate the localization of DEK in the cell, we performed fractionation studies. We found that DEK is an abundant protein in the cell and is eluted from the nuclei with 250 mM salt. Treatment of nuclei with RNase released only ~10% of the DEK protein, whereas DNase treatment released most of the DEK protein, indicating that most of DEK is associated with chromatin in vivo. Treatment of nuclei with micrococcal nuclease followed by glycerol gradient sedimentation revealed that DEK is associated with oligonucleosomes. Chromatin fractionation studies demonstrated that DEK is more or less equally distributed on transcriptional active and inactive chromatin regions. The amount and localization of DEK does not change during the cell cycle.

EXPERIMENTAL PROCEDURES

Cell Culture and Cell Cycle Synchronization—Human HeLa S3 cells were grown on plastic dishes in Dulbecco’s modified Eagle’s medium with 5% fetal calf serum. Cells were synchronized by a double thymidine block at the beginning of S phase and released into thymidine-free medium (12). S phase was determined by cell counting and by pulse-labeling with [3H]thymidine and mitosis by mitotic indexes exactly as described recently (Fig. 1) (12).

Immunoblotting and Antibodies—Immunoblotting was carried out according to standard procedures. Proteins were separated by SDS-PAGE (13) and transferred to nitrocellulose. The membrane was blocked in Rotiblock solution (Rot) and incubated with different antibodies. Enhanced chemiluminescence reagents (ECL, Amersham Pharmacia Biotech) were used for detection. The polyclonal DEK antibodies (raised against His-DEK) were kindly provided by Gerald Grosfeld (St.

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Jude Hospital, Memphis, TN) and the anti-SR antibodies mAb NM4 were a gift from Benjamin Blencow (University of Toronto, Toronto, Ontario, Canada). The MCM5 antibodies have been described (12).

Cell Fractionation—Cells were washed three times on the plate with ice cold protein buffer A (20 mM HEPES pH 7.4, 20 mM NaCl, 5 mM MgCl2, 1 mg/ATP) and lysed by Dounce homogenization. After centrifugation on ice, the cytosolic supernatant was separated from the nuclear pellet by centrifugation (5 min, 600 × g). Nuclei were resuspended in buffer A supplemented with 0.5% Nonident P40 (Nonidet P-40) and kept on ice for 15 min to lyse the nuclear envelope. Centrifugation separated the free nucleosomal proteins from the pellet (5 min, 1000 × g). The pellet was resuspended for 15 min on ice in buffer B (20 mM HEPES, pH 7.4, 0.5 mM MgCl2, 1 mM ATP, 0.3 mM sucrose) plus NaCl in concentrations from 0.1 to 0.45 M to release structure-bound proteins. The final pellet was extracted in RIPA (50 mM Tris-HCl, pH 8, 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate). Proteins of each fraction were precipitated according to Wessel and Flugge (14) and analyzed by SDS-PAGE and Western blotting. Histone H1 was isolated from the individual fractions by perchloric acid extraction as described previously (15) and visualized by silver staining.

Micrococcal Nuclease Digestion, DNase I, and RNase Treatment—Nuclei were prepared as described above and finally washed in elution buffer (20 mM HEPES, pH 7.4, 0.5 mM MgCl2, 1 mM ATP, 0.3 mM sucrose) containing 100 mM NaCl. Nuclei (500 μg of DNA/ml) were incubated for 30 min at 37 °C with 17 units of DNase I/100 μl of cell nuclei or 40 units of RNase A/100 μl of cell nuclei; enzymes (Roche) were RNase- and DNase-free, respectively. The reaction was stopped on ice with 8 mM EDTA. Supernatant and pellet were separated by one centrifugation step. For micrococcal nuclease digestion, nuclei (500 μg of DNA/ml) were adjusted to 2 mM CaCl2 and digested with micrococcal nuclease with the concentration and time indicated under “Results.” The reaction was stopped on ice with 8 mM EDTA. Released chromatin fragments were separated from insoluble material by centrifugation (10 min, 12,000 × g, 4 °C). Proteins were removed from the DNA for 30 min at 50 °C in 2% SDS, extracted as described by Wessel and Flugge (14), and analyzed by SDS-PAGE and Western blotting. DNA was visualized on agarose gels by ethidium bromide staining.

Glycerol Gradient Sedimentation—The supernatant from the micrococcal nuclease digestion was separated for 14 h (40,000 rpm, SW40) on 5–40% glycerol gradients (20 mM HEPES, pH 7.4, 0.5 mM MgCl2, 100 mM NaCl, 1 mM ATP). Proteins and DNA from the individual fractions were analyzed as described above.

Chromatin Fractionation—The isolation of S1, S2, and P chromatin fractions was based on the procedure described by Rose and Garrard (16). 107 nuclei were isolated in HB buffer (0.5 mM sucrose, 15 mM Tris-HCl, pH 7.5, 60 mM KCl, 0.25 mM EDTA, pH 8, 0.125 mM EGTA, 0.5 mM spermidine, 0.15 mM spermine, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, supplemented with a protease inhibitor mixture (Complete, Roche)) and finally resuspended in 200 μl of nuclear buffer (20 mM Tris-HCl, pH 7.5, 70 mM NaCl, 20 mM KCl, 5 mM MgCl2, 3 mM CaCl2, supplemented with protease inhibitors). Nuclei were incubated with 300 units of micrococcal nuclease at 22 °C. The reaction was stopped on ice after 1, 2, and 4 min of digestion, and samples were centrifuged (12 min, 12,000 rpm, 4 °C). The first supernatant was designated the S1 fraction. The pellet was resuspended in 200 μl of 2 mM EDTA, incubated for 10 min on ice, and centrifuged again. The supernatant and the pellet were designated the S2 and P fractions, respectively. Equal aliquots of the fractions were deproteinized, and the DNA was investigated by agarose gel electrophoresis (0.8%) and ethidium bromide staining. Proteins were extracted according to Wessel and Flugge (14) and analyzed by SDS-PAGE and Western blotting with DEK-specific antibodies.

Immunofluorescence Analysis—HeLa S3 cells grown on coverslips were fixed for 15 min at room temperature in phosphate-buffered saline (PBS) containing 3.5% paraformaldehyde, followed by a 3 min permeabilization step in PBS containing 0.3% Triton X-100. Cells were then blocked for 60 min at room temperature in a humid chamber in PBS containing 3% BSA, followed by the incubation with the primary antibodies for 1 h at 37 °C. After three washing steps in PBS, cells were incubated for 30 min at 37 °C with species-specific secondary antibodies. For immunofluorescence microscopy, HeLa cells were stained with Oregon Green-labeled goat anti-mouse. After washing in PBS, the DNA was labeled with Hoechst 33258 in PBS containing 40% glycerol. For cell fractionation, cells were first permeabilized and then extracted with CSK (10 mM Pipes, pH 6.8, 0.3 mM sucrose, 3 mM MgCl2, 1 mM EGTA, 0, 5% Triton X-100) containing different NaCl concentrations.

Mitotic cells, obtained after double thymidine block, were permeabilized for 5 min in PHEM (60 mM Pipes, 25 mM HEPES, pH 7.4, 10 mM EGTA, 4 mM MgSO4, 0.5% Triton X-100) and then fixed in 3.5% paraformaldehyde followed by blocking in PBS with 3% BSA and incubation with the first and secondary antibodies. Immunofluorescence Labeling of Spread Chromosomes—Metaphase chromosome spreads were obtained from HeLa S3 cells treated for 14 h with 40 ng/ml nocodazole. Blocked cells were collected by centrifugation at 250 × g for 5 min, resuspended in hypotonic buffer (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 5 mM MgCl2), and kept for 5 min at room temperature. Hypotonically swollen cells were centrifuged at 250 × g for 5 min, resuspended in fixation buffer (methanol/acetic acid 3:1), and incubated for 30 min at room temperature. After another centrifugation step, the nuclei were resuspended in a few droplets fixation buffer and kept on ice. The solution was pipetted on coverslips and dried at 37 °C. Coverslips were washed for rehydration three times for 2 min in TEEN (1 mM triethanolamine, pH 8.5, 0.2 mM EDTA, 25 mM NaCl, 0, 1% BSA, 0.5% Triton X-100), then incubated with the primary antibodies for 1 h in TEEN at 37 °C. After washing in KB (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0, 1% BSA, 0, 1% Triton X-100), chromosomes were covered for 30 min at 37 °C with the secondary antibodies, diluted in KB-; DNA was stained with Hoechst 33258.

RESULTS

DEK Is an Abundant Protein That Elutes at 250 mM Salt from Cell Nuclei—The DEK protein changes the topology of chromatin in vitro at a ratio of two to three molecules of DEK/nucleosome by specifically interacting with histone H2A/H2B dimers (3). It was of interest to investigate the subcellular distribution of the protein. For that purpose, we performed cell fractionation experiments.

HeLa cells were disrupted in hypotonic buffer including 5 mM MgCl2 (17) to prepare a supernatant containing cytosolic proteins. The isolated nuclei were then lysed in 0.5% Nonidet P-40 and centrifuged again to obtain nucleosome proteins. The remaining nuclear structure including chromatin and the nuclear matrix was successively extracted with a buffer containing increasing NaCl concentrations and finally with RIPA. Most of the DEK protein dissociated at 0.25 mM NaCl, and only small amounts were also present in the nucleosol or extracted with 100 or 450 mM salt (Fig. 1A). The salt extractability of the DEK protein was compared with that of other nuclear proteins. We found that the DEK protein behaved much like typical chromatin-bound proteins, namely hCdc6 and hOrc2 protein (18, 19). The transcription factor SP1 was, however, found predominately in the nucleolus and only traces in the 250 mM and 450 mM extract. As already known, most of the linker histone H1 eluted from the nuclei with 450 mM salt (see, e.g., Ref. 20), a minor portion was also detected after extraction of the nuclei with RIPA. The distribution of nuclear proteins found by this cell fractionation technique reflects only the steady state level of a protein; however, proteins seem to move in the nucleus and to exchange continuously between chromatin regions (see “Discussion”).

Immunofluorescence studies revealed a strictly nuclear signal, whereby the nucleoli were excluded from staining (Fig. 1B, control). The signal disappeared upon extraction of nuclei with increasing salt concentrations, and most of the DEK protein was released between 250 and 400 mM salt (Fig. 1B), in agreement with the biochemical fractionation studies of Fig. 1A.

DEK could be linked to nuclear structures via RNA or DNA. To address this point, we treated nuclei with DNase I or RNase. The mobilization of DEK was determined by Western blotting (Fig. 2A) and by immunofluorescence (Fig. 2B). Treatment of nuclei with DNase I released a large fraction of DEK, whereas RNase released only a small portion of DEK (Fig. 2A). These findings were supported by immunofluorescence analysis: A bright nuclear signal remained after RNase treatment of nuclei, which was substantially reduced after DNase digestion (Fig. 2B). Residual DEK staining was detected after DNase treatment and could be due to the association of some DEK with splicing complexes (9). We found indeed that the DEK staining, remaining after
DNase digestion, overlapped with regions stained by SR-specific antibodies (21), indicating that this fraction of DEK might be associated with splicing complexes in vivo.

The cell fractionation experiment and the immunofluorescence data both implied that DEK is an abundant protein in the nucleus. To estimate the number of DEK molecules in the cell, we considered the possibility that at least some DEK may be bound to ribonucleoprotein, and treated the chromatin fragments to mononucleosomes, but failed to mobilize additional RNA from the chromatin pellet (Fig. 3A). This is consistent with earlier studies, which have reported that a fraction of chromatin is refractory to nuclease attack even at high enzyme concentrations (22–24). Approximately 50% of the DEK protein was released already at rather low enzyme concentrations (2 units, Fig. 3B). However, the second half of DEK remained associated with an insoluble nuclear structure, even at very high micrococcal nuclease concentrations (Fig. 3B) and after prolonged incubation times (data not shown). To quantify the immunoblots, the stained bands of Fig. 3B were investigated by NIH imager and plotted together with the fraction of solubilized DNA as a function of the enzyme concentration used (Fig. 2A). Proteins were extracted from the soluble and insoluble chromatin and investigated by Western blotting (Fig. 3B, S and P). With increasing amounts of micrococcal nuclease, up to 40% of DNA in chromatin became solubilized. Higher enzyme concentrations degraded the released oligonucleosomal fragments to mononucleosomes, but failed to mobilize additional DNA from the chromatin pellet (Fig. 3A). This is consistent with earlier studies, which have reported that a fraction of chromatin is refractory to nuclease attack even at high enzyme concentrations (22–24).

To obtain further information on the DEK-carrying chromatin, the products of micrococcal nuclease digestion were separated on glycerol gradients. Equal aliquots of the individual fractions were deproteinized and investigated by agarose gel electrophoresis, to determine the position of chromatin fragments in the gradient (Fig. 4, upper part). Other aliquots of the gradient fractions were used to localize the DEK protein (Fig. 4, untreated). We found that all of the DEK protein released after treatment of nuclei with micrococcal nuclease co-sedimentated with chromatin fragments of various lengths, suggesting that DEK remained associated with chromatin after nuclease digestion. We considered the possibility that at least some DEK may be bound to ribonucleoprotein, and treated the chromatin fragments with RNase before separation on glycerol gradients.

**Fig. 2.** The main portion of DEK is resistant to RNase treatment but is extracted from nuclei with DNase I. A, cell nuclei (500 μg of DNA/ml) were incubated for 30 min at 37 °C in the absence (control) or presence of DNase I or RNase. Supernatant (S) and pellet (P) were separated by one centrifugation step. Proteins were analyzed by SDS-PAGE and Western blotting with DEK-specific antibodies. B, immunolocalization of DEK protein in control nuclei and after DNase I and RNase treatment. Cells grown on coverslips were treated with DNase I or RNase for 40 min at 37 °C after permeabilization in PBS, 0.5% Triton X-100 and hypotonic extraction for 10 min. Cells were either labeled with DEK-specific antibodies or with anti-SR antibodies as described in Fig. 1 (bar, 5 μm).
Indeed, a small portion of DEK was released from fast sedimenting material and migrated after RNase treatment just like isolated soluble DEK (Fig. 4, RNase and DEK—chromatin).

However, the majority of DEK was clearly bound to chromatin because DNase I digestion of chromatin fragments resulted in their complete degradation (not shown) and the release of the DEK protein (Fig. 4, DNase). Our conclusion is that a major portion of DEK is associated with chromatin.

Rose and Garrard (16) have shown that differential extraction of micrococcal nuclease-treated chromatin allows a separation of active and inactive chromatin. We have used their procedure to investigate the partition of DEK to functionally different chromatin regions. Briefly, nuclei were digested for 1, 2, and 4 min with micrococcal nuclease and centrifuged to prepare the supernatant fraction S1. This fraction has been described to consist of transcriptionally active chromatin that is deficient in histone H1 and enriched in HMG proteins (16).

The pellet was resuspended in an EDTA-containing buffer and centrifuged to obtain supernatant fraction S2, which has been shown to be depleted of transcribed sequences (16). The remaining insoluble fraction P includes the nuclear matrix with actively transcribed genes. The resistance to nucleases may be due to associated protein complexes as RNA polymerase (16) or the SWI/SNF complex, which has also been detected in fraction P (25). As shown by DNA analysis on an agarose gel, the fractions represent differences in nuclease accessibility of chromatin (Fig. 5). Fraction S1, containing between 5% and 10% of the cellular DNA, is mainly composed of mononucleosomalized DNA, whereas fraction S2, containing ~50% of cellular DNA, showed a typical nucleosomal ladder of DNA fragments.

The P fraction, with ~40% of cellular DNA, consisted of heterogeneously sized DNA (Fig. 5A). Equal aliquots of fractions S1, S2, and P were subjected to SDS-PAGE, and the DEK protein was detected by immunoblotting with DEK-specific antibodies (Fig. 5B). We found that the DEK protein is present in all three fractions. However, taking into account that fraction S2 contains the major part of the DNA, the DEK protein is enriched approximately 5-fold in fraction S1 with respect to fraction S2. This indicates that the DEK protein is more abundant in transcriptionally active chromatin.

Localization of DEK during the Cell Cycle—In order to investigate the localization of DEK during the cell cycle, HeLa cells were arrested at the transition between G1 and S phase by a double thymidine block (26). Synthesis of DNA, as determined by the incorporation of labeled DNA precursors, started between 1 and 2 h after thymidine removal and continued for ~7 h (data not shown). Equal cell aliquots were taken at the indicated times after thymidine release and fractionated as described above (Fig. 1). Proteins were separated by SDS-PAGE, and the DEK protein was detected after immunoblotting with DEK-specific antibodies. As a control Western blots were also stained with MCM5-specific antibodies (Fig. 6). Minichromosome maintenance (MCM) proteins are known to dissociate from chromatin during S phase (27), but rapidly bind to chromatin again at the end of mitosis (12). As shown in Fig. 6, DEK remained on chromatin during all stages of the cell cycle. Furthermore, DEK in all cases could be eluted at 250 mM salt from chromatin, suggesting that the mode of DEK-chromatin interaction does not change during S phase when chromatin is newly assembled. The internal control here is the MCM5 protein, which was found on G1 phase chromatin (Fig. 6, 3 h), but not on chromatin from S phase and post-S phase cells (Fig. 6, 6–11 h). Similar results were obtained after release of HeLa cells from a nocodazole block, where no change in the amount and localization of the DEK protein occurred during the following G1 phase (data not shown).

Immunofluorescence was used to investigate the association of DEK with chromatin during mitosis. HeLa cells were released from a thymidine block and 9 h later permeabilized and fixed with formaldehyde. The localization of DEK was detected with DEK-specific antibodies and Texas Red-conjugated secondary antibodies (Fig. 7A). We found that DEK is associated with chromatin during all phases of mitosis, starting in prophase to metaphase, anaphase, and telophase (data not shown). In addition, we prepared metaphase chromosomes from nocodazole-treated cells (Fig. 7B). After treatment with DEK-specific antibodies, we found a homogenous staining of the metaphase chromosomes. Thus, DEK remains bound to mitotic chromatin.

DISCUSSION

Our recent experiments have shown that DEK changes the topology of DNA in chromatin in vivo (3). It was therefore of interest to know whether DEK is in contact with chromatin in vivo. In this work we present evidence that the DEK protein is associated with chromatin in vivo. We found that ~50% of the DEK protein can be released from nuclei with low micrococcal
nuclease concentrations. The resistant fraction remains in the nucleus even after prolonged incubation with high amounts of micrococcal nuclease. Analysis of released chromatin fragments on glycerol gradients revealed that DEK co-sediments with oligonucleosome-sized DNA fragments. After treatment of these preparations with RNase, a small fraction of DEK was released and appeared at the position of free DEK protein. This indicates that a minor fraction of DEK is associated with RNA-containing complexes in vivo, as has been shown previously (9).

A co-localization of a fraction of DEK protein with splicing complexes was also confirmed by immunostaining with anti-SR antibodies. In addition to the fractions of DEK, which are solubilized by short treatment with micrococcal nuclease and include chromatin- and RNP-bound DEK, a third fraction exists, which could not be released from nuclei by micrococcal nuclease or DNase treatment but is eluted with 250 mM salt and might be associated with heterochromatic regions. Whether the distribution of DEK in the nucleus reflects different forms of the protein has yet to be shown. In fact, DEK can be phosphorylated (11), but whether this or other post-translational modifications direct the protein to different chromatin compartments or to ribonucleoprotein is not known.

DEK is not unique among nuclear proteins that bind to chromatin and to RNA containing structures. Interestingly,
DEK is bound to chromatin during mitosis. A, HeLa cells grown on coverslips, were synchronized with thymidine. 7.5 h after release cells were permeabilized, fixed in 3.5% formaldehyde, and incubated with DEK-specific antibodies and then with Texas Red-conjugated secondary antibodies (bar, 5 µm). B, preparation of metaphase chromosomes. Mitotic cells were collected after nocodazole block, swollen in hypotonic buffer, washed once in methanol/acetic acid (3:1), and then dried on coverslips. After rehydration, chromosomes were incubated with DEK-specific antibodies and then with Texas Red-conjugated secondary antibodies. Cellular DNA was stained with Hoechst 33258 (bar, 2 µ m).

FIG. 7. DEK is bound to chromatin during mitosis. A, HeLa cells grown on coverslips, were synchronized with thymidine. 7.5 h after release cells were permeabilized, fixed in 3.5% formaldehyde, and incubated with DEK-specific antibodies and then with Texas Red-conjugated secondary antibodies (bar, 5 µm). B, preparation of metaphase chromosomes. Mitotic cells were collected after nocodazole block, swollen in hypotonic buffer, washed once in methanol/acetic acid (3:1), and then dried on coverslips. After rehydration, chromosomes were incubated with DEK-specific antibodies and then with Texas Red-conjugated secondary antibodies. Cellular DNA was stained with Hoechst 33258 (bar, 2 µm).

Our studies on the association of the DEK protein with nuclear structures have analyzed the steady-state level of the DEK protein. In light of the new data that most proteins constantly move in the nucleus, it seems, however, quite likely that the DEK protein may also move between chromatin regions or even between chromatin and RNA.

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DEK is an abundant protein with more than a million copies per nucleus. A comparison of the DEK amino acid sequence with sequences in data bases identified homologous sequences within predicted open reading frames of zebrafish, fly, and plant expressed sequence tag cDNAs, but did not reveal a significant homology with any characterized protein. Interestingly, amino acids 149–183 of the DEK protein show strong homology to the SAF (scaffold attachment factor) box (33, 34), also termed SAP domain (after SAF-AB, Acinus, and PIAS) (35), a DNA-binding motif involved in chromosomal organization. This 31-amino acid motif reveals a bipartite distribution of strongly conserved hydrophobic, polar, and bulky amino acids separated by a region that contains an invariant glycine (34). The positions enriched in positively charged amino acids might make contacts with the DNA backbone. SAF boxes have been found in many different proteins as, for example, poly-ADP-ribose polymerase, the Ku autoantigen, and the RAD18 protein, involved in DNA repair. Furthermore, the SAF box is associated with different proteins involved in the assembly of RNA-processing complexes (35) and might tether a diverse set of domains involved in pre-mRNA processing to transcriptionally active chromatin. It has also been shown that the SAF box organizes interphase chromosomes by binding to SAR regions, and its release by caspases causes the chromosomes to collapse during apoptotic cell death and facilitates chromatin degradation (33, 36). Thus, the SAF box targets a variety of proteins to specific chromosomal locations. In the case of the DEK protein, the association with chromatin might be mediated by the binding of the SAF box to DNA and by additional protein-protein interactions with histone H2A-H2B dimers (3).

In accordance with previous experiments (11), our immunofluorescence data show a co-localization of the DEK protein with chromatin during mitosis. In addition we could demonstrate that there is no change in the amount and localization of the DEK protein during the cell cycle, indicating that DEK is associated with chromatin during the whole cell cycle. This suggests that the DEK protein might be involved in maintaining architectural features of chromatin.

Recent experiments using photobleaching techniques have demonstrated that different proteins as the nucleosomal binding protein HMG-17, the pre-mRNA splicing factor SF2/ASF, and the rRNA processing protein fibrillarin, which are involved in diverse nuclear processes move rapidly throughout the entire nucleus (37). In addition it was found that almost the entire population of histone H1-green fluorescence protein is bound to chromatin at any one time, but is exchanged continuously between chromatin regions (20, 38). Thus, H1-green fluorescence protein molecules reside on chromatin in living cells for ~220 s before dissociating and rapidly rebind to an available binding site (20).

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