The ubiquitous chromatin protein DEK alters the structure of DNA by introducing positive supercoils

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Running title: Introduction of positive supercoils by the DEK protein
ABSTRACT

We have investigated the molecular mechanism by which the proto-oncogene protein DEK, an abundant chromatin-associated protein, changes the topology of DNA in chromatin in vitro. Band shift assays and electron microscopy revealed that DEK induces both intra- and intermolecular interactions between DNA molecules. Binding of the DEK protein introduces constrained positive supercoils both into protein-free DNA and into DNA in chromatin. The induced change in topology is reversible after removal of the DEK protein. As shown by sedimentation analysis and electron microscopy, the DEK-induced positive supercoiling causes distinct structural changes of DNA and chromatin. The observed direct effects of DEK on chromatin folding help to understand the function that this major chromatin protein performs in the nucleus.
INTRODUCTION

DNA in the eukaryotic nucleus is highly organized in a complex chromatin structure (reviewed in Ref. 1). The coiling of DNA by histones in nucleosomes is a barrier to the entry of proteins involved in transcription, replication and other DNA transactions. Changes in chromatin structure as triggered by the combined action of histone acetyltransferases and histone deacetylases as well as chromatin remodeling complexes have a significant influence on the processes occurring on DNA (reviewed in Ref. 2-5). In search for factors that change the structure of chromatin and the replicative activity of chromatin templates we have recently identified the proto-oncogene protein DEK as a candidate protein that changes the topology of DNA in chromatin in vitro (6).

The DEK protein was initially identified in a fusion with the CAN nucleoporin in a subtype of acute myeloid leukemias (AML) involving chromosomal translocations (7). DEK was later identified as an autoantigen in several autoimmune diseases such as juvenile rheumatoid arthritis (8) or systemic lupus erythematosus (9). Despite these disease associations the function of the DEK protein in the cell remains elusive. There are two recent reports demonstrating that DEK could be involved in RNA metabolism. It was shown that DEK associates with splicing complexes through interactions mediated by SR proteins. DEK associates with the SRm160 splicing coactivator in vitro and remains bound to the exon-product RNA after splicing. This association requires the prior formation of a spliceosome (10). In addition DEK has been found in a five-component-complex of ~ 335 kDa at a conserved position 20-24 nucleotides upstream of exon-exon junctions in mRNAs (11).
However, DEK also binds to DNA (12-14) and to metaphase chromosomes (15). In support of this, we determined that DEK is a constituent of oligonucleosomes, generated by micrococcal nuclease digestion of chromatin in isolated nuclei (16). Most of the DEK protein was released from nuclei after DNase treatment, whereas only around 10% was released after RNase treatment, indicating that the major fraction of DEK is associated with chromatin in vivo (16).

Isolated DEK changes the topology of DNA in viral minichromosomes and reduces the accessibility of chromatin to binding factors including components of the replication machinery (6). However, the mechanism of the DEK induced change in topology has not been investigated. We describe here, that DEK changes not only the topology of nucleosomal DNA, but of protein-free DNA as well.

Glycerol gradients and electron microscopy showed that DEK induces distinct alterations of the DNA structure due to an introduction of constrained positive supercoils. We believe that these findings have profound implications for the current understanding of chromatin functions.

**EXPERIMENTAL PROCEDURES**

*Purification of GST-DEK* - The DEK gene was cloned in the pGEX3 vector (Pharmacia) to generate a GST (Glutathione S Transferase) fusion protein. The GST-DEK fusion protein was expressed in BL21 (DE3) pLys S. A standard preparation of GST-DEK was prepared from 1 l liquid culture in 2 x YT media. Further purification steps were done according to the manufacturers protocol (Pharmacia).
**Purification of His-DEK** - Full length DEK cDNA was cloned into pBlueBac His2A and co-transfected with linearized AcMNPV DNA (Bac-N-Blue DNA) into SF9 cells as described in the manufacturers protocol (Invitrogen). Passage level three virus stocks were used to infect HighFive cells for protein expression. After two days post-infection the cells were washed twice with PBS and then lysed with lysis buffer (100 mM Tris, pH 7.5; 150 mM NaCl; 5 mM KCl; 0.5 mM MgCl2; 1 % NP-40 and 5 mM Imidazol). Soluble His-tagged DEK was purified by Ni-NTA-Agarose (Quiagen) chromatography according to the manufacturers protocol.

**DEK Assay** - Purified GST-DEK protein was dialyzed on Whatman Filters (Type VS, pore size 0.025 µm) against buffer A-100 (20 mM HEPES at pH 7.6, 100 mM NaCl, 10 mM sodium bisulfite, 1 mM EDTA) in the presence of 1µg/µl BSA (Biolabs) for 90 min at 4°C. Salt-treated SV40 minichromosomes or SV40 DNA (17) were incubated with the dialyzed GST-DEK protein for 1 hour at 37°C (molar ratios of DEK to DNA were ranging from 10 - 234 moles DEK/mole DNA) in presence of 12 ng of human topoisomerase I. The reactions were performed in buffer A-100, containing 0.2 µg/µl BSA in a total volume of 90 µl. Supercoiling reactions with E.coli topoisomerase I were done in the presence of 6 mM MgCl2. After Proteinase K digestion, DNA was precipitated and analyzed on 0.8 % agarose gels in 0.5 x TBE at 2 V/cm for 16 h.

**Sedimentation Analysis** - 1.5 µg salt-treated SV40 minichromosomes or SV40 DNA were incubated as described above, except that the reaction volumes were 500 µl. The samples were loaded on 10 to 35 % or 5 to 60 % glycerol gradients (for chromatin and DNA, respectively) (20 mM HEPES at pH 7.6, 10 mM sodium bisulfite, 1 mM
EDTA, 100 mM or 700 mM NaCl as indicated) and run in a SW40 rotor (Beckman) for 4.5 h (minichromosomes) or 4 h (DNA) at 40 000 rpm at 4°C. The gradients were fractionated in 600 µl fractions; proteins were precipitated according to the Wessel-Flügge method (18) and analyzed by 10% SDS-PAGE followed by Western blot analysis and immuno-staining with a DEK-specific antibody. The DNA was purified and analyzed on a 0.8% agarose gel in 0.5 x TBE at 2 V/cm for 16 h.

**Chromatin reconstitution** - Nucleosomal cores were reconstituted onto a 1000 bp fragment of SV40 DNA by using a modification of the salt dilution method (19). In a standard reconstitution reaction histone octamers were mixed in an initial volume of 10 µl with 6 µg of the EcoRI/EcoRV fragment of SV40 DNA (1000 bp) in 2 M NaCl, 10 mM Tris-HCl, pH 7.4, 0.5 mM EDTA. Samples were diluted at successive intervals of 60 min to contain 1.12 M, 0.8 M, 0.6 M, 0.4 M, and finally 0.12 M NaCl in the same buffer at R.T. Samples were centrifuged at 12000 g for 10 min to remove aggregated material and stored on ice until use.

**Electron Microscopy** - SV40 DNA and SV40 minichromosomes were incubated with increasing amounts of DEK and human topoisomerase I. Unbound protein was removed by gel filtration through a Biogel A-5 column and samples were fixed with glutaraldehyde (0.1% v/v) for 15 minutes at 37°C. Protein-DNA complexes were spread by the BAC (alkyl benzyl dimethyl ammonium chloride) technique of Vollenweider (20). Electron micrographs were taken with a Zeiss EM 900 electron microscope. The enclosed area of nucleoprotein complexes was determined with the NIH Image program, length measurements were done with the AutoCAD program.

**Gel Mobility Shift Assays** - 500 ng of SV40 DNA (form I, II, III) or SV40
minichromosomes were incubated with increasing amounts of GST-DEK for 1 h at 37°C. Standard reactions contained buffer A-100 in a total volume of 30 µl. Binding of GST-DEK was analyzed on 0.6 % agarose gels in 0.5 x TBE at 3.5 V/cm for 4 h.

Two-dimensional Gel Electrophoresis - Supercoiling reactions were performed as described above and run in the first dimension on a 0.8 % agarose gel in 0.5 x TBE at 2 V/cm for 16 h. The second dimension was run in the presence of 0.25 µg/µl chloroquine on a 0.8 % agarose gel in 0.5 x TBE plus 0.25 µg/µl chloroquine at 2 V/cm for 16 h. DNA was analyzed by Southern blot and hybridization with [32P]dATP-labeled Hinf I digested SV40 DNA, followed by autoradiography.

Purification of E. coli Topoisomerase I - XL-1 blue cells were transformed with pJW312Sal. Purification of topo I was done from a 500 ml liquid culture exactly as described (21).

RESULTS

DEK binds to chromatin and DNA - Alexiadis et al. (6) have shown that incubation of SV40 minichromosomes with DEK, purified from human HeLa cells in the presence of topoisomerase I causes a drastic change in superhelical density. However, in our initial experiments, we failed to detect DEK-induced changes of the topology of relaxed protein-free DNA and concluded that DEK acts in a chromatin-specific manner (6). This was clearly in contradiction to the experiments from the Markovitz group (12-14), who had demonstrated that DEK binds to DNA in a site-specific
manner. To reinvestigate this point, we used mobility shift assays to compare the binding of DEK to protein-free DNA and to chromatin (Fig. 1A). DNA or chromatin was incubated with increasing amounts of the DEK protein and the resulting nucleoprotein complexes were separated by agarose gel electrophoresis (Fig. 1A). We found that DEK binds to DNA and chromatin substrates with similar affinities producing multiple complexes whose mobility decreased with increasing protein concentration until saturation was reached. With protein-free DNA we found at higher DEK amounts nucleoprotein complexes, which did not enter the gel, indicating that DEK directs the formation of multimeric DNA complexes (compare also Fig. 6A, e and Fig. 7).

To determine whether DEK is able to bind different topological forms of DNA, we compared the binding of the DEK protein to supercoiled form I DNA, relaxed form II DNA and linear form III DNA (Fig. 1B). In three independent experiments, we found that DEK binds with similar affinities to the three DNA structures tested.

We next asked whether DEK is able to change the topology of protein-free DNA. For this purpose, we used increasing amounts of DEK, comparing protein-free SV40 DNA and SV40 minichromosomes as substrates in the presence of topoisomerase I. Deproteinized DNA was investigated by agarose gel electrophoresis and ethidium bromide staining (Fig. 1C). As in our previous experiments, we found no significant change in the topology of protein-free DNA at low DEK/DNA ratios. At that ratios a topological alteration of DEK-treated SV40 minichromosomes was observed. However, topological changes of protein-free DNA occurred at higher DEK/DNA ratios, reaching a maximal value at ratios between 52 and 78 moles DEK/mole DNA. Thus, about 2 fold higher amounts of DEK are necessary to change the topology of
protein-free DNA compared to minichromosomes. Titration of topoisomerase I revealed that we used saturating amounts of topoisomerase I in our assays, a further increase did not effect the topoisomer ladder (data not shown). In kinetic experiments, we found that rates at which linking-number changes were introduced into DNA were similar for protein-free DNA and SV40 minichromosomes (data not shown).

The experiments shown here were performed with GST-tagged DEK protein, purified from bacteria. To exclude an effect of the GST-tag alone, we repeated the assays with recombinant His-tagged DEK, expressed and purified from insect cells (Fig. 1D). We found that the His-tagged DEK protein exhibited the same activity as the GST-tagged DEK protein, both in mobility shift assays (Fig 1D, left part) and in topology assays (Fig. 1D, right part). Together with the experiments performed with untagged human DEK protein (6), these data clearly demonstrate that the observed effects are solely due to the DEK protein itself.

The DEK induced change in DNA topology is reversible and caused by the introduction of positive supercoils - We addressed the question of whether the DEK-induced change in DNA topology is reversible after removal of the DEK protein. For this purpose, we incubated SV40 minichromosomes with DEK and topoisomerase I and separated the chromatin on glycerol gradients containing either 100 mM or 700 mM NaCl (Fig. 2). Histones were still present in stoichiometric amounts on chromatin purified on 700 mM gradients (data not shown). As determined by Western blot analysis, DEK was associated with chromatin at 100 mM NaCl but not at 700 mM NaCl. The interesting point here is that the topology of DNA did not change after removal of DEK protein, as shown by a similar distribution of DNA topoisomers in
the 100 mM NaCl and the 700 mM NaCl gradient. Thus, once introduced, the change in topology is maintained.

An explanation for this effect could be the introduction of constrained positive supercoils by the DEK protein as outlined in the model in Fig. 3A. The hypothetical minichromosome in Fig. 3A contains 8 nucleosomes, corresponding to 8 negative supercoils after removal of the nucleosomes by Proteinase K. The five positive supercoils, introduced by DEK, would neutralize the same number of negative supercoils. This model is consistent with the data of Fig. 2, because sedimentation through 700 mM NaCl removes not only DEK, but topoisomerase as well (data not shown) with the consequence that the DEK induced topoisomer ladder remains after removal of the DEK protein. In this case, a re-addition of topoisomerase to DEK depleted chromatin templates should revert the topoisomer ladder to form I DNA. To test this hypothesis we isolated DEK-associated chromatin from 100 mM (Fig. 3B, +DEK) and 700 mM gradients (Fig. 3B, -DEK) and treated the molecules with human topoisomerase I. As predicted, the topoisomers of DEK-depleted chromatin were completely converted into form I DNA after treatment with human topoisomerase I, whereas no effect was observed on the topoisomer ladder of DEK-associated chromatin. Thus the DEK induced change in DNA topology is reversible after removal of the DEK protein in the presence of human topoisomerase I.

We have repeated the experiment with protein-free DNA as substrate (Fig. 3C) except that the DEK protein was removed from the DNA by Proteinase K and not by salt-wash as in Fig. 3B. DEK-associated (Fig. 3C, +DEK) and DEK-depleted DNA (Fig. 3C, -DEK) was then treated with human topoisomerase I. We found that the topology of DEK-associated DNA was not changed by topoisomerase I whereas the
topoisomer ladder of DEK-depleted DNA was reverted to form II DNA.

To directly demonstrate the presence of positively supercoiled DNA, the reaction products were treated with E.coli topoisomerase I (Fig. 4A) and analyzed by two-dimensional gel electrophoresis (Fig. 4B). Bacterial topoisomerase, in contrast to eukaryotic topoisomerase I, which removes both negative and positive supercoils, can only remove negative but not positive supercoils (22). SV40 DNA was incubated with DEK and topoisomerase I for one hour under DEK assay conditions. The DNA was deproteinized with Proteinase K, precipitated and used as substrate for the topoisomerase assay and incubated either with human topoisomerase I or E.coli topoisomerase I. DNA was again deproteinized, precipitated and analyzed by agarose gel electrophoresis (Fig. 4A). The activity of the topoisomerases was checked with protein-free DNA (Fig. 4A, control). While human topoisomerase I reverted the topoisomer ladder to form II (compare Fig. 3C), E.coli topoisomerase did not change the topoisomer ladder, indicating that DEK introduces constrained positive supercoils into the DNA.

We then analyzed the supercoiling state of the reaction products by two-dimensional gel electrophoresis with the second dimension in the presence of chloroquine (Fig. 4B). Chloroquine changes the topology of closed circular DNA molecules by introducing positive superhelical turns. For orientation, we mark the position of fully relaxed closed circular DNA as a reference point ($\Delta Lk = 0$). DNA topoisomers on the arc extending leftwards from $\Delta Lk = 0$ have negative, and DNA topoisomers on the right arc have positive supercoils. We found that DNA in chromatin possessed around 25 negative supercoils, in accordance with the known number of 25 nucleosomes of SV40 minichromosomes (23). In contrast, DEK-treated DNA in chromatin had only
an average of –12 to –13 negative supercoils, demonstrating that DEK together with topoisomerase I changes the topology of DNA in chromatin. This change is most likely due to an introduction of positive supercoils, which compensate the negative supercoils. This could be clearly demonstrated by studying the topology of protein-free DNA by two-dimensional gel electrophoresis. We found that the linking number of protein-free DNA centers around 0, with a few positive and a few negative supercoils due to thermal fluctuation. After incubation with DEK protein, there was a significant increase in positive supercoiling. By counting the number of topoisomers we determined that on average 1 positive supercoil is introduced per 6 to 8 DEK molecules. We conclude that the DEK-induced change in DNA topology both in protein-free DNA and in chromatin is caused by the introduction of constrained positive supercoils.

*The introduction of positive supercoils by the DEK protein causes an alteration of DNA and chromatin structure* - To determine the effects of DEK-induced positive supercoils on DNA and chromatin structure, we performed sedimentation analysis (Fig. 5) and electron microscopy (Fig. 6). DNA or chromatin were incubated with increasing amounts of DEK (plus human topoisomerase I) and then analyzed by glycerol gradient centrifugation. The position and topology of the DNA were investigated on agarose gels and the position of the DEK protein was determined by Western blotting (Fig. 5). DEK influenced the sedimentation properties of both protein-free DNA and chromatin. However, while DEK at ratios of 45 mole DEK/mole DNA caused a precipitation of free DNA, high DEK/chromatin ratios increased the sedimentation rate, but did not precipitate chromatin. Furthermore, at a
DEK/DNA ratio of ~ 90, corresponding to a ratio of 3.6 molecules DEK per nucleosome, free DEK protein appeared on top of the gradients, indicating that the substrates were saturated with DEK. The sedimentation behavior in the presence of DEK indicated that DEK protein induced structural alterations of DNA and chromatin.

To further investigate the structural changes induced by DEK we examined SV40 DNA or SV40 minichromosomes by electron microscopy (Fig. 6). With increasing amounts of DEK we observed different types of structures. At low DEK/DNA ratios, most DNA molecules possessed one single protein dot. The size of the dot was similar to that of nucleosomes indicating that the DEK protein multimerizes when bound to DNA (Fig. 6A, b). At increasing DEK/DNA ratios, individual protein dots fused to form loops of variable sizes, indicating that DEK molecules on different sites on the DNA interact with each other (Fig. 6A, c) until large portions of DNA were covered by the DEK protein (Fig. 6A, d). In addition, DNA networks containing several SV40 DNA molecules connected by the DEK protein through intermolecular interactions were formed (Fig. 6A, e). Statistical evaluation revealed a heterogeneous population of molecules at all DEK concentrations used, which were never completely covered by the DEK protein (Fig. 6B). This supports biochemical data showing that saturating DEK concentrations lead to a spectrum of DNA topoisomers but never to fully superhelical DNA. A likely explanation is that DEK induces topological strain in the molecule, which prevents further loading of DEK protein.

The DEK-induced alterations of SV40 chromatin were analyzed in the same way (Fig. 6C). Without DEK, the chromatin carried around 23 - 25 regular spaced nucleosomes (Fig. 6C, a). At increasing DEK/chromatin ratios, the structure became
more compact, until the area of the nucleoprotein complexes measured 10-30% of the 
DEK-free control (Fig. 6C, b-e). Nucleosomes on DEK-treated chromatin appeared 
in clusters, indicating that DEK induced internucleosomal interactions. To exclude 
artefacts due to spreading problems, protein-free DNA was included in all samples as 
a control, and was found in an extended configuration (Fig. 6C, f).

To gain further insights into the mode of DEK/DNA interaction, a linear DNA 
fragment of 1000 bp was incubated in the presence of increasing amounts of the DEK 
protein and topoisomerase I and analyzed by electron microscopy (Fig. 7). One 
possibility is that right-handed wrapping of the DNA around the DEK protein causes 
the introduction of positive supercoils. Thus at moderate DEK concentrations we 
expected that wrapping of the DNA around the DEK protein should cause a 
shortening of the molecules. We found however that molecules were around 10% 
longer in the presence of moderate DEK amounts (Fig 7, upper panel). At higher DEK 
concentrations, different types of molecules were observed (Fig. 7, middle panel). 
They consisted of molecules arising by multiple intra- and intermolecular DEK-DEK 
interactions resulting in different forms of compacted molecules. In a control 
experiment, we used purified histone octamers and salt gradient dialysis and 
reconstituted the 1000 bp fragment into chromatin (Fig. 7, lower panel). Wrapping of 
the DNA around nucleosomes resulted in a significant shortening of the molecules. 
Taken together, these data clearly demonstrate that the DEK induced change in 
topology is not caused by wrapping of the DNA around the DEK protein.
DISCUSSION

DEK is an ubiquitous and abundant nuclear protein with ~ 5x10^6 copies/nucleus. A fraction of DEK is probably bound to nuclear RNA, but the majority of 80 – 90% of DEK is a constituent of chromatin (16). In spite of its abundance and wide distribution, surprisingly little is known about the functions that DEK may perform in chromatin. We show here that DEK in vitro does not only bind to chromatin as previously thought (6) but to protein-free DNA as well. However, around three times more DEK is necessary to reach saturation in the mobility shift assay compared to the amount necessary to completely change the topology of DNA or chromatin. In either case, the initial binding of DEK introduces positive supercoils up to a point where no further supercoils can be introduced due to sterical hindrance, resulting in the observed topoisomer ladder. In addition, DNA-bound DEK is able to interact with each other (Fig. 6 and 7). Therefore, additional DEK molecules can bind to the DNA by protein-protein interactions and thus cause a further change in the mobility shift assay.

DEK introduces positive supercoils into topologically fixed circular DNA substrates. This reaction is specific as other DNA binding proteins such as the SV40 T-Ag or histone binding proteins such as NAP1 do not change the topology of DNA at the same molar ratios (data not shown). The conclusion that DEK introduces positive supercoils into the DNA is based on the following observations. First, we found that the DEK induced change in topology is reversible after removal of DEK by eucaryotic but not bacterial topoisomerase I. Second, analysis by two-dimensional gel
electrophoresis directly showed the presence of positive supercoils.

How could DEK introduce positive supercoils into DNA? So-called helix tracking proteins, which translocate on the DNA, generate local positive supercoils ahead of the moving protein and negative supercoils behind them (24). When the substrate for DEK is protein-free-DNA, any positive and negative superhelicity generated by tracking would probably be self-canceling or relaxed by topoisomerase. This appears not to be the case in the presence of the DEK protein. Another possibility is that DEK wraps DNA in a right-handed direction, generating positive supercoils. The torsional stress would lead to an accumulation of unconstrained negative supercoils. Topoisomerase I is able to remove negative but not the positive supercoils that are constrained by DEK binding. Subsequent deproteinization would result in the formation of positively supercoiled DNA. However, electron microscopical analysis with linear DNA clearly demonstrated that DEK caused no shortening of the molecules, excluding a wrapping of the DNA around DEK. Another possibility is that protein-protein interactions between DNA-bound DEK molecules cause a change in helical twist by bending the DNA and thus introduce positive supercoils. Compensatory negative supercoils would be relaxed by topoisomerase I, positive supercoils might be stabilized by a framework of DEK molecules (compare Fig. 6). Further studies have to clarify the exact mechanism of positive supercoil induction by the DEK protein.

DNA supercoiling is ubiquitous in living cells and is known to participate in many DNA transactions (25). Previous examples of right-handed DNA wrapping proteins include DNA gyrase (26) and the archaeal histone HMf (27). DNA wrapping by these two proteins does not require ATP. Both positive and negative supercoiling activity
has also been associated with the yeast recombinase complex Rad 51/Rad 54 during recombination (28). In addition, a novel non-specific DNA binding protein Smj 12 has recently been identified in Sulfolobus solfataricus, which stabilizes the DNA double helix and induces positive supercoiling (29). Positive supercoiling activity has also been shown for condensins (30), multiprotein complexes, which contain the evolutionary conserved SMC (structural maintenance of chromosomes) proteins (31). The eukaryotic SMC proteins form two kinds of heterodimers, which are involved in chromatin and DNA dynamics. The two most prominent and best-characterized complexes are cohesin and condensin, necessary for sister chromatid cohesion and mitotic chromosome condensation (32). SMC proteins show high affinity for cruciform DNA (33), a property that is shared with a group of architectural proteins (34). Interestingly, we also found binding of the DEK protein to four-way junction DNA (Waldmann, manuscript in preparation), suggesting a possible function of the DEK protein in chromatin architecture. This possibility is supported by our sedimentation studies and electron microscopical analysis, which showed that DEK protein induces distinct structural alterations in the structure of both chromatin and DNA substrates.

The amount and localization of the DEK protein does not change during the cell cycle (16). The calculated amount of the DEK protein in the nucleus equals 1 molecule of DEK per 3-4 nucleosomes and this would be sufficient to exert a general effect on chromatin structure. Protein-protein interactions of DEK molecules might enhance and stabilize the compaction process (Scholten, manuscript in preparation). Since many proteins are highly mobile in the cell nucleus (35-37) it seems quite likely that the DEK protein exchanges between chromatin regions and thus might influence the
chromatin structure at different loci. The mechanisms by which these reactions are regulated remain an interesting topic for further research.

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FIGURE LEGENDS

Fig. 1. **Gel mobility shift assay with protein-free DNA and SV40 minichromosomes.**

Equal amounts of form I DNA or SV40 minichromosomes (A) or form I, II and III DNA (B) were incubated in the absence (-) or presence of increasing amounts of DEK and loaded directly on an 0.6% agarose gel. The molar ratios of DEK to DNA present in the reaction mixtures were in (A) lanes 1-10: 0, 8.5, 17, 25, 50, 78, 100, 130, 180, and 234, and in (B) lanes 1-5: 0, 50, 100, 130, and 180 moles DEK/mole DNA.

DEK assay. C, form I SV40 DNA (175 ng) or SV40 minichromosomes (175 ng) were incubated for 1 h in the absence (- DEK) or presence of increasing amounts of DEK protein and human topoisomerase I. The topology of deproteinized DNA was analyzed by agarose gel electrophoresis and ethidium bromide staining. The molar ratios of DEK to DNA were in lanes 1-11: 0, 4.5, 8.5, 17, 25, 50, 78, 100, 130, 182 and 234 moles DEK/mole DNA. D, gel mobility shift assay with His-tagged DEK. Equal amounts of DNA (form I, II and III) were incubated in the absence (-) or presence (+) of His-tagged DEK and loaded directly on an 0.6% agarose gel (first panel). DEK-assay with protein free DNA (second panel) and SV40 minichromosomes (third panel) in absence (-) or presence (+) of His-tagged DEK. The molar ratio of DEK to DNA 0 (-) and 182 (+). Sample analysis was as in (C). (I) supercoiled; (II) relaxed closed circular and nicked DNA).

FIG. 2. **DNA topology after removal of the DEK protein.** SV40 chromatin was incubated in the absence (mc without DEK) or presence of DEK (mc with DEK) and
human topoisomerase I and separated on glycerol gradients containing 100 mM or 700 mM salt. The position and topology of the purified DNA was analyzed by agarose gel electrophoresis, the position of the DEK protein was determined by Western analysis and compared with the sedimentation behavior of free DEK protein (free DEK). Note, that free DEK protein shows a faster sedimentation in 100 mM gradients compared to 700 mM, indicating that DEK forms multimers at low salt. The position of form I (supercoiled) and form II DNA (relaxed closed circular and nicked DNA) and of molecular weight markers (kDa) are given in the middle.

FIG. 3. Treatment of DEK-depleted DNA and chromatin with human topoisomerase I. A, model for DEK action on chromatin (see text fur further explanation). B, chromatin separated on 100 mM (+DEK) or 700 mM (-DEK) salt gradients (see Fig. 2) was incubated in the absence (-) or presence (+) of human topoisomerase I. As a control for topoisomerase activity protein-free SV40 DNA was incubated under identical conditions (control). Deproteinized DNA was investigated by agarose gel electrophoresis. C, DEK-associated DNA was first incubated in the absence (+ DEK) or presence (- DEK) of Proteinase K and then in the absence (-) or presence (+) of human topoisomerase I. As a control protein-free DNA was incubated under identical conditions (control). (input -DEK, means SV40 DNA was incubated under the same conditions in the absence of DEK). Purified DNA was separated by agarose gel electrophoresis. The position of form I (supercoiled) and form II DNA (relaxed closed circular and nicked DNA) is indicated.

FIG. 4. Analysis of the supercoiling state. A, DEK-associated DNA was treated with
Proteinase K (-DEK) and then incubated in the absence (-) or presence (+) of either human topoisomerase I or E.coli topoisomerase. The activity of the topoisomerases was tested with protein-free DNA (control). Purified DNA was analyzed by agarose gel electrophoresis. The position of form I (supercoiled) and form II DNA (relaxed closed circular and nicked DNA) is indicated. B, SV40 minichromosomes (a, b) and SV40 DNA (c, d) were incubated in the absence (a, c) or presence (b, d) of DEK (at a molar ratio of DEK to DNA of 50) and topoisomerase I. The purified DNA was separated by two-dimensional gel electrophoresis with the second dimension in the presence of 0.25 µg/µl chloroquine. DNA was investigated by Southern blotting with 32P -labeled Hinf I digested SV40 DNA. The most relaxed topoisomer is defined as LK=0, the positions of positive (LK >0) and negative (LK<0) topoisomers are indicated.

FIG. 5. Sedimentation behavior of DNA and chromatin in the presence of increasing amounts of DEK. 1.5 µg of SV40 DNA (A) or SV40 minichromosomes (B) were incubated in the presence of increasing amounts of the DEK protein and topoisomerase I. Nucleoprotein complexes were separated on glycerol gradients. Proteins were extracted from individual fractions and analyzed on 10% SDS-PAGE followed by Western blot analysis. The DNA was purified and analyzed on a 0.8 % agarose gel. Control gradients were run in the absence of DEK (DEK/DNA ratio = 0) or in the absence of a DNA template (free DEK). The molar ratios of DEK to DNA are 15, 45, 75 and 90. The position of sedimentation markers (S), molecular weight markers (kDa) and form I and from II DNA are indicated.
FIG. 6. **Electronmicroscopical analysis.** SV40 DNA (A) and SV40 minichromosomes (C) were incubated in the absence (A, C, a) or presence of increasing amounts of DEK and visualized by electron microscopy (DEK/DNA ratios compare Fig. 5). Bar represents 100 nm. Note that the graph in C, f is taken at another magnification to visualize compacted chromatin together with protein-free DNA as spreading control. 

**B,** subdivision of the types of DNA molecules observed at a given DEK/DNA ratio are given as percent of the total number of molecules per ratio (around 100).

FIG. 7. **Interaction of DEK with linear DNA.** A 1000 bp DNA fragment was incubated in the absence (-) or presence (+) of DEK (molar ratio: 27 pmole DEK/mole DNA) and topoisomerase I and visualized by electron microscopy. The length of molecules without DEK was measured (= 153,03 ± 7.81; 29 molecules) and compared with DNA molecules uniformly covered by DEK (= 169,68 ± 31,52; 41 molecules) (upper panel). At a molar ratio of 54 moles DEK/mole DNA a heterogeneous population of molecules was observed due to intra- and intermolecular interactions induced by the DEK protein (middle panel). The 1000 bp fragment was reconstituted into chromatin by salt gradient dialysis and the length of the resulting DNA fragments was measured (= 100,84 ± 20,9; 34 molecules) (lower panel). The length of the molecules is given in scan units. Bar represents 100 nm.
Fig. 2
Fig. 3
Fig. 5

Ratio
DEK/DNA

0

15

45

75

90

Free DEK

Sedimentation

Sedimentation

94 kDa
67 kDa

94 kDa
67 kDa

94 kDa
67 kDa

94 kDa
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94 kDa
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94 kDa
67 kDa

94 kDa
67 kDa
Fig. 6

A

B

<table>
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C
The ubiquitous chromatin protein DEK alters the structure of DNA by introducing positive supercoils
Tanja Waldmann, Carmen Eckerich, Martina Baack and Claudia Gruss

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